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VECTOR CONTROL IN HONDURAS:
A REPORT ON
A FIELD TRIAL OF
PROGRAMMATIC ENVIRONMENTAL ASSESSMENT

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PREFACE

The original scope of the consultants' assignment was expanded tremendously after the team's arrival in Honduras. This was done to meet the programming needs of the AID mission in the country; the expanded work had to be summarized on site, in AID format, so that USAID/Honduras could submit on time to AID/Washington the complete program and budget request. A complete countrywide vector control program, including organization, training, methodology, logistics, and budget, had to be designed and detailed in this report as a basis for the new operation and with the understanding that experience and training in basic mosquito control are virtually non-existent in Honduras.

This attempt to provide a safe, efficient, and cost-effective working plan for a complicated long-term program, which would be implemented by people who, although talented and dedicated, have no background in the field, was necessarily time-consuming. The team leader recognizes and accepts full responsibility for the delayed submission of this report and apologizes for those gaps that may still exist in this proposal when it is implemented.

ACKNOWLEDGMENTS

The authors of this report, John Stivers (team leader) and Fred Roberts, acknowledge with profound gratitude the courtesy and cooperation of the APHA Washington staff. They note with awe the staff's success in accomplishing the seemingly impossible task of obtaining visas and airline reservations and tickets, and of providing, within the available time of three working days, necessary instructions, administrative forms, and travel advances to team members at opposite ends of the country.

Further thanks are extended to the overworked USAID/Honduras Human Resources Development staff, who found time to provide background information and to discuss the team's findings and recommendations during one of the mission's busiest and most hectic periods of activity.

The team also wishes to express its gratification for the candor of GOH Vector Control Division (VCD) personnel in discussing their technical problems, and their lack of training and experience in solving them. The team was impressed with their eagerness to acquire such knowledge and experience.

Finally, the team feels that recognition is due to the scores of Hondurans, of all stations, rural and urban, official and private, who, without exception, made the team welcome, frankly discussed their experiences with vectors and vector-borne diseases, and offered their personal opinions on the services provided by the GOH anti-malaria programs, past and present.

EXECUTIVE SUMMARY

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Scope of Work

The original purposes of this consultation were to assist the mission in designing the malaria control portion of its Health Section I Project; to prepare an environmental assessment of the malaria control portion of the project; and to fieldtest the AID malaria control programmatic environmental assessment (PEA) system. The consultants assigned were John Stivers, a medical entomologist with 20 years of experience in malaria eradication program management, much of it in Latin America, and 15 years in vector control at all levels, and Fred Roberts, a medical entomologist with 15 years of experience in vector control program management and one of the designers of the PEA system.

Inasmuch as USAID had had no direct involvement in the Government of Honduras (GOH) malaria program since 1969, other than a short-term emergency relief operation following a hurricane in 1974, it was felt that the current operations of the MOH Vector Control Division (VCD) should be assessed before the effort to design a Health Section I assistance proposal was undertaken.

Constraints

The only constraints on the assignment were total time available and lack of time for consultation. The VCD staff were involved in an emergency program to train 150 soldiers as temporary spraymen and the USAID health staff were occupied with meeting the deadline for the Health Sector project. Consequently, neither group was always able to spare the team the time it needed for direct consultation. Undoubtedly, this would not have been the case under other circumstances.

Observations and Findings

At this time, the VCD is operating as though it were a firehouse responding to alarms and attempting to beat out one brushfire while sparks are igniting two more. Few staff members have had any experience or training, and what little has been acquired is found among mid-level supervisory personnel--a legacy of the former SNEM program, the National Malaria Eradication Service. Unfortunately, vector control techniques, tools, and insecticides are, for the most part, different from and require more precise use than those of a malaria eradication program. Attempting, whether deliberately or subconsciously, to apply the latter to the former is not only counterproductive; it also can be hazardous.

The two key executives of the VCD, the director and the chief of entomology, are both medical doctors who are new to their posts and who have a combined total of less than two months of training in vector control. The entire staff is far short of its authorized strength, especially at the lowest level, the vector control auxiliary (ACV). The ACV is a new position that demands great responsibility, flexibility, and judgment, but it is set at such a low salary scale that only a sixth grade (primary school) education can be required legally. This precludes any hope of future promotion, and men of the caliber required are not attracted to the job.

Because of the manpower shortage, the general lack of experience and training, and the pressure of recurring emergencies, expensive and rather delicate machinery is misused and abused. Dangerously concentrated chemicals are wasted, handled haphazardly, stored where the public has access to them, and applied in a manner that can result in severe damage to health and the environment. For simple lack of knowledge, no attempt has been made to culture or encourage natural predators and parasites of mosquitoes, many potential examples of which the team saw while in the field.

Given these circumstances, it would be foolhardy to recommend that AID assist this program by providing only funds and commodities. This kind of assistance would only expand and perpetuate the existing inefficient and risky operations. However, if correctly handled, AID participation could help the VCD become a model program, a training ground, and a source of information on the value of the "Integrated Pest Management" approach to vector and malaria control for all of Latin America.

Although they have almost no vector control training and experience, the upper- and mid-level personnel of the VCD with whom the team consulted are highly intelligent and dedicated, and they are hungry for knowledge of both the theories of integrated vector control and the "tricks of the trade" which old hands in the profession take for granted. Brought to full numerical strength, given the opportunity for training, and, even more important, given day-to-day guidance in applying this new knowledge to operations, the VCD staff would have all but unlimited potential as human resources for the program. Outside the VCD, the team encountered a number of knowledgeable Hondurans in the government and in the private sector who are mines of information and resources of value to the program. Furthermore, they are eager to share their experience and knowledge.

Conclusions

The team concludes that, given the potential of the in-country staff, AID should provide to the VCD the maximum assistance possible, but that this assistance must be selected and channeled carefully.

Recommendations

1. A full-time technical adviser to the director of the VCD should be provided for at least two, and preferably four, years. This man should be a professional entomologist, be reasonably proficient in Spanish, and have wide experience in U.S.-type integrated vector control programs. He should be knowledgeable in the use of modern insecticide-dispersing devices and the handling and use of potentially hazardous chemicals; be thoroughly familiar with biological control and vector source management, reduction, and elimination; and understand the relatively new concept of vector population management, which uses a current census of vectors and relies on a judgment as to time, place, and method, or combination of methods, used to attack a given population of vectors. (See Exhibit F, a position description, in Chapter III.)

2. A short-term (four months) vector control adviser should be provided to help organize and provide basic theoretical and operational training courses to VCD personnel at all levels.

3. A short-term consultant (or consultants) should be engaged to perform a countrywide survey of indigenous potential parasites and predators of mosquito larvae.

4. A Honduran administrative adviser to the director of the VCD should be contracted for at least one year to provide assistance in improving inventory control, maintenance of vehicles and equipment, communications, paper flow, etc.

5. Commodities such as adulticiding, larviciding, and entomological equipment and supplies should be provided for initial use in basic program-wide training courses and later distribution throughout the country for operational use. A limited number of vehicles and boats should be provided to allow for expansion of the program into areas now being neglected. In addition, provision should be made for some future-year insecticide supplies or earth-moving equipment. The need for these would become evident as the program progresses.

6. Upper-level VCD personnel should receive training outside Honduras and have an opportunity to inspect operating U.S. vector control agencies.

ABBREVIATIONS

ACV	Vector Control Auxiliary
CDC	Center for Disease Control
EPA	Environmental Protection Agency
GOH	Government of Honduras
HRD/H	Human Resources Development/Health Section
IEE	Initial Environmental Evaluation
LECO	Loundes Engineering Co., Inc.
ME	Malaria Eradication
MOH	Ministry of Health
PAHO	Pan American Health Organization
PEA	Programmatic Environmental Assessment
PID	Project Identification Document
SCISP	Interamerican Cooperative Public Health Service
SNEM	National Malaria Eradication Service
ULV	Ultra Low Volume
UNICEF	United Nations Children's Fund
USAID	United States Agency for International Development
USPHS/CDC	United States Public Health Service/Center for Disease Control
VCD	Vector Control Division
WHO	World Health Organization

I. INTRODUCTION AND BACKGROUND

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Purpose of the Assignment and Scope of Work

The original purpose of the assignment, as given by APHA in the initial phone conversations with the consultant team leader and later confirmed by letter, was "to assist the mission in Honduras in an initial environmental evaluation (IEE) of the malaria control segment of the Health Section I Project." The time allowed to accomplish this task was a reasonable three weeks.

As indicated in a previous exchange of communications between the Washington office of the Agency for International Development (AID) and the USAID/Honduras mission, a much wider scope of work was required. It included:

a. assistance to the mission in the design of the malaria control portion of its Health Section I Project;

b. preparation of an IEE on said project;

c. fieldtesting of the AID malaria control Programmatic Environmental Assessment (PEA); and,

d. an estimated four- to six-week period, as necessary, to attain these ends.

On arrival in Tegucigalpa, initial discussions with USAID personnel revealed that, because no one on the mission staff was experienced or knowledgeable in malaria and/or vector control, a wider scope of work was required (see above description). Furthermore, it had to be expanded further to include the following:

e. a rapid evaluation of the existing GOH program to determine the extent of the problem, existing resources, and the methods being applied;

f. determination of whether U.S. assistance is required and, if so, the most effective role AID can play in providing such assistance; and,

g. direct assistance to the mission by the team in the preparation of a Project Implementation Document, an internal project briefing presentation paper, position descriptions, and a preliminary budget for the project.

To accomplish the latter, a training program had to be outlined and, on return to the U.S., the team had to contact a number of commercial firms to obtain or confirm commodity costs.

Mission personnel recognized that the time allowed was totally inadequate for the accomplishment of these multitudinous tasks. They would have been pleased to extend the consultation period to allow for more intensive coverage, but were themselves operating under the constraint of a June 9 deadline for the complete Health Section I Program Submission.

History of Malaria/Vector Control Programs in Honduras

Official campaigns to control malaria or other vectors of human disease in Honduras date back to 1942, when the Interamerican Cooperative Public Health Service (SCISP), an organization jointly managed and funded by AID's predecessor agency and the GOH, initiated epidemiological and entomological studies of the problem. These continued and were supplemented by vector control activities in selected areas through 1948. At that time, available chemical weapons were limited to oil and Paris Green larvicides. Greater reliance was placed on two more permanent control methods: draining and filling vector sources. Some of the lined trunk drains that were constructed in Choloteca during this campaign are still in good condition and functioning. (Interestingly, both the VCD officials who pointed them out to the team and a local newspaper editor, who stopped to see what was happening, mentioned and gave full credit to the joint U.S./GOH agency responsible for their construction.)

In 1949, PAHO, UNICEF, and the GOH, through SCISP, signed a multilateral agreement by which the country began a combined malaria control and *Aedes aegypti* eradication program based on household spraying with DDT and the administration of anti-malaria drugs. Partial coverage was achieved in each of the succeeding years through 1955.

In 1956, the GOH legally created the National Malaria Eradication Service (SNEM), a semi-autonomous, vertically-organized agency under SCISP administrative control. PAHO provided technical assistance and UNICEF contributed equipment and supplies.

SNEM's operations were programmed under the standard WHO eight-year malaria eradication scheme. This plan called for completion, in 1951, of mapping, baseline entomological and epidemiological studies, recruitment, training, and deployment of equipment and supplies. These tasks were accomplished. Maintenance of a lethal residual of insecticide on the interior walls of all dwellings in the malarious areas and the establishment of a case-finding and treatment network to measure the results were programmed for the following four years, 1958-1961. In theory, the spraying could have been stopped at the end of the period of attack and an intensified early detection and treatment program (consolidation) then implemented to mop up the few remaining indigenous and imported cases that occurred in the final three years, 1962-1964. After this, the country could have been declared malaria-free.

In fact, for a number of reasons covered elsewhere in this report, the 1961 objective was never reached. Some areas were passed to consolidation in 1963 and 1964; some of these returned to attack and others advanced to consolidation in 1965. Mass drug prophylaxis was attempted in some areas in 1965, but attaining and maintaining the necessary complete coverage of the population were found to be impossible. Officially, the GOH attributes this failure to a reduction in AID grant funding in the years 1963-1965.

In 1964, a three-year plan was conceived to be funded by an AID loan. During the two years of negotiation which followed, the problem worsened, and by the time the loan was approved (1966), it was already obvious that the funds would be insufficient. The three-year program finally began in 1967. An increase in the loan was approved in 1968. An external evaluation in 1969 indicated that, nationwide, the malaria situation was, if anything, worse than it had been three years earlier.

In 1970, a new seven-year plan of operations was begun with technical assistance from PAHO. The insecticide supply ran out in 1974, spraying was stopped, and malaria rates began to rise again. Hurricane Fifi hit the north coast that year and AID provided some insecticides as an emergency-relief measure. When this supply ran out in 1975, field operations were again interrupted until late 1976. The majority of SNEM's trained and experienced personnel was lost as a result of these repeated layoffs, and the quality of the work deteriorated accordingly.

In 1978, 21 years after the inception of SNEM's original malaria eradication campaign and 13 years after the country was scheduled to have been declared malaria-free, the official malaria rates were slightly higher than they had been at the start of the program (see Table 1). However, in their December 1979 report to the MOH, epidemiologists Campbell, of the Center for Disease Control (CDC), and Lopez, of the MOH, estimated that, in fact, for every case reported in 1978, four additional cases went unreported. If this is true, there must have been nearly 175,000 cases in 1978, and an even larger number would be occurring now.

During the active period of the malaria eradication campaign, a network of village volunteers was established to take blood samples and administer presumptive doses of anti-malaria drugs. These volunteers were recruited, trained, and serviced by SNEM evaluators who frequently picked up blood smears for delivery to SNEM diagnostic labs; replenished drugs, slides, and other supplies; and reported the results of samples diagnosed since their previous visit. The data thus generated were used by SNEM to evaluate progress, delineate potential trouble spots, and follow up positive cases for curative treatment. This system deteriorated with the rest of the SNEM organization, and the size of the sample and the validity of the data decreased accordingly.

For the past several years, a wave of dengue virus has been moving northward through Central America. From its slow, steady progress, it is, apparently, being spread by overlapping host and vector populations and is not "leap frogging" (being transmitted by infected humans as they travel long distances

Table 1

MALARIA RATES FOR HONDURAS*
1958 - 1978

Year	Cases	Cases/100,000 Inhabitants
1958	16,850	921.7
1959	14,650	776.4
1960	6,303	323.2
1961	5,796	306.2
1962	6,750	446.7
1963	7,077	349.7
1964	6,663	535.6
1965	6,952	304.4
1966	17,172	724.8
1967	16,152	660.6
1968	15,666	618.0
1969	29,584	,815.7
1970	34,537	,337.6
1971	48,586	,843.9
1972	18,651	694.2
1973	8,862	340.6
1974	7,503	280.3
1975	30,292	1,100.5
1976	48,804	1,723.7
1977	39,414	1,353.3
1978**	34,606	1,000.1

*VCD 1979 program submission to MOH.

**1978 figures estimated by CDC/MOH epidemiological team to reflect only 20% of actual cases.

or transport accidentally infected vectors--methods of disease transmission that always constitute a potential threat.) This wave-front, which is now being reported in Mexican cities along the Texas border, caused a serious epidemic in Honduras in 1978. Official morbidity and mortality data on this outbreak are scanty, but the epidemic was serious enough that the MOH organized a separate section for emergency control of the vector Aedes aegypti in and around the urban centers. This operation, carried out by a small unit in the MOH, consisted mostly of ULV adulticiding and a minor amount of larviciding.

Thus, in 1978, the incidence of malaria in Honduras was higher than ever before. The large semi-independent organization known as SNEM had failed, after more than 20 years, to reach its eight-year goal, was beset with technical problems not covered in the WHO malaria eradication handbook, was bereft of most of the original trained and highly motivated personnel, and was under-budgeted. At PAHO's recommendation, in 1979, the GOH changed its goal from eradication to control of malaria. To implement this new approach, SNEM was officially disbanded and a Vector Control Dision (VCD) created within the MOH. A one-year trial program was adopted by the MOH with the stated intention of using the experience gained during this year as the basis for programming a four-year operation. The GOH has requested that AID assist the MOH in formulating and funding the latter program.

II. OBSERVATIONS AND FINDINGS

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Current Problems

Honduras has a serious malaria problem, and the potential for a malaria epidemic as well as an epidemic of yellow fever and dengue, should these viruses reappear, is great. There are various levels of endemicity of malaria in 90.4 percent of the geographical area of the country; 87.4 percent of the population are at risk. P. falciparum infections are particularly high in the areas along the northern Atlantic and southern Pacific coasts, indicating that transmission is virtually unchecked. As is usually the case with malaria, those most often and most seriously infected are the rural poor, although in the city of Choluteca sources of Anopheles were observed also; these would readily support transmission to urban areas as well.

In the Atlantic regions, where the vectors are still susceptible to the chlorinated hydrocarbons, residual spraying of the interior walls of dwellings is an effective measure to stop transmission and lower the malaria rates. However, as has been learned through bitter experience, such treatment is of little use unless virtually all dwellings are covered on a timely basis. The program lacks the manpower to accomplish complete coverage. At the time of the team's inspection, 150 soldiers were being trained in this technique; under the supervision of the VCD, they will be expected to provide at least one spray cycle of coverage.

On the Pacific side, vector resistance to the existing residual insecticides is almost total, largely as a result of overuse of the sprays in the cotton and rice fields. Though farmers have shifted to the organo-phosphate insecticides, the vector populations are becoming resistant to these as well.

As a matter of policy, the vertically-organized, semi-autonomous former National Malaria Eradication Service has been disbanded, after several years of insufficient funding and relative inactivity. It has been replaced by the Vector Control Division in the Ministry of Health. Most of the SNEM personnel have left the program, and the VCD is still not completely organized; has many vacant positions, untrained and inexperienced personnel, and an unclear chain of authority; and lacks many of the basic tools and supplies required for a vector control campaign.

Technical Aspects

In the Atlantic coastal regions, residual spraying of dwellings is still effective, but new official policy calls for a reduction of the malaria rates to a tolerable level by decreasing the total population of vectors so that it can no longer support the disease. This method, which predates DDT by almost half a century, is slower in demonstrating initial results; it also is open-ended, unlike the SNEM operation, which was scheduled as a one-time, eight-year project. It is, however, a proven method; the most striking examples of its effectiveness in this

hemisphere are the programs in the Panama Canal and the United States. Among its advantages: the method can be used to control all mosquito-borne diseases (it need not be specifically aimed at malaria); the general annoyance caused by mosquitoes can be reduced greatly; and lands previously uninhabitable because of the mosquitoes or the prevalence of the diseases they transmit can be opened for economic use. There is no reason why such an approach, properly applied and supplemented by an efficient case-detection and treatment system, should not succeed in Honduras.

An efficient mosquito control program uses a three-pronged attack, which is aimed at reducing mosquito sources, larval populations, and adult populations.

Source reduction is normally the preferred method, because it has the longest-lasting effect; is, in the long run, the least expensive; and, where applicable, can reduce to a minimum the need for larviciding and adulticiding. It is accomplished by draining or filling swamps, manipulating the salinity of salt marshes and of water-levels of reservoirs, clearing emergent vegetation, removing artificial containers of water, and stocking with predators or parasites (or otherwise changing) the aquatic environment to make it inhospitable to mosquito larvae. The method of choice varies with the particular source involved, and it requires a study of the specific situation. (For example, drainage of a swamp created by runoff from rice culturing would be the indicated method, provided there was an outlet for the water; however, farmers could hardly be expected to drain the rice field themselves.)

Source reduction is the method of choice, where applicable, because it needs to be done only once per source; only minor maintenance in following years is required.

If the source itself cannot be eliminated or altered, the next best strategy is to attack the larvae, when the mosquito population is still concentrated and has not begun to affect the human population. If the short-lived larvicides now available are used, applications must be timed to hit peak larval densities. This requires constant inspection of sources, so that treatment can be scheduled for maximum benefit, and constant monitoring of the larval populations to determine their resistance to the available larvicides. This treatment should <u>not</u> be applied routinely at fixed times. Larviciding according to the calendar rather than the larval density is costly, may miss complete generations of larvae, and can hasten the development of resistance to the larvicide. (One exception to this general rule may be the storm-drainage system in Choluteca, where the sources are small, easily accessible to spraymen, but almost impossible to inspect.)

The final line of attack is against the adult mosquito. This strategy is the least efficient, because the vector population has dispersed from the source and a much greater territory must be treated in order to control it. Its effectiveness is also transitory, since large populations of vectors can fly into an area a few hours after treatment. However, once adult mosquitoes appear in numbers in populated areas, this is the only means of protection left, and it may be the method of choice against dengue and yellow fever vectors that emerge from a multitude of small sources in urban areas. Modern ultra-low-volume (ULV) machines can be used

efficiently to control adult mosquitoes, but they must be well calibrated and correctly operated, which is not currently the case in Honduras. (See following section on insecticide storage and use.)

As with larviciding, adulticiding should be applied in response to build-ups in a vector population; it should not be applied at scheduled cycles. A continuous census of adult vectors must be maintained in order to detect increases. For such a census, mechanical traps are preferable to hand collections because their use eliminates variations resulting from different individuals' attractiveness to and skill in capturing mosquitoes.

The foregoing basic facts on vector control, though they seem extraneous in a report such as this, were put in the record because the professionals responsible for carrying out the vector control program in Honduras have only a general theoretical awareness of them and no practical experience in applying them.

Insecticide Storage and Use

DDT and propoxur water suspensions are still being used for household interior wall spraying in the Atlantic coast province, where the vectors are still susceptible to the chemicals. The VCD has long experience in this operation, which has been the standard malaria eradication technique throughout the world for over 30 years, with no notable hazard to the spraymen or the householders. Without specifying the exact amount on hand, the director of the VCD assured the team that there is an ample reserve of this material to cover program needs in the foreseeable future. AID will not, therefore, be supplying this commodity. The spread or development of resistant strains of vectors will probably dictate an early end to the use of this technique in any case.

Malathion, in the form of ultra-low-volume mechanically-produced aerosol mist, is the chemical most widely used by the VCD for urban adulticiding treatments. Correctly applied at controlled dosages and in droplets, this material presents no hazard to humans. (See chapter on IEE and Apendices C-E.) However, in bulk, the high (96 percent) concentrate used in ULV application can be extremely hazardous if handled carelessly, and current safety practices of the VCD leave much to be desired. Approximately 100 barrels of this material are stored on a veranda at the VCD headquarters, which is located in a MOH hospital in Tegucigalpa. (The exact amount was impossible to determine because of the manner in which the barrels are stored; barrels of other insecticides are mixed in with the supply.) The barrel currently in use to fill the tanks of the ULV spray machines (LECOs) is positioned on its side, with one end extending over the edge of the veranda. An ordinary unlockable brass water faucet has been screwed into the bung on the overhanging end. There is no nearby water supply for clean-up or emergency wash-down. An obviously seldom cleaned funnel and a bucket used to fill the LECO tanks are left on top of the barrels when not in use. The soil under the barrel tap is saturated from spillage or leakage (there is a puddle of the insecticide on the surface). (A pickup truck which had backed into the area to load several drums of the insecticide bogged down in the mud and had to be pushed out.) The material is freely accessible to hospital personnel, ambulatory patients, and visitors.

The team was disturbed at the time to note that, although some of the barrels (the shipping stencils indicated they were purchased by the VCD) bore the correct EPA-approved ULV concentrate labels, most (those apparently purchased by or through PAHO) were unlabeled and simply bore a stencil stating: "Malathion technical insecticide, for use only by manufacturers in the preparation of malathion insecticides." VCD personnel had not noted the difference and were using interchangeably material from barrels with either marking to fill the tanks of the spray machines. Both are 96 percent concentrates. The team was uncertain whether the remaining 4 percent might contain different solvents or surfactants. On return to the U.S., the chief of American Cyanamid's Research and Development Department was contacted. He assured the team that, according to EPA regulations, although use of the material in the unlabeled barrels for ULV applications would be illegal in the U.S., the material in the barrels with a different marking is identical.

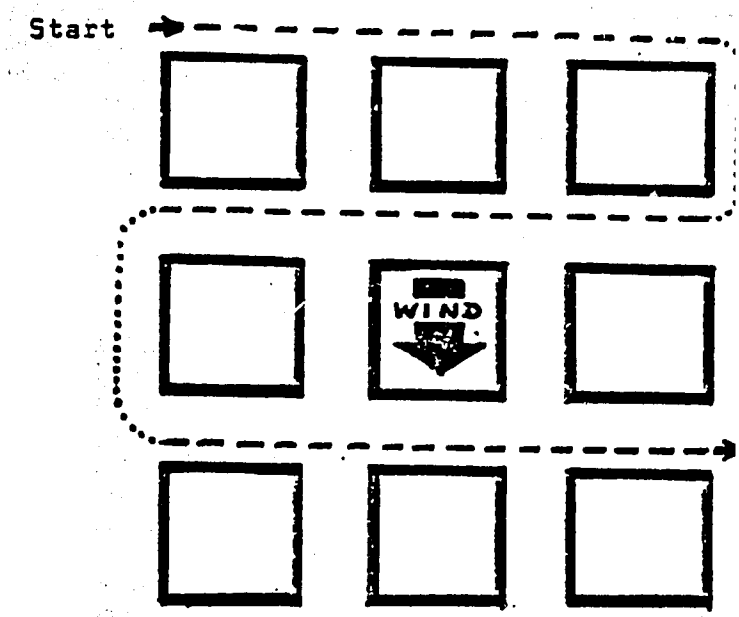
The LECOs themselves are being used incorrectly and perhaps hazardously. Given the extremely low dosages of the highly concentrated material and the need to fill large volumes of air with minute droplets, precise calibration of output and microscopic measurement of the droplets produced are constantly needed. Apparently, the machines used by the VCD have not been calibrated (nor have the sizes of the droplets been measured) since they left the factory, because the program staff lack the knowledge, supplies, and special equipment needed to do the job.

The recommended technique for truck-mounted LECO ULV application is to operate the vehicle at 10 mph-20 mph on a path as nearly at right angles to the wind direction as the layout of the street allows (see Exhibit A). The discharge nozzle is supposed to be pointed straight back, down the path of the truck, and elevated at a 45-degree angle from the horizontal. The objective is to create a cylindrical cloud of fine droplets at a 10-15 foot elevation behind the truck. Before it dissipates, this cloud is moved sideways by the 5 mph-10 mph wind to kill all flying mosquitoes in the block downwind of the city. The miniscule size of the droplets allows them to drift through the tiniest openings, agitates resting mosquitoes into flight, and impinges on and kills mosquitoes in the air.

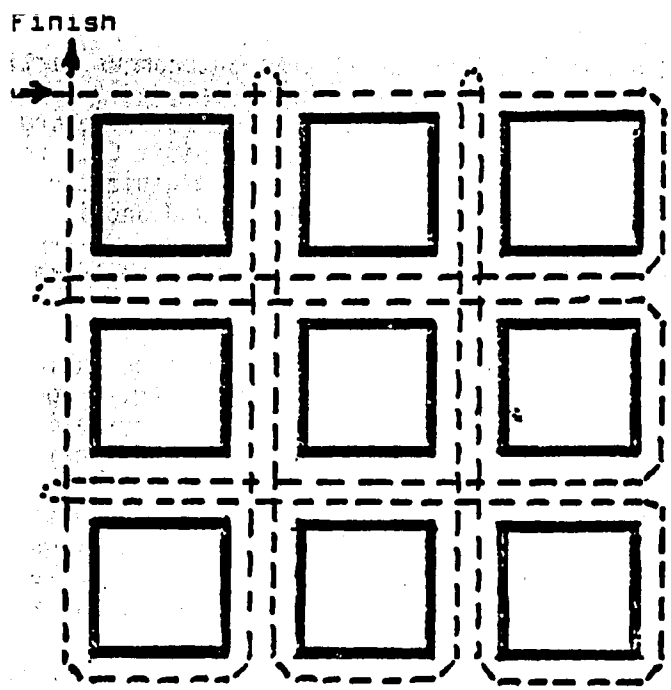
Apparently, the malaria eradication technique of covering interior walls with a residual-action insecticide, which will kill mosquitoes which repeatedly rest upon it, is still being used. The PAHO adviser to the VCD has recommended that the nozzles be turned 45 degrees to the right of the vehicle path so that the insecticide is blown at an angle at the exterior walls of the houses on the right side of the street. He also recommends advising householders along the truck's route to open their doors and windows as the machine passes so that the insecticide can be blown directly inside. (This is a truly thankless task at 4:00 a.m., and one which residents have refused to carry out.) The effect of the wind is disregarded. The adviser's recommendations call for operating the machines first up one side and then down the other side of all streets in the area being treated. Such a pattern wastes the insecticide being blown on house walls, overdoses downwind blocks when there is a crosswind, and sends the droplets straight down the street or back onto the truck when the operator is traveling into or against the wind--an obvious waste of 75 percent of the insecticide,

Exhibit A

ULV APPLICATION PATTERNS



Approved pattern: to treat nine normal city blocks, truck travels 1300 feet, with ULV machine operating during 2700 feet of travel.



Pattern in use by VCD: to treat same area, ULV machine and truck operate through 10,800 feet of travel.

truck and LECO fuel, and manhours. In practice, the operators have in some cases carried these recommendations even further, aiming the nozzle horizontally and directly to the right. The result is that a significant proportion of the insecticide is driven, at high velocity, directly onto exterior house walls, parked cars, and any pedestrians unlucky enough to be abroad. Under such conditions, the advantages of the fine mist are lost because the droplets coalesce on the struck surfaces and run off. This wastes an even greater percentage of the material, damages automobile paint, and introduces a human toxicity hazard (see Exhibit B).

Epidemiology

Under the former SNEM, an efficient network of volunteer collaborators who took blood samples of fever cases in their localities and dispensed presumptive doses of anti-malaria drugs was organized. These volunteers were recruited and trained by the program's evaluators, who kept them supplied, picked up blood samples, and returned the results of the laboratory examinations to them. The program thus received a body of significant data which could be used to evaluate program progress and to indicate areas in need of special attention. As SNEM operations ran down, the collaborator network deteriorated, and the measurement of malaria rates suffered accordingly. The United States Public Health Service (USPHS) and the CDC estimate that, in recent years, for every case diagnosed in Honduras, there are probably four other cases which go undetected.

Organizational Aspects

The former SNEM followed the usual semi-autonomous vertical structure of most eradication programs. It had its own budget, administration, logistics systems, headquarters, and area offices and staffs. The organization was more or less parallel to the Ministry of Health (see Exhibit C). For a temporary crash program, such as an eradication campaign, such organization is ideal in that it permits complete concentration on the task at hand and is not encumbered by politics or diverted by other health responsibilities. However, a permanent national program to control vector-borne diseases should rationally be integrated into the permanent structure of the Ministry.

As the proposed table of organization shows (see Exhibit D), the responsibility for vector-borne disease control policymaking, programming, budgeting, and technical backstopping is placed on the VCD. Operational responsibility is given to the regional and area health offices and local health centers.

In theory, the VCD's supply and transport responsibilities should be delegated to appropriate MOH administrative sections, and blood diagnosis to the MOH Laboratory Division. In practice, this would almost double the workloads of these personnel. It is planned to make the transition in these areas gradually over a number of years.

Ultra Low Volume Aerial Application where automobiles, trailers, trucks and pleasure boats are present

IMPORTANT NOTICE

TO BE APPLIED ONLY BY TRAINED PERSONNEL OF PUBLIC HEALTH ORGANIZATIONS, MOSQUITO ABATEMENT DISTRICTS OR PEST CONTROL OPERATORS.

DIRECTIONS FOR USE

ADULT MOSQUITO CONTROL OVER CITIES, TOWNS, AND OTHER AREAS WHERE AUTOMOBILES, TRAILERS, TRUCKS AND PLEASURE BOATS ARE PRESENT: Apply 2.6 to 3.0 fluid ounces of CYTHION or MALATHION ULV per acre. Aerial spraying should not be attempted when the wind is at or above 10 mph or temperatures are above 82°F.

IMPORTANT—Undiluted spray droplets of CYTHION or MALATHION ULV will permanently damage vehicle paint finishes unless the aircraft used for the ultra low volume application meets all of the specifications listed below:

FIXED WING AIRCRAFT

1. Aircraft is operated at 150 mph or more.
2. There are no leaks in the ultra low volume spray system.
3. Nozzles are placed on the boom at a 45° angle down and into the wind.
4. Diaphragm check valves are used on all nozzles to insure positive cut-off of the spray.
5. Dosage of CYTHION or MALATHION ULV does not exceed 3 fluid ounces per acre.
6. The spray system produces droplets of this product in the 50 to 60 mass median diameter (MMD) micron range, with no more than 10% of the droplets exceeding 100 microns, as determined by readings made from microscope slides coated with DRI-FILM* or TEFLON*.

HELICOPTER

Equipment specifications

1. Rotary nozzle equivalent to Beecomist Spray Head Assembly Model No. 350 equipped with:
   a. a direct reading RPM tachometer or low RPM signal light readily visible to operator;
   b. a stainless steel porous metal sleeve, 20 micron pore size, dynamically balanced to the nozzle;
   c. a diaphragm check valve as near to the rotary nozzle as possible to insure positive cut off of the spray;
   d. nozzle on-off switch separate from main switch and pump switch.
2. Minimum no-load nozzle speed of 10,500 RPM.
3. A continuous nonpulsating metered flow must be maintained by a variable speed metering pump equipped with:
   a. a positive cut off valve between tank and pump;
   b. a flow gauge or tachometer visible to operator;
   c. a pump on-off switch separate from main switch and nozzle switch.
4. Maximum flow rate of 0.5 gallon per minute per nozzle.
5. Rotary nozzle must be mounted behind and below the boom with the sleeve directed toward the rear of the aircraft and parallel to the ground during flight. Nozzle must be positioned to minimize air turbulence and the collection of CYTHION or MALATHION ULV droplets on mounting brackets, feed lines, fittings, etc., or any part of the aircraft.

Operating Procedures

6. CYTHION or MALATHION ULV must be prefiltered through a 10 micron filter prior to transfer into helicopter tank. A 50 mesh stainless steel line strainer must be installed in the pump feed line.
7. Entire system, including tank, pump, nozzle and feed lines, to be used only for application of CYTHION or MALATHION ULV.
8. Entire system must be inspected daily to insure that there are no leaks.
9. Sleeve must be removed and cleaned immediately after each use by washing with hot water and blowing dry from outside in with clean air.
10. Rotating nozzle must be turned on and operating before turning on pump. For shut off, pump must be shut off and lines cleared prior to stopping nozzle rotation.
11. Dosage of CYTHION or MALATHION ULV does not exceed 3 fluid ounces per acre.
12. The spray system must produce droplets of CYTHION or MALATHION ULV with a mass median diameter (MMD) of less than 50 microns, with no more than 2.5% of the droplets exceeding 100 microns, as determined by readings made from microscope slides coated with DRI-FLIM or TEFLON.

*Trademark of General Electric Company

*Registered Trademark of E. I. duPont de Nemours & Co., Inc.

Ultra Low Volume Application
NONTHERMAL AEROSOLS
Applied by
GROUND EQUIPMENT

IMPORTANT NOTICE

TO BE APPLIED ONLY BY TRAINED PERSONNEL OF PUBLIC HEALTH ORGANIZATIONS, MOSQUITO ABATEMENT DISTRICTS OR PEST CONTROL OPERATIONS.

DIRECTIONS FOR USE

Adult Mosquito Control—For control of adult mosquitoes over a 300-foot swath with nonthermal aerosols of CYTHION or MALATHION ULV using the following rates at the indicated vehicle speeds:

Vehicle Speed Rate per Hour	Flow Rate of MALATHION Fluid Ounces per Minute	Maximum Flow Rate per Hour
5	1.0 to 2.1 fluid ounces	1 gallon
10	2.0 to 4.3 fluid ounces	2 gallons

Adult Stable Fly Control—For controlled adult stable flies over a 300-foot swath with nonthermal aerosols of CYTHION or MALATHION ULV using the ultra low volume method, use the following flow rates at the indicated vehicle speeds:

Vehicle Speed Miles per Hour	Flow Rate of MALATHION Fluid Ounces per Minute	Maximum Flow Rate per Hour
5	2.1 fluid ounces	1 gallon
10	4.3 fluid ounces	2 gallons

DROPLET SIZE

1. The Mass Median Diameter (MMD) of the droplets should not exceed 17 microns. The MMD is the drop diameter which divides the spray volume into two equal parts; i.e., 50% of the volume is in the drop sizes below the MMD and 50% is above the MMD.
2. Spray droplets should not exceed 32 microns in size. Three percent of the spray droplets (6 droplets out of 200) can exceed 32 microns providing the MMD does not exceed 17 microns and no droplets exceed a maximum of 48 microns. Larger droplets, when transported by natural air currents, impinge more readily on objects in their pathway and will permanently damage automobile-type paints.
3. More than one-half of the total spray mass must consist of droplets in the 6 to 16 micron range to achieve adequate dispersal of insecticide over a 300-foot swath.
4. A minimum of two-thirds, preferably four-fifths of the total spray mass must consist of droplets not exceeding 24 microns in range.

OPERATING EQUIPMENT

Each Nonthermal Aerosol Generator used for dispersal of CYTHION or MALATHION ULV to control adult mosquitos must have minimum capability of producing the droplet spectrum described under DROPLET SIZE. The initial determination of droplet size is made after the unit is installed in a vehicle and prior to its use in mosquito control operations. The unit should be rechecked as frequently as necessary to insure that proper droplet size is maintained for each operation. Determination of droplet size every two months is usually sufficient if the unit has been maintained in good operating condition. Equipment manufacturer's instructions setting forth cleaning and maintenance of the unit must be followed. The unit must be inspected before each operation to correct any leaks or obstructions in the spray system; to detect whether the nozzle, hoses, or other parts are worn and need replacement; to insure that the flow meter is properly calibrated; and to determine that the pressure recommended by the manufacturer is being maintained.

Flow Rate—must be regulated by accurate flow meter.
—not greater than 1 gallon per hour at 5 mph or 2 gallons per hour at 10 mph.

Nozzle Direction—rear of the vehicle.
—upward at an angle of 45° or more.

Vehicle Speed—not greater than 10 miles per hour.
—shut off spray equipment when vehicle is stopped.

IMPORTANT—Spray droplets of undiluted CYTHION or MALATHION ULV will permanently damage automobile paint unless all the conditions described and recommended in this leaflet are met.

Directions for Determining the droplet size of MALATHION Ultra Low Volume nonthermal aerosols

Permanent records of each droplet size determination must be kept and made available to American Cyanamid upon request.

I. Preparation of Slides with DRI-FILM

CYTHION or MALATHION ULV droplet sizes are determined by depositing a sample of the aerosol on a coated glass slide and measuring the droplets under a high-power microscope. Ordinary 3" x 1" glass slides must be coated with silicone (General Electric SC-87 DRI-FILM) prior to sampling to prevent excessive spreading or coalescence of the droplets. The slides are dipped into a 10 percent solution of DRI-FILM in toluene, drained and dried at about 200°F. for 30 minutes, after which they are dipped in acetone, allowed to dry and stored in a tight slide box. Coating solution must be freshly prepared. Do not store coating solution because it will deteriorate. Slides are lightly polished with a soft tissue before using to remove any foreign particles.

II. Deposition of CYTHION or MALATHION ULV Droplets on Slides

Droplets should be collected under ideal operating conditions to insure representative sampling of droplets in the aerosol. A sample of the CYTHION or MALATHION ULV aerosol is deposited on a slide by waving the slide as rapidly as possible perpendicular through the aerosol cloud at a distance of 25 feet from the point of discharge. The slide velocity may be increased by attaching it to a 3 or 4 foot stick by means of a spring paper clip. At least two slides should be exposed to insure an adequate sample. Store slides in a tight slide box for transfer to a location where measurements can be made. Avoid excessive heat during transit and store in a cool place until measurements can be made.

Although label specifications require the aerosol nozzle to be angled upward at 45° or more during operation, it is more convenient to position the nozzle parallel to the ground for droplet sampling. If this is not possible it will be necessary to be positioned at a sufficient height to obtain a representative sample of the aerosol.

III. Determination of CYTHION or MALATHION ULV Droplet Sizes

A microscope with mechanical stage and an eyepiece micrometer are used to determine the size of the individual aerosol droplets. Prior to taking measurements, the divisions of the eyepiece micrometer must be calibrated into microns by means of a stage micrometer. In the example represented in Table 1, droplets were measured at 400x magnification. At that magnification each division of the eyepiece was calibrated to equal 3.5 microns.

At least 200 droplets should be measured. Usually this is easily accomplished on one slide. An accurate method is to measure all droplets that pass through the micrometer scale as the slide is moved from one edge to the other by using the mechanical stage. Measurements should not be taken along the margins of the slide. It is more convenient to measure in terms of the divisions of the eyepiece micrometer and then convert these divisions into microns.

The measurements converted into microns must then be corrected for the amount of spread that occurred on the slides. The CYTHION or MALATHION ULV spread factor for silicone-coated slides is 0.5. Therefore, in Table 1 each division of the eyepiece actually equals 1.75 microns (3.5 microns times the 0.5 spread factor).

The spread factor for TEFLON-coated slides is 0.69. The following procedure as given for silicone-coated slides, would be the same for TEFLON-coated slides once the value for each eyepiece division has been determined.

The measurements are tabulated and processed as in Table 1. The Maximum Diameter is calculated by converting the diameter of the largest droplet measured into microns. In Table 1, the largest droplet measured had a diameter of 19 eyepiece divisions. Therefore, the Maximum Diameter is 33.3 microns (19 x 1.75 = 33.3).

To determine the Mass Median Diameter (MMD), the accumulative percentages from the last column in Table 1 are plotted against the eyepiece divisions (D) on arithmetic probability paper as in Figure 1. Directly across from the 50 percent point on the line is the median droplet size in eyepiece divisions which must be converted to microns. In Figure 1, 9.2 eyepiece divisions times the conversion factor of 1.75 equals a Mass Median Diameter of 16.1 microns.

Figure 1.
Percentage of the total volume of aerosol samples below each stated droplet size (from Table 1). The Mass Median Diameter is determined from the 50 percent point on the line. The Mass Median Diameter (MMD) = 9.2 divisions times 1.75 = 16.1 microns.

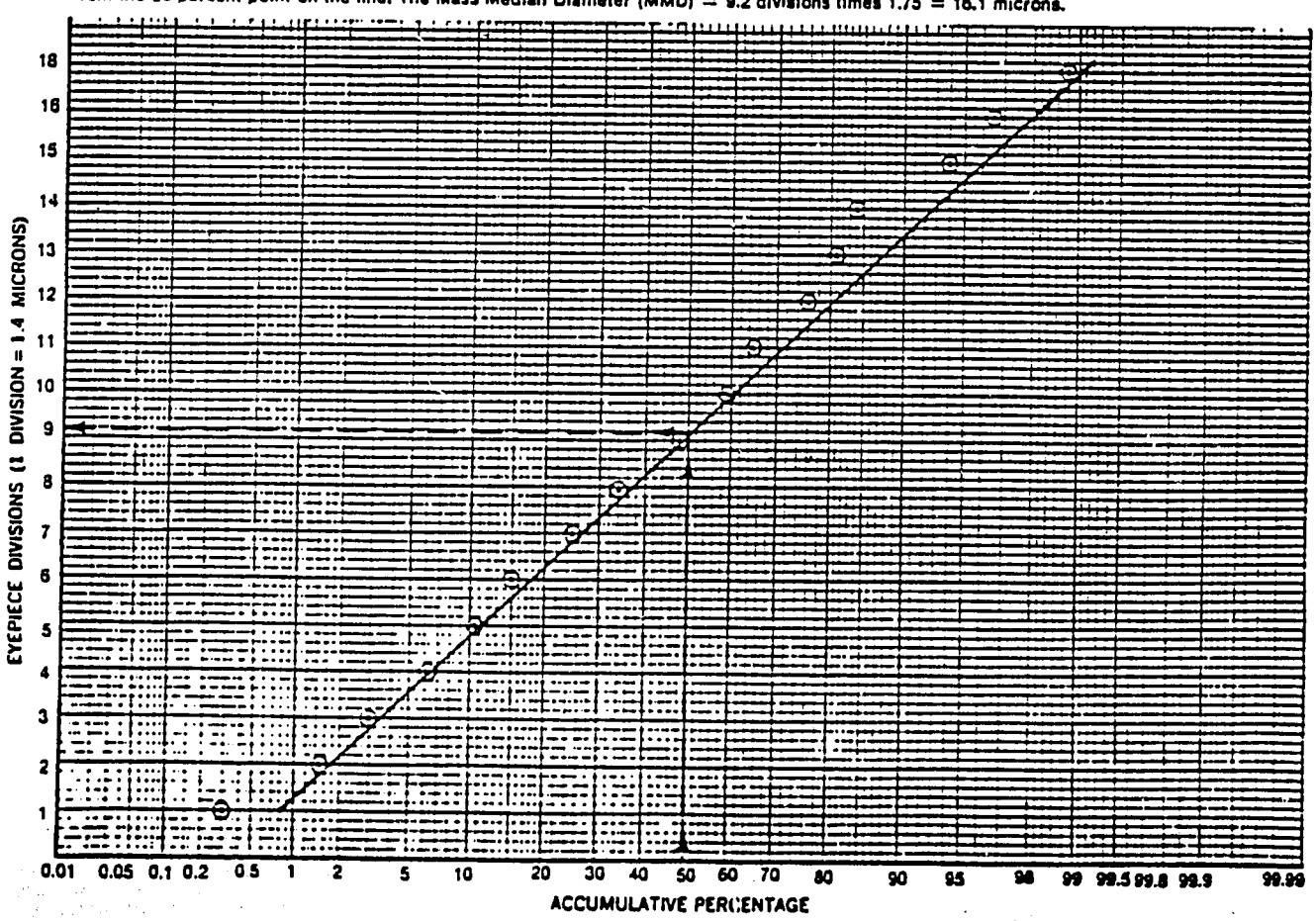


Exhibit C

FORMER SNEM ORGANIZATION

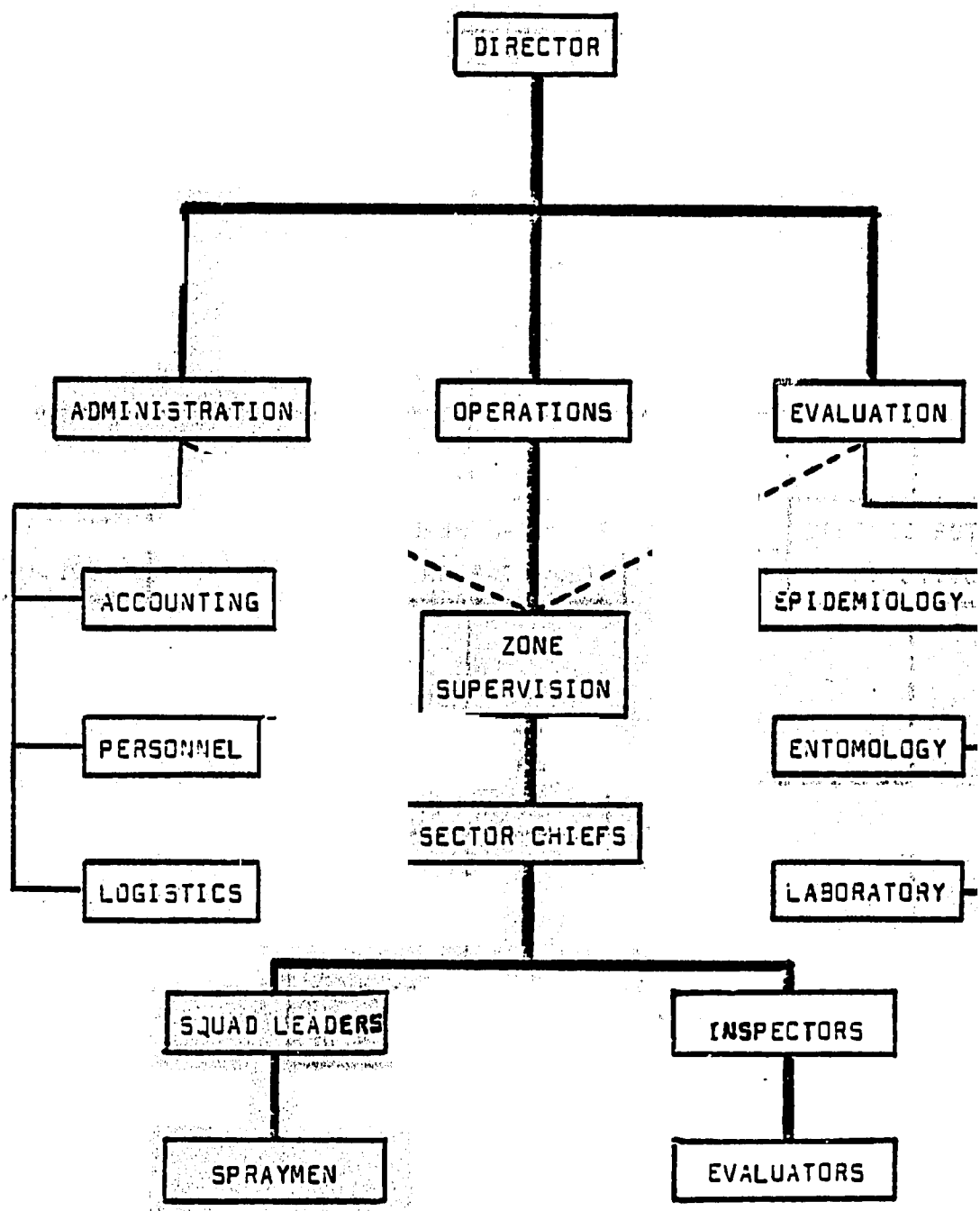
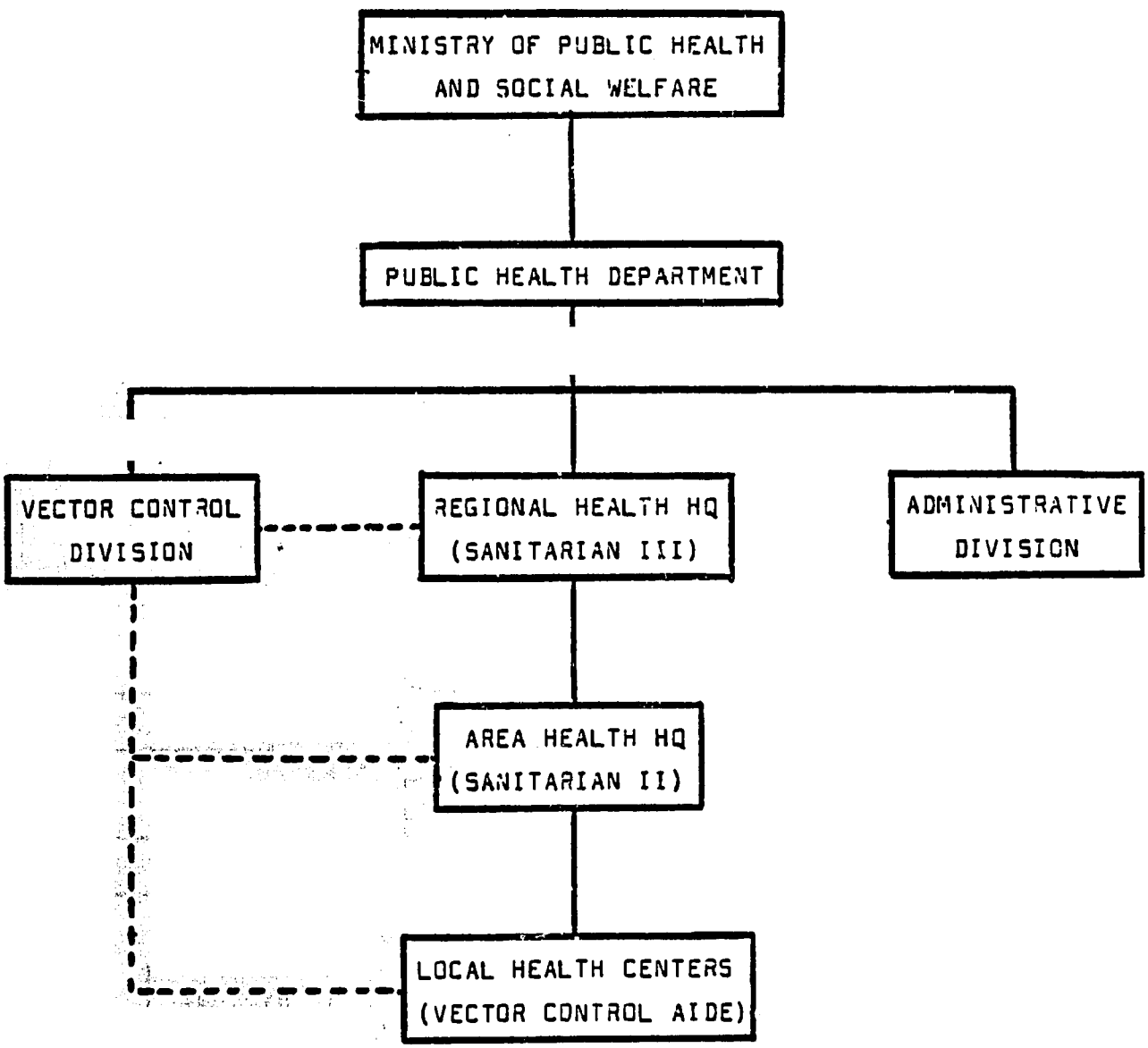


Exhibit D

ORGANIZATIONAL POSITION OF VECTOR CONTROL DIVISION



Personnel and Training

The program's weakest area at this time is personnel and training. If these components are not strengthened, the program has little chance of successfully achieving its goal of efficient vector control.

Several years of underfunding of the malaria eradication program have resulted in the loss of almost all of the program's personnel at all levels. Under the proposed reorganization, MOH personnel will be responsible for implementing malaria control in their respective regions and areas. But they have had little or nothing to do with control of this disease for the past 20 years because of the existence of the parallel organization, SNEM. At the regional, area, and health center levels of the MOH, new positions have been created to assume these added duties and responsibilities, but, for the most part, the positions are vacant. The key top-level executives of the VCD (the director, chief of entomology, and chief of *Aedes aegypti* control) are well qualified medical doctors, intelligent, and highly motivated. Unfortunately, none has any experience or training in vector control. Fortunately, all three recognize and are anxious to remedy this problem, but there is no one at present in Honduras who has the practical and theoretical know-how to help them.

At the field level, a new type of employee, the vector control aide (ACV)* has been proposed. The concept is new, and if the right people can be found to fill these positions, Honduras could become a leader and model for other countries also trying to combat increasing malaria rates that are due to the failure of malaria eradication (ME) techniques.

Under this plan, the ACV would be fully responsible for vector control and case-finding and treatment in an area containing 1,000-1,500 households in 15-25 localities within 15 kilometers of the health center at which he is based. Geographical size would be determined by population density, endemicity, chosen method of control, etc. Each locality would be visited at least twice a month. Obviously, one man cannot perform all the necessary services in such a territory, nor can he maintain vigilance with such infrequent visits. The plan, therefore, is to enlist the community itself in day-to-day operations, with the ACV acting as an educator, trainer, and supervisor of the ongoing work of citizen-volunteers. For projects that are either too large, complicated, or hazardous, the ACV would request temporary help from VCD headquarters.

Past experience with the volunteer blood samplers and medication dispensers indicates that such a plan is workable, but that it depends entirely on the initiative and motivation of the ACV. Unfortunately, this position has been set at the bottom rung of the civil service system; the pay is low and only a sixth grade education is required. Since all but manual laborers in the system require a high school education, such an employee is at a dead end; he cannot look forward to the possibility of promotion. This not only discourages initiative and ambition; it also deprives the higher levels of the knowledge and experience gained by working in the field and made available when vacancies at regional and area levels are filled through promotion.

Also known as Vector Control Auxiliary.

Eleven training courses have been held for prospective ACVs. One hundred eighty-five aspirants started these courses, but only 78 finished, and nine more quit before going to work. Thus, only 69 of the proposed 280 posts have been filled, and the training and capability of these 69 persons are questionable.

One of the reasons for this high attrition rate is that recruiting and training were done in the larger cities, and the largely urban-dwelling student body simply was not interested in moving to some other part of the country and working in a rural area.

Ninety-six light motorcycles were purchased to provide mobility for ACVs with large territories to cover. These have remained in a warehouse, because none of the ACVs knows how to operate them. Also, during the team's visit, 150 soldiers were being trained in house-spraying to provide a cycle of coverage in the Atlantic coast regions, where this ME method still works. In order to train the men in this simple mechanical technique, which has been in use in Honduras for years, it was necessary to bring in a PAHO adviser from Mexico. He had to leave to attend to a more urgent problem in another country. The course was not completed.

Equipment

Except for the few uncalibrated ULV machines of the *Aedes aegypti* program and the remainder of the SNEM vehicle fleet, manual sprayers, and serology microscopes, the VCD is virtually unequipped. Coastal islands with high endemicity and large vector populations are neglected because there is a lack of boats; pickup trucks and jeeps are in short supply and poor condition; and entomological equipment is virtually non-existent. There appears to be a sufficient supply of residual insecticides, but development of vector resistance could render these useless. The VCD has no drainage equipment and no equipment or supplies for collecting, rearing, and distributing vector parasites and predators. At this time, the need for and variety of equipment in these categories are unknown and cannot be determined without further study of the situation.

III. RECOMMENDATIONS FOR VECTOR CONTROL PROGRAM

IV. RECOMMENDATIONS FOR VECTOR CONTROL PROGRAM

1. A negative determination (a complete Environmental Impact Statement is not required) should be reported in the USAID/Honduras Malaria Control Project Initial Environmental Examination (IEE).

2. The Programmatic Environmental Assessment (PEA) of Malaria Control Programs should be adopted, with the modifications noted, by AID as a standard method for making this determination. Appropriate AID personnel should be trained in its use.

3. USAID should provide long-term (four-year) technical assistance to VCD/Honduras that includes provision of a full-time entomologist-adviser to the program. This will facilitate the reorganization and implementation of vector control operations during the critical early years (see Exhibit E, a recommended position description).

4. Short-term (a total of four months) technical assistance should be provided by USAID for the purposes of organizing and teaching a series of vector control training courses for new staff of the VCD and of briefing existing MOH personnel who will be assuming vector control responsibilities.

5. Short-term technical assistance should be provided for the purpose of preparing a country inventory of indigenous potential parasites and predators of vectors.

6. USAID should underwrite the cost of a local administrative adviser to the director of the VCD for one year. This person should assist in improving inventory control, vehicle and equipment maintenance, communications, forms control, etc.

7. USAID should provide the following commodities: adulticiding, larviciding, and entomological equipment and supplies. These commodities should be used initially in the personnel training courses and then distributed throughout the country for use in operations. A limited number of vehicles and boats should be provided to allow for expansion of the program into areas that are not being covered at this time. Provision also should be made for future-year insecticide supplies or earth-moving equipment, the need for which will become evident as the program develops.

8. Upper-level VCD personnel should receive out-of-country training and have an opportunity to observe operating U.S. vector control programs.

9. In order to implement Recommendations 3-7, the detailed four-year budget, which was prepared jointly by this team and the USAID Director of Health and which is summarized in this document, should be approved and funded. (see Exhibit F).

Exhibit E

JOB DESCRIPTION FOR VECTOR CONTROL ADVISER

Assignment

Full-time; minimum two-years duration, to be extended as long as USAID provides significant financial support to program.

Scope of Work

Act as direct technical and operational adviser to director of Ministry of Health Vector Control Division (VCD) and chiefs of entomology and operations sections of the Division in all matters pertaining to insect control operations. These would include training of personnel; safe handling of insecticides; environmental impact; applied field research; continuous routine census of vector populations; evaluation of effect of project operations on these populations; choice of methods, equipment, and materials, etc.

Act as technical adviser to chief of USAID Human Resources Development/ Health Section (HRD/H), providing periodic reports of VCD progress and activities, assisting in preparation of required AID programming and budgetary documents, backstopping, and assisting short-term AID vector and/or malaria control consultants.

The operating base will be located at VCD national headquarters in Tegucigalpa, and not at USAID, and approximately 50 percent of time should be spent in the field. Permanent assignment of 4-wheel drive vehicle will be required to provide mobility throughout country.

Coordination and cooperation with other agencies of GOH and with international organizations will be expected to resolve mutual problems.

Requirements

1. Degree(s) in entomology.
2. Experience in vector control operations and training of personnel.
3. Fluency in Spanish, or willingness and ability to learn the language before the assignment.
4. In good health and physically active.

Exhibit E (cont.)

5. Highly adaptable in personal contacts in order to empathize and communicate with uneducated farm workers on one hand and Ministry of Health, PAHO, GOH, and USAID executives on the other.

6. Interested in adapting and using biological or source reduction methods of vector control and IPM approach of maintaining census of vector and predator populations in order to be able to anticipate the emergence of vectors and to determine and time the method of control that is most efficient in killing the vector but that has the least effect on the environment.

Exhibit F

BUDGET SUMMARY
(4 Years)

	AID	GOH
In-country Training of VCD Personnel (Technical Assistance)	$ 27,000	$123,640
Out-of-country Training of VCD Personnel	26,200	15,900
Field Investigation/Intervention Determination (Technical Assistance)	245,000	166,000
Entomological Survey Equipment	27,100	
Operational Equipment and Supplies	574,400	320,000
Subtotal	$899,700	$625,540*
TOTAL	$1,525,240	

*In addition to these matching categories, MOH funding will also cover all payrolls, local procurement, etc.

IV FIELD TEST OF PROGRAMMATIC ENVIRONMENTAL ASSESSMENT

IV. FIELD TEST OF PROGRAMMATIC ENVIRONMENTAL ASSESSMENT

Introduction

The Programmatic Environmental Assessment (PEA) of Malaria Control Programs is authored by Harvey I. Scudder and Fred C. Roberts under Contract Number AID/SOD/PDC-C-0163, dated August 30, 1979. It consists of a one-volume manual and a guidebook divided into four sections. The manual contains a series of interrelated algorithms supported by the guidebook, which provides resource literature useful in making decisions in the algorithms. The PEA is designed for USAID mission staff to help them select the most effective methods of malaria control with the least impact on the environment. If it is successful, the PEA assists staff in selecting enough cost-effective control methods to ensure long-term control while simultaneously avoiding methods with significant negative impact on the human or natural environment. Ideally, a program formulated through the PEA results in a "negative determination" in the Initial Environmental Examination (IEE).

The field test of the PEA was scheduled for Honduras following a positive determination in an IEE. The determination was made, however, without benefit of specifying the insecticides to be used as larvicides or the types of sources that would be controlled by the physical modifications specified. In light of these circumstances, and following discussions with the concerned officers in the Honduras mission, it was considered most practical to start the process again, formulating a specific program and reissuing an IEE consistent with that program.

A malaria control program was formulated and an IEE on the program has been completed (see Appendix D).

Testing Methods

The PEA was tested by three methods. First, it was used as a supplemental tool in formulating the control methods to be used in the Malaria Control Program. Second, it was the major tool used to develop the IEE on the program. Third, the PEA was critiqued by two individuals who saw the document for the first time: John Stivers, the team leader on this project, and Dr. Thomas Hyslop, chief of the Health Section, AID/Honduras.

The PEA as a Tool to Select Control Methods

The selection of control methods was, for the most part, based on the team's experience. The guidebook was extremely useful because it provided specific and thorough information on areas in which the team lacked experience or where supportive literature was useful. For example, a great deal of useful information about natural and biological control of rice fields was found in the

guidebook. The Honduras mission is now encountering problems in the rice fields. Given this fact and the limitations of control methodologies because of vector resistance to insecticides, this information was valuable in formulating the program. Copies of the literature were well received by MOH staff who were dealing with the problems. Literature in the guidebook on the design and management of reservoirs to prevent production of anophelines also proved to be valuable.

The guidebook was found to be lacking in the areas of adulticiding and ultra-low-volume spray techniques. Also, some technical errors were found in the algorithms. Suggested corrections were written in the margins of the master copy.

The PEA as a Tool to Conduct the IEE

The PEA was used to produce the IEE document. A negative determination was recommended. Its acceptance or rejection in Washington may be the most valid test of the PEA system. The manual was useful in producing the IEE, since it required a detailed step-by-step determination of the safety or environmental hazards of each proposed methodology. In this way, the PEA indicated where safety measures, insecticide application procedures, or other activities of the program needed safeguards.

The guidebook again proved to be helpful by supplying technical information on insecticides and biological control agents and on their impacts on the environment.

The major problem in using the PEA as a tool for an environmental review was that it did not prescribe a clear method of documenting the decisions made in the algorithms. This was needed so that others could follow the logic.

Another problem was that the PEA did not automatically generate a document that would be in a form consistent with current AID documents.

Critique of the PEA

Dr. Hyslop found the algorithms confusing, too complex, and too complete. He felt that there should be some point in the beginning of the algorithms where the mission's program or environmental officer, recognizing that he was entering areas outside his own field of expertise, would be offered an alternative to request specialized assistance in preparing the IEE. It appeared that Dr. Hyslop viewed the PEA, in its present form, as an impediment, and not as a useful tool.

Mr. Stivers, to the contrary, found the PEA quite useful and feels it would be a valuable tool in the hands of technically trained personnel in malaria control programs. He did, however, indicate that the algorithms appeared, at first glance, to be rather formidable and that non-technical personnel unfamiliar with the system and the terminology would, as a result, avoid them.

Conclusions

The PEA appears to be a very useful tool for technically trained personnel in malaria control. Even if it can be simplified, it may not be a useful document for those not trained in biology, environmental sciences, or malaria/vector control. This probably is the result of the inherent complexity of malaria/vector control, and is not necessarily a shortcoming of the PEA itself. If the document is oversimplified, malaria control programs might well fail and significant environmental problems could result.

Recommendations

1. The manual (in particular, the algorithms) should be simplified wherever possible without sacrificing important considerations.

2. Additional information should be included in the guidebook to fill existing gaps (ULV, adulticiding, EPA label information on insecticides, information on *Anopheles* species).

3. The few minor adjustments to correct errors found in the algorithms should be made.

4. The algorithms should be modified as necessary to coordinate more closely with AID's environmental policies and supporting documents.

5. A cross-section of technical, administrative, and programming personnel in the missions should be trained in the use of the document. Its acceptance by these persons should be reviewed.

APPENDICES

Appendix A

LIST OF PERSONS CONTACTED

Appendix A

LIST OF PERSONS CONTACTED

USAID/Tegucigalpa

Thomas Fallon, Financial Analyst, Controllers Office

Thomas Hyslop, M.D., Chief, Human Resources/Health

Lars Klassen, Program and Capital Resources Officer

James Leo, Management Officer

Ronald Meers, Rice Culture Consultant

George Moore, Health Planning Adviser

Marvin Raglan, Rice Culture Consultant

Roger Russel, Engineer, Environmental Evaluation Officer

GOH/Ministry of Health/Vector Control Division

Arturo Maradiago, M.D., Chief, *Aedes aegypti* Section

Carlos A. Pineda, M.D., Chief, VCD

Luis Rivera, M.D., Chief, Entomology Section

GOH/MOH/Other Divisions

Juan de Dios Paredes, M.D., Chief, Sanitary Region II

Juan de Leon Paz, M.D., Chief, Evaluation

GOH/Ministry of Renewable Natural Resources

Gustaro A. Cruz D., Biologist, Wildlife Department

Pan American Health Organization (PAHO)

Alberto Amat S., Engineer, Adviser to VCD/Malaria

Cesar Lizarzaburu, AMRO/Mexico, Training Consultant

Other Agencies and Commercial Suppliers

Robert Taylor, Chief, Vector Biology and Control Division, Bureau of Tropical Diseases, Center for Disease Control, Atlanta

R. E. Deems, Director, Agricultural Research Division, American Cyanamid Co.

Richard J. Nielsson, Director, Latin America/Far East Plant Products Research and Development, American Cyanamid Co.

Howard Barker, President, Environmental Aviation Services, Inc.

William C. Suter, Export Agent, Loundes Engineering Co., Inc. (LECO)

Ben Rainville, Export Sales Manager, Wellcraft Boats

Appendix B

DOCUMENTATION AND CALCULATIONS OF FORESEEABLE EFFECTS OF THE PROJECT COMPONENT ON THE HUMAN ENVIRONMENT (Addenda I-IV)

Appendix B

ADDENDUM I

DOCUMENTATION AND CALCULATIONS OF POSITIVE FORESEEABLE EFFECTS OF THE PROJECT COMPONENT ON THE HUMAN ENVIRONMENT

Positive Effects

1. Prevention of more than 3,650,000 man-days of incapacitation per year.

The population of Honduras in malarious areas totaled 2,670,000 in 1977, representing 91 percent of the total population in that year (Cox and Weissman, 1977). The current rate of malaria cannot be established because of the marked decrease in surveillance efforts. Specialists from the USPHS Communicable Disease Center (AID/Honduras Mission, 1980) estimated that, for every case treated in 1978 (34,606), four cases went unreported. This implies that about 173,030 cases actually occurred in 1980.

An appreciation of the magnitude of the incapacitation caused by malaria can be gained by making the following assumptions:

a. The estimate by malaria control specialists of 173,030 cases of malaria in 1979 is reasonable.

b. An estimated total of 138,424 cases went untreated (173,030 cases - 34,606 treated cases = 138,424).

c. An untreated case of malaria would be expected to incapacitate an individual for 28 days in a year (assuming an average of 14 days in the initial attack and at least one relapse of 14 days) (AID, 1977).

d. The total number of days of incapacitation in 1978 could be estimated to have been: 138,424 x 28 days = 3,875,872 man-days. This is a reasonably conservative figure, since any number of additional work days were probably lost by people who were suffering from malaria and received treatment.

e. The proposed project would reduce malaria cases at least to the level attained in the intensified efforts of 1973-1974, which means the number of cases would be reduced to about 8,000/year (Ministry of Public Health and Social Assistance, 1979).

f. The project would reduce days of incapacitation to 224,000 man-days per year (8,000 cases x 2 days incapacitation = 224,000 man-days of incapacitation (MDI) in one year).

Using the days of incapacitation in 1978 as an estimate of the level expected to occur in the absence of the proposed project, and subtracting the expected MDI level that would occur with implementation of the project, one can derive an estimate of the MDI level that could be prevented by the proposed project:

Prevented Incapacitation: 3,875,872 MDI without program

- 224,000 MDI with program

= 3,651,872 MDI

The above figure represents a conservative estimate, since the estimated number of cases was selected from data collected in 1978. Malaria control has been deteriorating in scope and quality since 1978. This trend is expected to continue in the absence of the proposed project, suggesting that a higher estimate of untreated cases could be justified for the years 1979 and 1980. The number of relapses suffered by untreated patients in a year is also likely to exceed the estimate of one relapse per year for each untreated malaria victim indicating additional days of incapacitation.

An additional component of incapacitation could be added because some cases would probably have relapses in the years beyond that of the initial attack.

2. Creation of valuable human resources by training malaria control specialists.

The beginning phases of the proposed project include a thorough training program to reach the personnel in vital positions, including: the entomologist, supervisors, promoters, and some vector control auxiliaries. The educational program will cover areas necessary to conduct a safe and effective vector control program (safety procedures, monitoring, chemical control procedures, biological control methodologies, physical control, etc.). These trained individuals will become the foundation on which an effective long-term malaria control program can be built.

3. Prevention of the expansion of malaria or higher malaria rates accompanying hydroelectric projects, irrigation, or other water-related development.

At least one hydroelectric project is being planned for Honduras in the near future. This would create a large reservoir in the area of El Cajón. Reservoirs of this kind can create extensive areas suitable as an aquatic habitat for anopheline mosquitoes unless proper preventive measures

are incorporated into its design and management (TVA, 1947). In the Choluteca area, land is being converted to wet production of rice; this has great potential for the production of anopheline mosquitoes. Techniques of design and management might be introduced in the early developmental stages, where it may be possible to assist the rice growers in avoiding excessive anopheline production. The techniques would enhance the natural control components (predatory fish and insects) already known to exist in some local rice fields.

4. Phasing out of interior wall spraying of DDT and/or Baygon and mass drug prophylaxis.

Interior spraying is now done in the northern areas, particularly in the Department of Atlántida. In 1979, a total of 239,454 interior treatments with DDT and/or Baygon were accomplished, exposing 1,213,145 persons to residuals of these insecticides on the walls of their homes (Ministry of Public Health and Social Assistance, 1979).

5. Reduction of existing risks of insecticide poisoning by upgrading safety procedures used in the storing, handling, and application of insecticides used in the malaria control program.

The program would train and equip the Ministry to store, mix, handle, and apply insecticides in a safe manner by:

A. Upgrading existing procedures for insecticide storage and mixing:

1. Use of protective clothing (mask, gloves, etc.)
2. Equipment cleaning procedures
3. Disposal techniques
4. Restricted access to and control of insecticide work area
5. Shower and hand-washing requirements
6. Emergency procedures
7. Insecticide-testing procedures
8. Hazard characteristics of each insecticide and special handling and mixing requirements
9. Ingestion hazards.

B. Upgrading existing larviciding and adulticiding procedures:

1. Use of protective clothing
2. Emergency procedures (field)
3. Label requirements (or special requirements)
4. Calibration of application equipment
5. Pre-treatment sampling (larval densities, biological control)
6. Treatment decisions
7. Post-treatment sampling (detecting field failures)
8. Non-target considerations (drift hazards, etc.).

C. Informing medical staff to provide emergency care to employees or citizens as specified.

D. Upgrading existing facilities (provide showers, hand-washing facilities, limited access where required).

E. Conducting continued training and review.

F. Establishing baseline cholinesterase tests for employees exposed to organophosphate insecticides and requiring routine testing.

6. Reduction of disease and discomfort in the human population now caused by biting and disease-transmitting insects.

The current program of wall spraying is primarily directed at the adult anopheline mosquitoes resting on the walls of houses. A significantly larger number of other pests or vector mosquitoes would be eliminated by the proposed program. Larval control by chemical, physical, or biological methods directed at the source would reduce levels of cohabiting mosquitoes, as well as the anophelines. The adulticiding by ULV would be expected to reduce numbers of pests and vector insects in the terrestrial environment as well as control anopheline mosquitoes. The proposed malaria control program could, therefore, provide additional benefits by interrupting transmission of dengue, yellow fever, encephalitis, and chagas.

7. Indirect effects by avoiding 3,650,000 man-days per year of incapacitation due to malaria

a. Net annual income would increase.

The intermittent disability associated with malaria would be expected to reduce human labor productivity and to have an adverse impact on the individual as well as society in general. The reduced capacity to work leaves the already poor laborer in a continuing state of poverty (Cox and Weissman, 1979). Avoidance of more than 3,650,000 man-days of incapacitation should significantly increase individual income and productivity.

b. Medical costs to society would be reduced (associated with 138,424 fewer cases of malaria).

If the malaria programs were to treat detected cases of malaria and if the number of cases predicted previously actually occurred (138,424 cases), labor for diagnosis and treatment would be required to cure this level of case load. Drugs, transportation, and facilities would also be needed to support a radical cure program. Also, if the individual chose to cure himself, he would incur the costs associated with obtaining the drugs.

The proposed program would reduce the medical costs of labor, equipment, facilities, and drugs that would be required to cure an additional 138,424 cases.

c. The demand on the general health delivery systems would be reduced.

In the absence of an effective malaria program, the additional 138,424 cases would add an additional burden to the general health program. Many of the malaria cases could be expected to join patients with other illnesses at already overburdened hospitals and regiona regional health care centers. The proposed program should significantly reduce the case load at regional hospitals and regional health care centers where malaria would be prevalent.

d. Productivity of the educational system would be increased by increasing teacher and student attendance.

The Government of Honduras is now beginning a campaign to increase its current literacy level. The current rate of literacy in Honduras is reported at 42 percent (Ministry of Health and Social Assistance, 1979). Since 47.9 percent of the population is 14 years or younger (AID/Honduras Mission, 1980) it can be expected that 47.9 percent of the 3,651,873 man-days of incapacitation per year would be incurred by those less than 14 years of age. Undoubtedly, this would have a significant impact on school attendance in malarious areas.

ADDENDUM II

DOCUMENTATION AND CALCULATIONS OF FORESEEABLE EFFECTS OF THE PROPOSED PROJECT COMPONENT ON THE HUMAN ENVIRONMENT

Negative Effects

None.

Malathion will be applied as ultra-low-volume (ULV) insecticide to reduce levels of adult anopheline mosquitoes. The ULV adulticiding will be necessary where interior wall spraying is not effective because of insecticide resistance and until other control methods can be implemented.

The ULV formulation (96 percent malathion) is applied by truck-mounted equipment at a rate of 1.0-2.1 oz./minute at 5 miles per hour. The application is made during a mild cross-wind to produce a swath-width of 300 feet. Droplet sizes are produced in the range of 6-32 microns which readily impinge upon irregular surfaces of small insects.

The application of ULV malathion by these and other methods has been accomplished safely in many mosquito programs in the United States. A comfortable margin of safety is provided. An application of an extremely low volume of insecticide will produce mortality in adult mosquitoes.

The margin of safety provided through ULV methods can be appreciated when one traces the distribution of the amount of ULV malathion that would be required to cause mortality in one person. The lethal dose for an individual weighing 120 pounds would be approximately 55 grams (Oral LD 50 = 1000 mg/kg; lethal dose of 55 kg person = 55 kgs x 1000 mg/kg = 550 mg/1000 mg/1 g = 55 g). This potentially lethal dose, which would have to be ingested to cause mortality, is dispersed by the previously described ULV methods into a volume of approximately 1,070,000 ft^3. (1 oz/35g x 55g = 1.57 oz lethal dose; 1.57 oz/2.1 oz x 440 ft = 328 lineal feet, meaning a 1.57 oz lethal dose is dispersed in 329 lineal feet of application. Assuming a minimum height of 10', the volume would be distributed to $(328)^2 \times 10$ = 1,075,840 ft^3.)

Abate insecticide will be applied as an emulsifiable concentrate diluted to proper field strength, or as 1 percent granules. The maximum application rates are set at 0.5 lbs AI/acre. The insecticide is only moderately toxic with an oral LD 50 of 1000 mg/kg to 1,650 mg/kg. The insecticide was tolerated at levels of 256 mg/man-day for 6 days without effect and has been proven safe for use in drinking water (AID, 1977).

The proposed project component will provide training to upgrade safety procedures and application methods for control of larval and adult mosquitoes (see outline in Addendum 1, Number 5).

ADDENDUM III

DOCUMENTATION OF FORESEEABLE EFFECTS OF THE PROPOSED PROJECT COMPONENT ON ORGANISMS IN THE BIOSPHERE

Positive Effects

The freshwater systems of Honduras contain numerous species of fish in the family poeciliidae, including members of the genus Gambusia and Poecilia (Martin, 1972; Diaz, personal communication, 1980). It is likely that one or more species of these native fishes will possess the desirable characteristics to be utilized as useful biological control agents in the malaria control program. Production of anopheline mosquitoes in many aquatic sources, such as rice fields, ditches, seepages, and stock ponds, might be controlled by the translocation of these native fishes. The sources would, for the most part, be enhanced environmentally by such an approach, since the species diversity is increased and, in some cases, an additional trophic level is added to the system.

Although the IEE is not requesting clearance for these procedures at this time, the proposed project component includes funding for a biological control inventory which would assist in determining the feasibility of such an approach.

ADDENDUM IV

DOCUMENTATION OF FORESEEABLE EFFECTS OF THE PROPOSED PROJECT COMPONENT ON ORGANISMS IN THE BIOSPHERE

Negative Effects

Our investigations of riverine, rice, and back-yard sources of anopheline mosquitoes revealed a significant number and diversity of insect predators (Odonata, Notonectidae, Dytiscidae, Gerridae (?) Hydrophilidae (?)). Applications of larvicide (abate) and ULV adulticide (malathion) may reduce numbers of these predators.

Strict procedures are outlined in the mitigation measures to avoid direct mortality to beneficial insects or disruption of natural biological control (see mitigation measures).

Both abate and malathion insecticides degrade in the environment with no evidence of bioaccumulation (AID, 1977).

Appendix C

CHECKLIST FOR INITIAL ENVIRONMENTAL EVALUATION

Appendix C

Health Sector Project

IMPACT IDENTIFICATION AND EVALUATION FORM

CHECKLIST

Impact Areas and Sub-areas	Impact Identification and Evaluation 1/
A. LAND USE	
1. Changing the character of the land by:	
a. Increasing the population.	N
b. Extracting natural resources	N
c. Land clearing.	N
d. Changing soil character.	N
2. Altering natural defenses.	N
3. Foreclosing important uses	N
4. Jeopardizing man or his works.	
5. Other factors	

1/ Explanation of Symbols

N - No environmental impact
L - Little environmental impact
M - Moderate environmental impact
H - High environmental impact
U - Unknown environmental impact

IMPACT IDENTIFICATION AND EVALUATION FORM

B. WATER QUALITY

1. Physical state of water. . . . . N
2. Chemical and biological states . . . . . L (see Part II)
3. Ecological balance . . . . . L (see Part II)
4. Other factors

_____ _____

_____ _____

C. ATMOSPHERIC

1. Air additives. . . . . N
2. Air pollution. . . . . N
3. Noise pollution. . . . . N
4. Other factors

_____ _____

_____ _____

D. NATURAL RESOURCES

1. Diversion, altered use of water. . . . . L (see Part III)
2. Irreversible, inefficient commitments. . . . . N
3. Other factors

_____ _____

_____ _____

E. CULTURAL

1. Altering physical symbols. . . . . N

IMPACT IDENTIFICATION AND EVALUATION FORM

E. CULTURAL (continued)

2. Dilution of cultural traditions. . . . . N

3. Other factors

F. SOCIOECONOMIC

1. Changes in economic/employment patterns. . . . . H (See Part III, 1, 2, 7)

2. Changes in population. . . . . N

3. Changes in cultural patterns . . . . . N

4. Other factors

G. HEALTH

1. Changing a natural environment . . . . . N

2. Eliminating an ecosystem element . . . . . N

3. Other factors

Reduced Malaria Rates H (see Part III)

H. GENERAL

1. International impact . . . . . N

2. Controversial impacts. . . . . N

3. Larger program impacts . . . . . N

IMPACT IDENTIFICATION AND EVALUATION FORM

H. GENERAL (continued)

4. Other factors

_____ _____

_____ _____

I. OTHER POSSIBLE IMPACTS (not listed above)

_____ _____

_____ _____

_____ _____

Appendix D

INITIAL ENVIRONMENTAL EXAMINATION
(Parts I-IV)

Appendix D

Part I

INITIAL ENVIRONMENTAL EXAMINATION

Project Location:	HONDURAS
Project Title:	HEALTH SECTOR I (522-0153)
Funding:	$8.0 Million Total 6.0 Million Loan 2.0 Million Grant FY 80 $4.34 Million FY 81 $3.66 Million
Life of the Project:	4 Years, CY 1980-1983
Malaria Control Section Prepared By:	John Stivers Consultant, APHA Fred C. Roberts Consultant, APHA Lawrence J. Klassen Assistant Capital Development Officer
	Date: June 5, 1980

Threshold Decision:

a. Recommendation:

Negative Determination
(Full Environmental Impact Statement Not Required)

b. Concurrence:

DATE:

John R. Oleson, Mission Director, USAID/Honduras

Part II

DESCRIPTION OF THE PROJECT COMPONENT

The object of the malaria control component is to assist the Honduras Ministry of Health in developing a program capable of providing long-term, effective malaria control to the rural and marginal urban areas. Malaria is now found in 90 percent of the country containing about 87 percent of the population (AID/Honduras Mission, 1980). The existing program consists of malaria eradication efforts (wall spraying with DDT and Baygon) and emergency responses to epidemics by mass drug prophylaxis programs, radical cures of cases, and insecticidal control of adult stages of the malaria vectors. The rate of malaria is increasing rapidly because of administrative problems in the malaria program and because the malaria vectors have developed resistance to insecticides in some areas of the country (Ministry of Public Health and Social Assistance, 1980).

The project component is designed to provide training, technical assistance, and equipment to upgrade the existing malaria control program. The objective would be to reduce malaria rates to acceptable levels by integrating additional vector control measures into the program to suppress the vector population.* The type and number of control methods to be employed will be determined by consideration of numerous factors, including costs, environmental risks, safety hazards, health benefits, insecticide resistance, etc. The project component would provide the training and equipment necessary to support an integrated approach to malaria control consisting of the following measures:

1. Space-spraying of adult vectors with ultra-low-volume (ULV) spray equipment using malathion insecticide.

2. Application of a larval insecticide (abate) to prevent emergence of anopheline mosquitoes at the aquatic source.

3. Cooperative planning efforts to prevent vector sources that might otherwise be created as a byproduct of development projects (highways, hydroelectric projects, etc.)

4. Recognition of existing natural control of the vectors and employment of methods to enhance natural control and to introduce biological control.

5. Physical modification or elimination of the aquatic sources of the anopheline vectors.

---

* An acceptable level of malaria is considered to be that level of malaria that can be effectively detected and cured without detrimental impact on other health programs.

The integrated program would reduce the high malaria rates by the immediate employment of "short-term" methods using insecticides (numbers 1 and 2 above). These measures would be supplanted, and possibly replaced, by long-term methods as the program evolves (numbers 3-5 above). The integrated program would be expected eventually to phase out the need for wall spraying (DDT and Baygon) and for the mass drug prophylaxis programs now being employed.

The initial environmental analysis has been accomplished by a procedure titled the Programmatic Environmental Assessment of Malaria Control Programs (Scudder and Roberts, 1980). Two insecticides have been reviewed by the process: abate, to be used as a larvicide, and Malathion, to be used as a ULV adulticide. As the vector control program is further developed, selected chemical, biological, and physical control methodologies will also require environmental review. At that time, an environmental determination (positive or negative) will be made by the field adviser, using the programmatic assessment methods (see attached algorithms), which will be reviewed as required.

Part III

IDENTIFICATION AND EVALUATION OF ENVIRONMENTAL IMPACT

Reasonable Foreseeable Effects of the Proposed Project Component on the Human Environment - Nature, Scope, and Magnitude

Positive Effects

The proposed project component, if successful, will have a substantial positive impact on the human environment. The following is a list of positive effects; scope and magnitude are described where possible (see Part I for calculations and documentation):

1. The project component would prevent the loss of more than 3,640,000 man-days of incapacitation because of malaria.

2. The project component would create valuable human resources by training malaria control specialists to function as an integral part of the general health delivery system.

3. The project component would prevent the creation of endemic foci, or higher malaria rates, that could accompany hydroelectric projects, irrigation, or other water-related developments.

4. The proposed project component would reduce risks associated with insecticides and drugs by phasing out the household spraying (DDT and Baygon) and mass drug prophylaxis now employed in some areas of Honduras.

5. The project component would decrease the existing risks of insecticide use by upgrading safety procedures used in the storing, handling, and application of insecticides in malaria control efforts

6. The control of vectors of malaria by the proposed project component would not only result in a reduction of malaria rates, but should also significantly reduce other vector-borne diseases and discomfort in the human population now caused by biting mosquitoes.

7. Numerous positive, indirect effects of the project would be accrued by the avoidance of more than 3,650,000 man-days of incapacitation per year caused by malaria.

   a. Net annual income would increase as a result of avoidance of incapacitation by malaria, thereby increasing general productivity.

b. Medical costs to society would be significantly reduced as costs associated with 138,424 treated cases would be avoided.

c. Demand on the general health delivery system would be reduced by the avoidance of 138,424 cases of malaria.

d. The productivity of the educational system would be enhanced by increasing teacher and pupil attendance by an amount equal to that which would be lost to teachers and students incapacitated by malaria.

Negative Effects

None. (See Part II.)

.easonable Foreseeable Effects of the Proposed Project Component on Organisms in the Biosphere

Positive Effects

1. Numerous aquatic habitats characterized by simple and truncated food webs and high production of anopheline mosquitoes will be diversified by the addition of predatory fish indigenous to Honduras. (See Part III.)

Negative Effects

1. Application of malathion at ultra low volume (ULV) to control adult anopheline mosquitoes may reduce numbers of beneficial insects (bees, adult dragon flies, hymenopteran parasites, insect predators, etc.) (See Part IV.)

2. Applications of abate as a larval insecticide to control anopheline larvae in the aquatic habitats may reduce levels of cohabiting insect predators, causing disruption of natural control. (See Part IV.)

Mitigation Measures

1. Strict procedures will be established in the project component to ensure that preventive measures are taken before ULV is applied.

a. A survey of the target area to detect domestic or agricultural bee-keeping facilities.

b. A survey of the target area to determine the general level of beneficial and pest insects.

c. Systematic warning to bee-keepers in the target area to protect or remove their bee colonies.

d. Periodic surveys of indicator species in the target area to detect significant increases in pest insects or decreases in beneficial insects associated with the application of ULV malathion.

2. Training will be conducted and operational procedures established to enable operational personnel to detect and measure the natural control of anophelines that may be provided by insect predators in the larval sources. In this way, the operational personnel can avoid applications of abate larvicide that might disrupt significant natural control being accomplished by insect predators.

Part IV

RECOMMENDATION FOR ENVIRONMENTAL ACTION

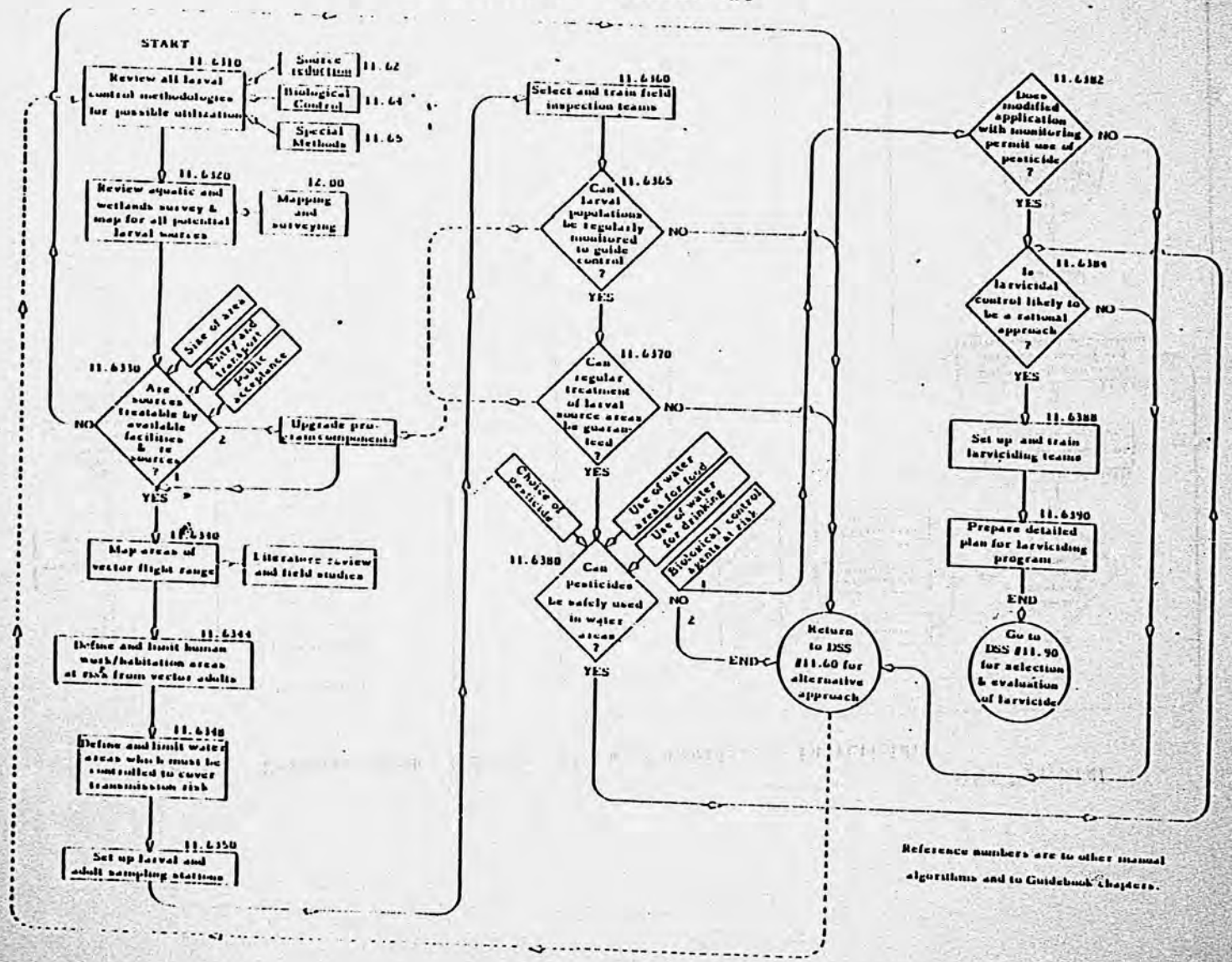
A negative determination is recommended for the proposed malaria control program. The Initial Environmental Examination indicates that the human environment will gain substantially by the implementation of the project component and that the minor environmental impacts will be mitigated or avoided by measures that are to be incorporated into the program.

Appendix E

ILLUSTRATED PROCEDURES FOR VECTOR CONTROL

Select Chemical Larvicide Methodology

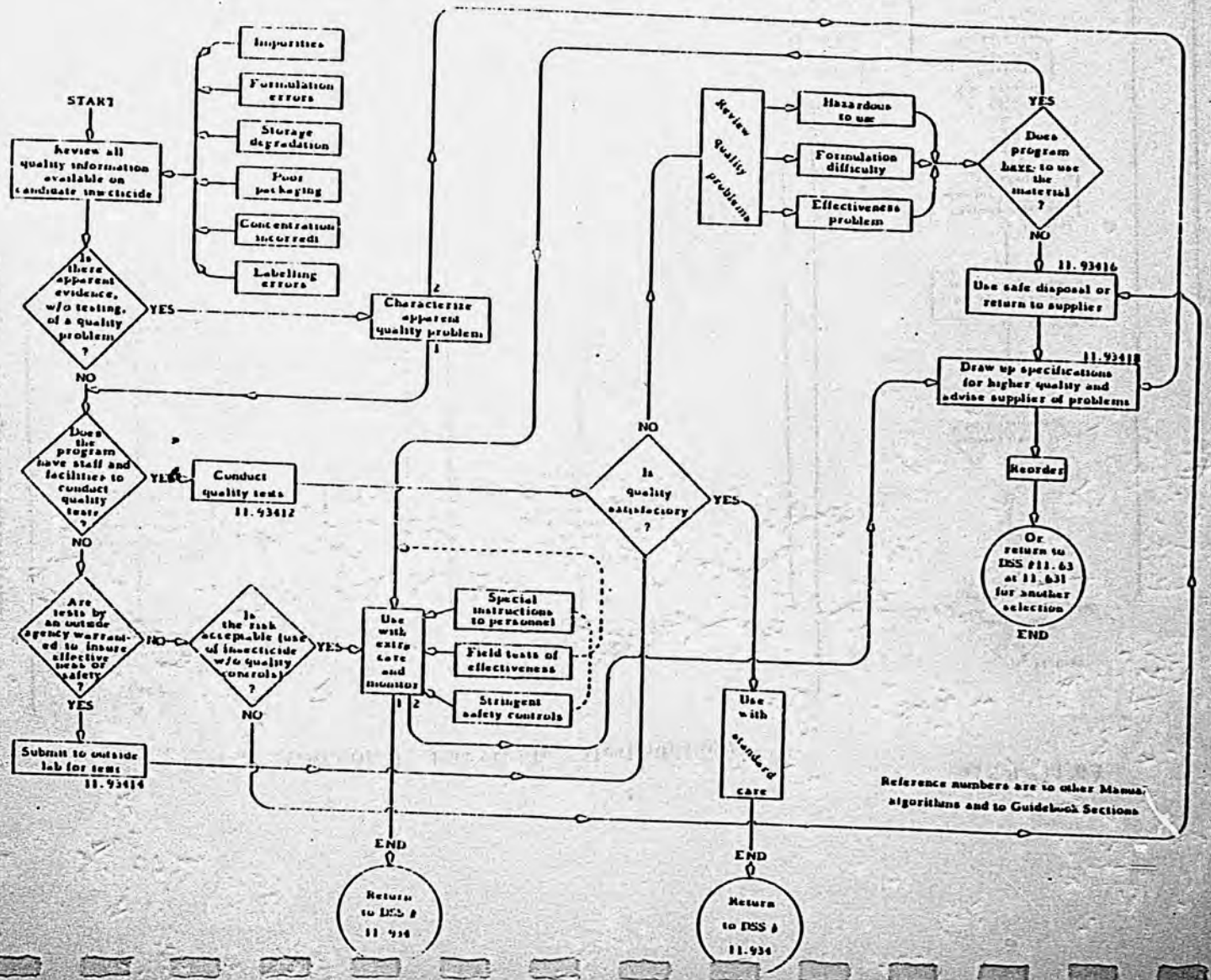
D.S.S. #11.63



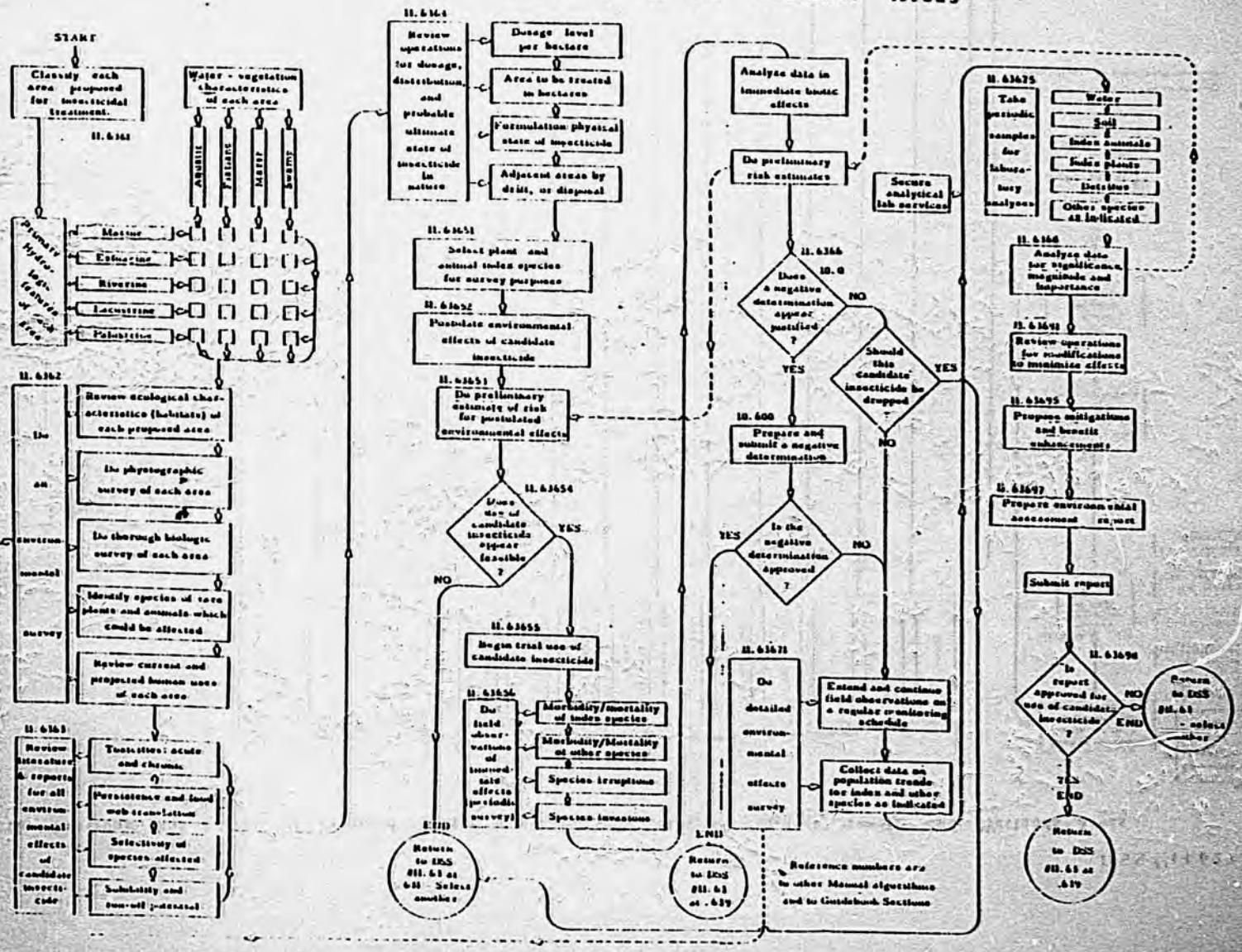
Reference numbers are to other manual algorithms and to Guidebook chapters.

Review Problems With Quality of a Candidate Insecticide

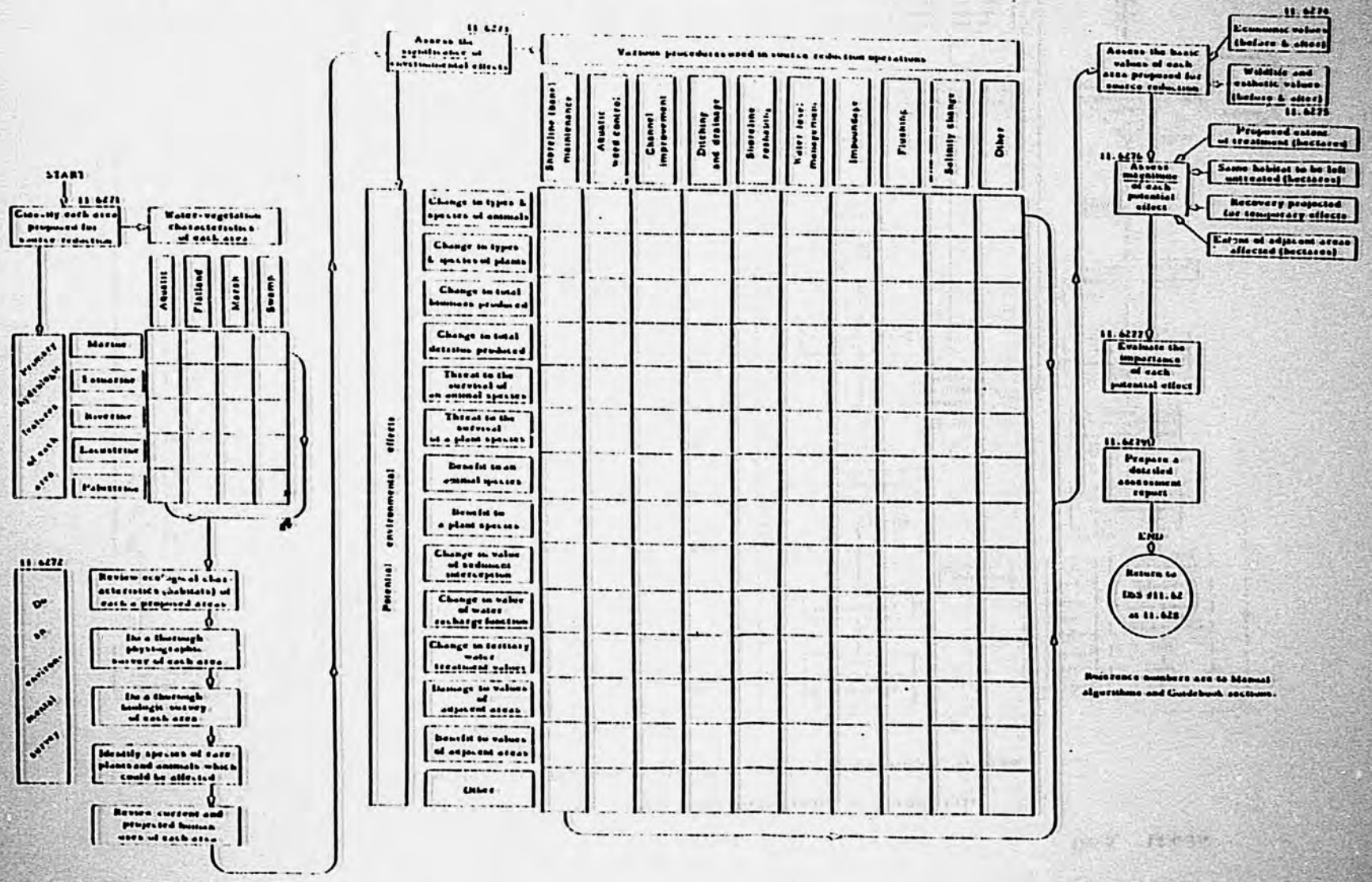
DSS #11.9341



Assess the Environmental Effects of a Candidate Insecticide on Natural Aquatic and Wetlands Areas

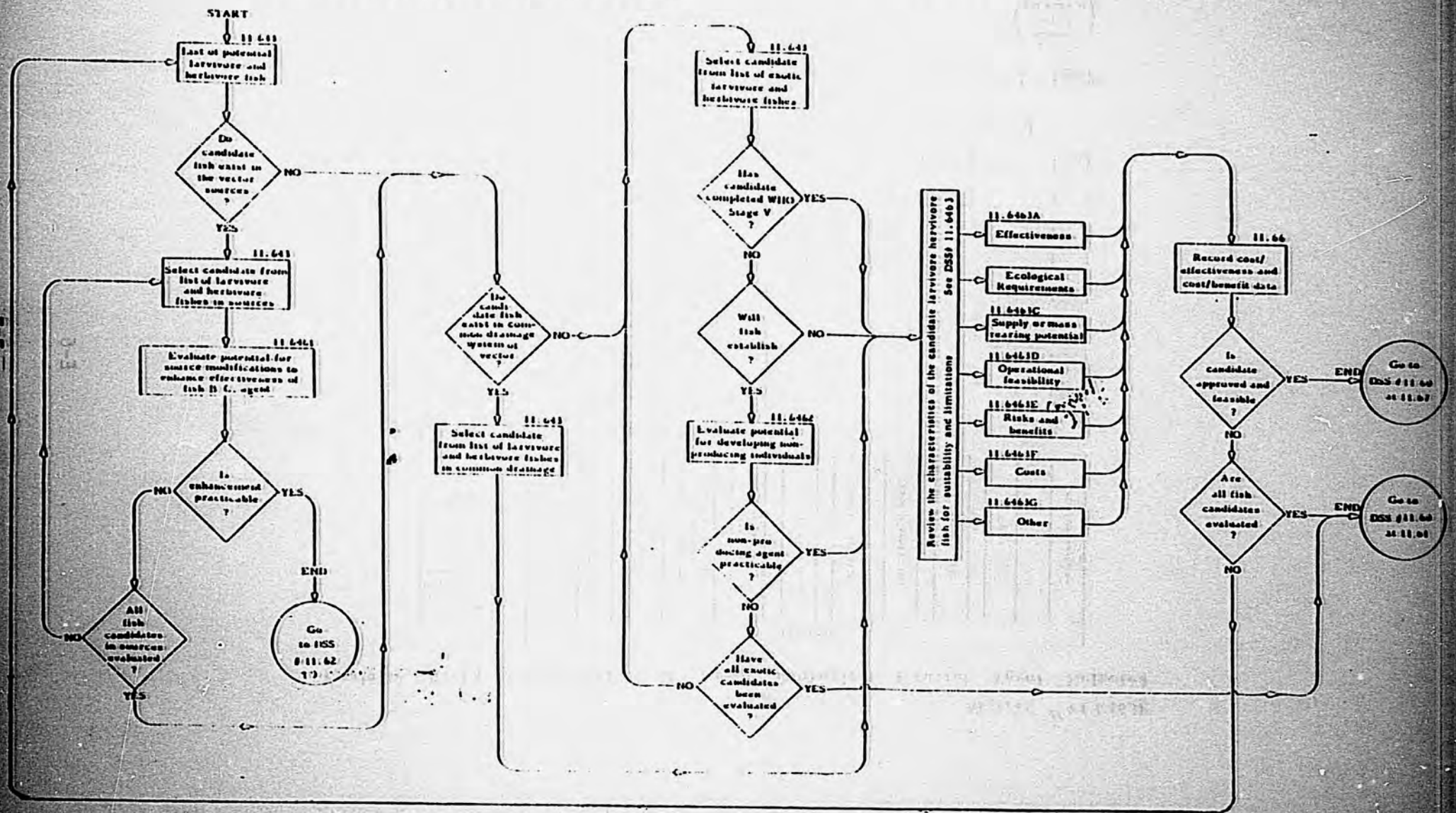


Assess the Environmental Effects of Proposed Source Reduction Measures on Natural Aquatic and Wetlands Areas



To Select Larvivore or Herbivore Fish

D.S.S. #11.646



Matrix #11.6463E

Evaluate Risks and Benefits of Local Biological Control Agent Selected

Evaluate the significance, magnitude & importance of the biological control agent in its local ecological effects

Will it:

INTERACTIONS	Rare and endangered plant or animal species	Sport or commercial fishes	Productive plant species	Pest or noxious animal or plant spp.	Resident or migratory birds	Food chains or webs	Species diversity	Rate of productivity	Nutrient budget	Other	Total/interactions
Prey upon											
Compete with											
Hybridize											
Infect											
Become prey for											
Decrease											
Increase											
Disrupt											
Stabilize											
Invade other habitats	Repeat above for each ecologically different Anopheline source area										
Total/targets											END

Reference numbers are to Guidebook Sections.

Return to DSS #11.6463 at 11.6463E

Appendix F

BIOCONTROL AGENT DATA SHEET

Appendix F

BIOCONTROL AGENT DATA SHEET

I. Candidate Biocontrol Agent: *Romanomermis culicivorax*

II. Targets: *Anopheles quadrimaculatus*, *An. crucians*, *An. freeborni*, *An. albimanus*, other mosquitoes.

III. Sources: Rice fields, semi-permanent and permanent freshwater, sewage-settling tanks.

IV. Feasibility Factors:

A. Enhancement Methods: Used in conjunction with an insect growth regulator, Altosid, enhances kill to 100% and enhances recycling of mermithids (Finney et al., 1977); control reduced in sites with dense vegetation or algal mats (Platzer, 1979), suggesting vegetation or algal control would enhance effectiveness.

B. Stage in WHO Scheme: Completed Stage V (Laird, 1977).

C. Establishment Potential: Recycling possible (Levy et al., 1979).

D. Ecological Requirements: Low salinity freshwater required (Levy et al., 1979); high concentrations of calcium, nitrates, nitrites, and phosphates particularly toxic to preparasites (Platzer, 1979); parasites resurged after source dry for five weeks (Peterson and Willis, 1975); contradiction on effectiveness in polluted water--one study said highly polluted water had no adverse effect (Levy and Miller, 1977), while another suggested low oxygen levels reduce infectivity and control, can overwinter in ricefields (Platzer 1979). Optimum temperature of water for infectivity is 21°C-33°C.

E. Level of Effectiveness: 43.8 percent - 66.7 percent parasitism rate, physiologic resistance evidenced, search range a few feet (Levy et al., 1979); 45 percent - 85 percent infection rates, anophelines more susceptible than culicines, dense vegetation or algal mats reduces control, high levels of predator copepods reduce effectiveness (Platzer, 1979). Efficiency beyond that of *Gambusia* and *Toxorhynchites* (Laird, 1979).

F. Supply and Availability: U.S. registered product, "Skeeter Doom," Fairfax Biological Laboratory, Clinton Corners, New York (Laird, 1979).

G. Mass Rearing Technology: *In vivo* rearing, six days; *in vitro*, six weeks (Laird, 1977).

H. Application Methods: Readily adaptable to current mosquito control technology (Platzer, 1979); aerial or ground spray systems usable (Levy et al., 1977).

I. Field Detection Techniques

J. Other: Product viability problems remedied by Nutrilite Products, Inc., Lakeview, California (Laird, 1979).

V. Environmental Impacts:

Completed WHO five-stage scheme (Laird, 1977); specifically parasitizes only mosquito larvae (Platzer, 1979); establishes (Levy et al., 1979); virtually host-specific for mosquitoes (Levy and Miller, 1977); non-pathogenic to rodents, fish, and aquatic crustaceans; cases of association of mermithid larvae with man in mouth, urinary passages and faeces, but none found in tissues of man (Brown, 1974).

VI. Environmental Indicators

VII. References:

Brown, 1974; Finney et al., 1977; Laird, 1977; Laird, 1979; Levy and Miller, 1977; Levy et al., 1979; Petersen, 1978; Platzer and Stirling, 1978; Platzer, 1979; Washino and Fukushima, 1978.

BIOCONTROL AGENT DATA SHEET

I. Candidate Biocontrol Agent: *Poecilia reticulata* (The Guppy)

II. Targets: Primarily for culicines, may be useful for anophelines.

III. Sources: Urban streams, ponds, sewage oxidation ponds, ditches.

IV. Feasibility Factors:

A. Enhancement Methods

B. Stage in WHO Scheme: No indications that tests have begun.

C. Establishment Potential: Potential for establishment high in sources with favorable ecological parameters, will not establish in presence of piscivorous fish (Bay, 1972).

D. Ecological Requirements: Requires relatively warm waters, demonstrates high pollution tolerance (Bay, 1972).

E. Level of Effectiveness: Full-grown females may consume up to 150 third and fourth instar mosquito larvae per day, males up to 20 larvae per day (Mallars and Fowler, 1970).

F. Supply and Availability: Guppies are native to Central and South America and may be obtained from mosquito control agencies using fishes (introduced to Hawaii, Malaysia, Bangkok, Thailand, Burma, and the Philippines). Guppies may also be obtained from commercial fish suppliers.

G. Mass-Rearing Technology: Reproductive rate surpasses *Gambusia* (Mallars and Fowler, 1970), suggesting cultural methods similar to *Gambusia* can be used.

H. Application Methods: Collecting, rearing, and stocking methods would be similar to those of *Gambusia* (Sholdt et al., 1972).

I. Field Detection Techniques: Aquatic or dip nets, minnow seine nets, and fish traps may be used (Sholdt, et al., 1972).

J. Other

V. Environmental Factors:

May out-compete native fishes but will not establish itself in presence of piscivorous fish (Bay, 1972); may be useful in waters with intermittent or permanent pollution problems that are intolerable to other fish.

VI. Environmental Indicators:

Presence of non-piscivorous fishes occupying similar niche may result in competitive displacement.

VII. References:

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Appendix G

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A NOTE TO READERS

The following are 10 reprints of works listed in the bibliography. They were selected because they are directly applicable in Honduras and are representative of the wealth of literature available to the program.

ADVANCEMENTS IN THE USE OF FLATWORMS FOR BIOLOGICAL MOSQUITO CONTROL

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ABSTRACT

Studies in an increasing number of localities with diverse climates show the usefulness of flatworms for biological mosquito control. Mass production techniques are developed for one species, *Dugesia dorotocephala* (Woodworth).

Flatworms (Platyhelminthes:Turbellaria) hold a unique potential in the biological control of juvenile mosquitoes largely because they can be mass produced easily (Legner and Tsai 1978), unlike predaceous insects, some can be confined at high densities without cannibalism.

Flatworms have been shown experimentally to devastate laboratory mosquito cultures, killing far more larvae than they consume (Jenkins 1964; Legner and Medved 1972, 1974; Medved and Legner 1974, I. N. McDaniel, personal communication). They have been reported equally effective in natural habitats (Legner and Medved 1974; Yu and Legner 1976). Among those habitats in which flatworms could be usefully deployed against mosquitoes are rice fields, roadside ditches, water sloughs, agricultural and construction drain depressions, catch basins, canal seepages, treated sewage effluents, and snowmelt and rainwater pools.

Other attributes making these organisms especially amenable are their semi-dormant "winter" eggs or embryos (Borradaile and Potts 1958), their slow rate of dispersal which causes them to be effective near the place of treatment, and their effectiveness in shallow water with emergent vegetation (Yu and Legner 1976).

The extraordinary regenerative capabilities of flatworms were known since the early 1800's, some investigators considering them almost immortal under the edge of a knife (Bronsted 1969). Their ability to devour mosquitoes among other prey was soon discovered (Lischetti 1919); and Stage and Yates (1939) reported that *Dugesia tigrina* (Girard) effectively consumed *Culex* and *Aedes* larvae. The Turbellaria are nearly all free-living, non-parasitic worms (Borradaile and Potts 1958, Kenk 1972). Among the six orders are species that have great potential as predators of mosquitoes in freshwater habitats. Some genera in the order Rhabdocoela such as *Microstomum*, retain nematocysts in their ectoderm which are derived from Coelenterates on which they have fed (Kepner and Barker 1924). Such structures are useful in warding off predators, but also may be useful in killing mosquitoes.

Recent studies on the destructive capacity of *Dugesia dorotocephala* showed this species to be very effective against eggs, larvae and pupae of *Culex peus* Speiser, *Cx. pipiens quinquefasciatus* Say, *Cx. tarsalis* Coquillett in California at the application rate of 25 mature worms / m² of water surface (Legner and Medved 1972, 1974; Medved and Legner 1974; Yu and Legner 1976). In Florida additional mosquitoes killed by this predator were *Anopheles quadrimaculatus* Say, *An. crucians* Wiedemann, *Culex nigripalpus* Theobald, *Aedes taeniorhynchus* (Wiedemann), *Culex erraticus* (Dyar and Knab), *Cx. salinarius* Coquillett, *Aedes sollicitans* (Walker), *Psorophora confinnis* (Lynch-Arribalzaga), and *P. ciliata* (Fab.) (Levy and Miller 1978). Field studies with *Dugesia tigrina* to control feral *Culex restuans* Theobald and *Cx. pipiens pipiens* L. were performed by L. Learned in North Dakota (L. Learned, personal communication), and several exceptionally potent species of *Mesostoma* spp. were found in California rice fields (Collins and Washino 1978, Case and Washino 1979). In Maine, *Mesostoma macroprostatum* Hyman offers good potential in the control of univoltine *Aedes* spp. (I. N. McDaniel, personal communication). Over 75% control was achieved through inoculation of 3-6 eggs of this rhabdocoel turbellarian; and once established, the worms continued to cycle with the mosquitoes year after year! They both hatched at the same time in early spring and the worms produced eggs and died off at about the same time that the mosquitoes emerged. These worms normally had one generation per year. R. D. Sjogren (personal communication) reports over 40 natural populations of *Phagocata velata* (Stringer) from central Minnesota, with some densities exceeding 50/200-ml mosquito dipper samples.

Ecological Impact. - - Flatworms are natural components of aquatic communities, and no record has been found of their being considered pests in nature. However, extremely high application rates (exceeding 2,000/m² of water surface) of *Dugesia dorotocephala* were found lethal to *Tilapia* fish fry in experimental indoor and outdoor aquaria (Mulla and Tsai 1978). The latter report appears to have confused a nest brooder *Tilapia sillii* Gervais, with the mouth brooder *T. mossambica* Peters, as the authors state that *T. sillii* were used "on the 2nd day after leaving their parents' mouth cavity" (see Legner 1978). In another study the abundance of natural insect predators in experimental ponds was unaffected by *D. dorotocephala* applications of 25 worms/m² of water surface (Legner 1977). Although it may be expected that very young fish fry can be damaged by high application rates of flatworms (Mulla and Tsai 1978), one would not expect the need for such applications in environments where such fish are abundant.

Adaptability. - - Flatworms are quite tolerant of varying water qualities (Legner, Tsai and Medved 1976), but are adversely affected by Cl^- concentrations exceeding 1500 ppm (Levy and Miller 1978b). Certain pesticides were found to stimulate planarian reproduction (Levy and Miller 1978a). One planarian, *D. dorotocephala*, is highly active under temperatures fluctuating between 20° and 24°C (Medved and Legner 1974).

Legner, 1979.

Mode of Attack. - - Some species of Turbellarians also apparently paralyze their prey prior to ingestion of body fluids (Case and Washino 1979, Legner and Yu 1975). A sticky mucus produced abundantly by epithelial glands in the head region serves as a "pseudomandible" which holds a mosquito long enough for toxins to exert an effect (Legner and Yu 1975). Only soft body contents of mosquitoes are consumed, the cuticle being discarded; and all developmental stages of mosquitoes are attacked (Legner and Yu 1975). Some microturbellarians in rice fields are thought to produce very potent toxins which enable killing of prey much larger than themselves (Collins and Washino 1978, Case and Washino 1979), their potency appearing unique (Coward and Piedilato 1972, Riser and Morse (eds.) 1974).

Propagation. - - Culture techniques for flatworms have been available for decades, with only small modifications required for mass propagation. The density of the planarian, *D. dorotocephala* was increased 88 times in 95 days through the development of a special biological filtration system using *Elodea densa* (Planchon) Caspary (Legner and Tsai 1978). Other procedures including the provision of various foods, host-predator densities, photoperiods, etc. are available for maximizing planarian yields (Jennings 1957; Legner, Tsai and Medved 1976; McConnell 1967; Tsai and Legner 1977; Wulzen 1923, 1924, 1927). *Artemia salina* (L.) has been the best alternate food to mosquito larvae (Tsai and Legner 1977).

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CAMPBELL

A SUGGESTED APPROACH TO MALARIA CONTROL AND TO THE METHODOLOGY APPLICABLE IN DIFFERENT EPIDEMIOLOGIC SITUATIONS, BASED ON EXPERIENCE IN THE AMERICAS[1]

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Most countries in the Americas affected by malaria now have areas where eradication cannot be achieved in a predictable limited time; hence it is necessary to plan in terms of lesser objectives attainable in the programable future. This article describes a number of the major changes needed to redirect the attentions of traditional malaria eradication programs toward these less ambitious but critically important goals.

Introduction

All the countries in the Americas with malarious areas have been engaged since the late 1950s or early 1960s in malaria eradication programs. According to the reports provided by these programs, as of 31 December 1975 areas containing 99 million people (48.3 per cent of those inhabiting the originally malarious areas) were in the maintenance phase; areas with 45 million people (21.7 per cent) were in the consolidation phase; and areas with 61 million people (29.7 per cent) were still in the attack phase.[3]

Examining the general evolution of these programs, it is evident that the rapid progress observed in their early years slowed down in the late 1960s, and that the malaria situation has remained practically stationary since 1970, advances in some areas being counterbalanced by setbacks in others. This evolution follows, perhaps with some delay, a pattern observed in other parts of the world. L. J. Bruce-Chwatt, reviewing the world malaria situation in 1969, remarked that "the remaining one-quarter of our unfinished task will probably be more difficult than all that we have done before" (5).

The reasons for this resurgence of malaria in many areas have been analyzed in a number of different studies (*4, 5, 30, 34, 36*). They include planning and strategic errors by the malaria eradication programs; inadequate appreciation of technical problems; changes in socioeconomic thought over the last decade that have affected the general outlook toward international assistance; and changes in the focus of biomedical interest in the developed countries that have been reflected in the selection of health priorities by developing countries. As a result, malaria programs are confronting a need to attack the most difficult areas for the control of the disease in the face of diminishing program priority, lack of adequate or continuous financial support, unattractive career prospects for professional

[1] Paper presented at the Consultative Meeting on Malaria held at New Delhi, India, on 21-23 April 1976. Also appearing in Spanish as an abstract in the *Boletín de la Oficina Sanitaria Panamericana* 85(4): 418-438, 1978.

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[3] See reference *32* for definition of terms.

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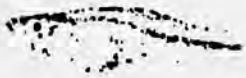
The before and after views, shown above, of the Northern Highway Bridge over the Tipitapa River in Nicaragua indicate the progress made in efforts to reduce the breeding places of anopheles. (Photos: Government of Nicaragua.)

Another proven measure in malaria control: the construction of canals to drain swamplands, as shown above being carried out in Haiti. (Photo: Dr. S. Palacios/PAHO.)



Good results have been obtained in the control of mosquitoes using larvivorous fish, and the one species proven effective under all conditions is the *Gambusia affinis*—the male (above) and female (below)

and technical malaria personnel—and hence a continuing decline in the numbers of such personnel. A vicious circle is thus being created in which lack of support prevents progress, lack of progress erodes confidence, and lack of confidence leads to further lack of support.

Obviously, these conditions apply in very different degrees to different countries; but it may be generally stated that no country with areas still in the attack phase has adequate financing to complete eradication as a time-limited operation. Against this background, resurgent malaria is an increasing problem.

General Strategic Considerations

The Governing Bodies of the Pan American Health Organization, which have followed this situation with great concern, asked the Meeting of Directors of the Malaria Programs of the Americas, held in Quito in April 1975, to review the problem and make recommendations with regard to priorities and strategy. This meeting recommended that countries in which malaria is an important actual or potential problem capable of hampering socioeconomic development should accord high priority to their antimalaria programs; that these programs should be included in the health sector of the national plan for socioeconomic development; and that the programs should be provided with an adequate structure and financing. It also requested that the Governments, PAHO/WHO, and other international and bilateral agencies intensify their efforts to promote research and improve training at all levels. These recommendations have been endorsed by the Directing Council of the Pan American Health Organization.

Overall, strategic considerations are being guided by the principles that the Twenty-second World Health Assembly adopted in 1969. Though all the countries of the Americas have reaffirmed that eradication is the final objective of their fight against malaria, most of them have areas where this objective cannot be achieved by a time-limited program and where it is necessary to plan in terms of less ambitious goals. In general, the priority of each program should be redefined in terms of the actual and potential problem that the disease represents in relation to other health problems and the expected efficacy of the available attack measures. Countries are encouraged to reexamine these matters in relation to their implications for socioeconomic development and to include the antimalaria program within the health sector of their national development plans.

The need for planning is obvious; it is also desirable to integrate all plans seeking to improve the quality of life into a general development plan for harmonious achievement of objectives. Despite the validity of this general principle, however, it is often necessary to adopt a pragmatic attitude and to start some desirable control action in the absence of a properly balanced plan, which is very frequently difficult to prepare and especially to implement, given the uncontrollable development drives prevalent in many developing countries.

A similar pragmatic attitude should guide the decision about whether and when to integrate existing malaria programs into the general public health services, as recommended by the sixteenth report of the WHO Expert Committee on Malaria (14). Different countries have adopted widely varying attitudes toward this matter. It is perhaps too early to attempt to evaluate the results of their actions, but there are indications that several countries are being successful in combining, under the direction of the malaria program, the control of selected vector-borne diseases and other high-priority public health actions that require an organization with deep rural penetration and an efficient, vertical, and centralized administrative structure. The success of such combination depends on making

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financial and personnel provisions commensurate with the increased activities assigned to the program. The malaria organization may also help considerably in providing guidance for programs extending primary health care to rural areas, programs with which it should later establish the closest possible cooperation.

The Ten-Year Health Plan for the Americas agreed upon and adopted in 1972 serves as a framework for national health plans. The Plan's main general target for malaria in the Region is to achieve or maintain eradication in areas inhabited by 84 per cent of the population of the originally malarious areas.

The areas inhabited by the remaining 16 per cent of the population involve highly heterogeneous sets of biocenoses that vary widely in their ecological, anthropological, epidemiologic, and socioeconomic characteristics. In fact, the only common denominator among these areas is that malaria transmission has persisted in each of them despite their inclusion for years in national malaria eradication programs. Therefore, it is being recognized that changes are necessary for successful handling of malaria problems in these areas, either to improve the epidemiologic situation or to avoid unnecessary waste.

The Approach to Malaria Control

Defining the Problem

The new approach should start by redefining the malaria problem in terms of variables relevant to control policies. The traditional strategy for malaria eradication—based on the use of an attack measure which was considered to be cheap, effective, and safe—insisted quite logically on security. Therefore, the delimitation of malarious areas became more an exclusion of proven nonmalarious areas, no attempt being made to study the quantitative distribution of the problem. This had the effect of actually extending the treatment coverage to many malaria-free areas, since in most instances malarious areas were delimited by including all areas at or below the altitude of the highest known malarious locality.

In contrast, the new plans for action have to be based on combinations of various measures that are seldom cheap, that are only partially effective, and that in some instances may require special safety precautions or special training for effective use. Under these circumstances, it is necessary to focus attention on identifying the actually malarious locality involved—and in a few instances, even the specific malarious house.

Study data from one American country covering a three-year period indicate that during that period only 7.5 per cent of the localities involved had been positive for malaria, and that only a considerably lower percentage had been repeatedly positive (24). Also, various studies of the typical malarious house show some characteristics that may serve as a basis for including specific home improvement actions or guidelines in health education programs geared to community participation. In particular, a study done in Colombia (29), found that malaria-positive houses tended to be small, overcrowded, isolated, and built in a rudimentary manner with incomplete walls. Entomological observations in Central America have indicated that the mosquito-bite rate experienced indoors by people in houses with incomplete walls is considerably higher than the rate in houses with complete walls.

Similar considerations should be applied to the transmission season. The season was once thought to be irrelevant, since it was felt possible to apply attack measures with whatever frequency was needed to maintain continuous effective action for the whole duration of the attack phase. Now that this is no longer true, it is necessary to seek out and take advantage of any seasonal interruption or decline in transmission due to

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climatic, meteorological, or other factors, as for example the seasonal application of insecticides by aerial spraying for the protection of cotton or other crops which periodically interrupts malaria transmission in certain areas (*20*).

As mentioned above, it is possible to find good examples in the Americas of most of the problems that have impeded normal development of malaria eradication programs (*22, 23*). Here, as elsewhere, the traditional distinction between financial, administrative, operational, and technical problems is becoming less relevant because problems belonging to these different categories often appear in combination. In this vein, if particular programs have been unable to obtain the financial, administrative, or operational facilities needed to achieve time-limited malaria eradication during the past 15 years or more, it does not seem logical to expect they can do so in the near future. Hence such programs are as much in need of a new approach as those with technical problems.

Also, as a general rule technical problems are closely associated with financial and operational ones as a result of interrelationships between many of their causative factors. In proposing solutions, these interrelated factors—not merely the technical problems involved—should be considered. Failure to take cognizance of the need for this new attitude has led in the past to further complication of the technical problems. The following provides a good example:

Along much of the portion of the Central American Pacific Coast stretching from southern Mexico to Costa Rica, the main malaria vector, *Anopheles albimanus*, developed resistance to DDT in the early 1960s. The mosquito's geographic distribution and the percentage of resistant individuals in the population correlated with the distribution of cotton cultivation and the intensity of insecticide applications—especially aerial sprayings—used to protect crops. The countries most affected by this problem are El Salvador, Guatemala, Honduras, and Nicaragua.

Because DDT was losing most of its effectiveness in these countries, its use was complemented between 1965 and 1970 by mass drug administration; and, as drug coverage progressively lost (or in many places never attained) effective levels, such coverage was supplanted after 1970 by indoor spraying with propoxur. The high cost of this latter insecticide forced the malaria programs to restrict its use to the areas of highest DDT resistance, and even to limit attack measures altogether to the areas of most intense transmission.

All treatments, with one or the other insecticide, followed the total coverage principles mentioned above, and the operations in question achieved a high degree of efficiency. As a result, in 1973 the four countries, taken as a single unit, enjoyed the lowest malaria incidence in their recorded history. Nevertheless, propoxur selection pressure in the treated areas was sufficiently high to promote rapid development of propoxur resistance. This was detected first in Nicaragua and El Salvador in 1971, thereafter in most of the central and eastern coastal plains of El Salvador, and later in Guatemala (1973), other areas of Nicaragua (1974), and Honduras (1975).

Obviously, the rapidity with which this resistance developed was due to the high level of protection afforded mosquitoes by the propoxur-resistant gene; to that gene's mode of inheritance; to the previous selection through agricultural sprayings with parathion, other organophosphorus compounds, and (in a few instances) other carbamates which cause a certain level of cross-resistance to propoxur; and to the high level of coverage afforded the protected areas. This development of resistance, combined with the persistence of low levels of transmission in untreated areas and intense population movements throughout the countries involved, led to a resurgence of

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malaria – a resurgence that in 1975 brought about the highest levels of malaria incidence recorded since the eradication campaign began.

It is reasonable to assume that application of propoxur represented a good technical solution to the problems facing the area's malaria eradication programs in 1969, when a meeting of directors of the malaria programs of Central America recommended its use, and that, if it could have been applied as part of a complete and properly carried out malaria eradication program, the insecticide would have achieved its objective during its period of effective life. Or, on the other hand, if it had been used for focal and seasonal treatments, as part of a properly planned control program, selection of resistant strains might have proceeded at a slower pace.

The foregoing example illustrates the dangers involved in underestimating the need to plan control programs on the basis of their own merits and their relevance to the problems involved. [redacted]

Collection of Information

This is another basic matter to consider when planning control or other actions not aimed at time-limited eradication in areas where a malaria eradication program is currently in operation. Most of the unsuccessful eradication programs have reached sometime in the past the late attack phase, and many have for some time been in the consolidation phase. As a result, they have developed a case detection system based on models designed, ideally, to detect and treat the last autochthonous case. Some doubts have been expressed (7) about the need for such perfect surveillance systems, even for eradication programs. In areas where transmission is still present, much of the information obtained by such systems may be at the same time redundant and insufficient as a guidance for control operations. These defects become particularly apparent when, due to financial difficulties, the surveillance system does not provide the degree of coverage – in terms of the time and area – required to meaningfully estimate the incidence of the disease.

Programs of nontime-limited eradication in the Americas are trying to maintain a surveillance system based on a widely distributed network of passive case-detection posts that will provide a rough but cheap indication of incidence variations complemented by periodic prevalence surveys in areas where changes in rates of transmission are expected, as a consequence of either control measures or other circumstances. According to the nature of the control strategy, these surveys may or may not be directed at specific age groups, and may or may not be complemented by other evaluation methods such as entomological studies or serologic surveys.

Goal Selection

A third basic problem is determining the levels of malaria that may be tolerated, or perhaps that should be tolerated, given the existence of other major social and health problems. The idea of making an *a priori* determination of levels of suffering "tolerable" for any population is obviously repellent. Nevertheless, the history of the malaria eradication effort in many countries has shown that when malaria incidence falls below a certain level the program breaks down, thereby failing to achieve or maintain an adequate consolidation phase. The problem has been defined as one of "near-success in an environment with an excess of problems clamoring for attention. As malaria recedes to a low level ones pressing health and social problems exert irresistible demands for available resources" (27). Under such circumstances

there may be good indications, and perhaps spontaneous definition, of these levels. Of course, whatever these levels may be, they will be subject to change as other problems approach solution or control feasibility improves.

Within this context, it would be necessary to monitor variations in incidence, in the manner that this is accomplished by use of endemo-epidemic indices, so that there would be a warning system to call for action should these levels be surpassed. This warning system should be complemented by the study of other factors that are relevant to the local epidemiology of malaria, and that may provide effective warnings of periodic epidemic waves—factors such as the "malaria epidemic index" (*13*), the "paraquinquenal cycle" (*6, 10*), or other meteorological, entomological, or parasitological variables that should be studied in order to arrive at a better predictive epidemiology.

In practical terms, these considerations are most applicable to areas with unstable malaria, as are most of the malarious areas found in the Americas, where there is some degree of correlation between intensification of the control effort and reduced incidence. In the areas with stable malaria, prevalent in rural tropical Africa, most attempts at limited control of malaria transmission may be futile.

Choice of Methods

As mentioned above, the first choices to be made in a control program are which areas or localities, and which sectors of the population, will require specific action. These are often difficult matters to decide, particularly when most areas have been under insecticide protection for years and the choices have to be based on estimation of potential risks. Furthermore, they require setting up criteria in order to select action priorities vis-à-vis a constellation of epidemiologic, entomological, operational, and socioeconomic factors.

The administrative and operational transformation of an eradication program into a control program has to be carried out with considerable care, in order not only to avoid or minimize the epidemiologic risks involved in discontinuing certain operations, but also to avoid wasting human, administrative, and operational resources. Actual methodological choices should be based on the guidelines set by the WHO Expert Committee on Malaria (*34*), the WHO Interregional Conference on Malaria Control (*36*), the specific technical manuals on larval control operations and personal and community protection against malaria (*33, 35*), as well as on new developments in mosquito control (*14, 21, 37*). Careful consideration should also be given to possible harmful consequences that particular antimalaria actions may have on man and the environment (*15*).

In accord with the foregoing strategy recommendations, the malaria programs of the Americas are being reoriented (*23*) toward the establishment of flexible local strategies, in which all available methods are considered and used, whenever applicable, in an approach that is becoming known as "integral control."

For some years indoor residual spraying with DDT will continue to be the method of widest applicability, and integrated control programs will still depend on this method for the control of most of the rural malaria. It is expected, nevertheless, that coverage may be reduced in both time and area.

In the presence of DDT resistance, it is necessary to ascertain the operational implications of this resistance under local epidemiologic conditions. For example, along the Pacific Coast of Central America and Mexico the history of malaria control and the evolution of resistance suggest that, even up to a point where 40 to 50 per cent of the *A. albimanus* population was resistant, DDT still attained useful malaria control.

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But as the proportion of resistant mosquitoes increased beyond that level, the effectiveness of DDT spraying dropped rapidly. It is also important to investigate the main causative factors behind the development of resistance and to determine whether or not indoor house spraying significantly contributes to that development. In the Pacific Coast areas previously referred to, where the vector, *A. albimanus*, is highly exophilic and zoophilic, development and maintenance of DDT and organophosphate resistance has depended almost exclusively on the use of aerial insecticide sprays to protect cotton. From the mid-1960s onward, the increase in the frequency of agricultural insecticide spraying led to a breakdown of malaria control in Central America, while in southern Mexico a decline in cotton cultivation over the same period was accompanied by a decline in the proportion of resistant *A. albimanus* and achievement of effective malaria control with DDT indoor spraying. A close watch over agricultural policies may have some predictive value under those circumstances (*11*).

Also, it should be noted that when DDT resistance occurs, the choice of replacement insecticides is quite limited. Malathion, fenitrothion, and propoxur are all considerably more expensive than DDT and resistance to all of them has developed rather quickly under the conditions prevailing in Central America.

[illegible]

Antilarval operations have well-recognized limitations but are attracting new attention as a way of dealing with problems of resurgent malaria in areas with high population densities.

Classical source reduction methods are being promoted, and are already being undertaken, by some malaria programs including those in the Dominican Republic, El Salvador, Haiti, and Nicaragua. In most cases malaria programs could profitably encourage and advise the authorities of municipalities or of development projects to apply these methods, either for the control of malaria or for their own economic advantages as the classical "bonifica integrale."

Larvicidal operations, used as a complement to source reduction and indoor spraying, are being employed by the above-mentioned programs. They are also being used to help cope with some occurrences of urban malaria in Brazil, Mexico, and Nicaragua, and with one instance of rural malaria in Guatemala.

Larvivorous fish are also attracting attention although the introduction of *Gambusia* is feared for its possible ecological implications. In some countries, such as El Salvador and Venezuela, an active search is being made for local larvivorous species that could be colonized and distributed.

Chemotherapy has an obvious place in any control program, and in many areas the only feasible action may be to make drugs widely available in order to prevent mortality and protect the most vulnerable population groups. Mass drug distribution has been successfully used to eliminate foci of transmission in many malaria eradication programs or to control outbreaks, especially in Colombia. But attempts to use this method as the main attack measure against malaria in a wide area of Central America, for a prolonged period of time, led to refusal of the population to accept it. In addition, resistance of *Plasmodium falciparum* to 4-aminoquinolines represents an important problem in many areas of Brazil, Colombia, Guyana, Panama, Suriname, and Venezuela.

Because a control program has to rely heavily on community participation, health

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education at all levels of the community has to play a decisive role. Past experience shows that when it has been expected that health education efforts would change the attitudes of the population in a short time, these efforts have often failed. Cooperation may nevertheless be expected for specific short-term projects with obvious advantage, such as drainage or other source-reduction projects, and through them an effort to convey a better understanding of the malaria problem to the population may be attempted.

It is obvious that any selective use of antimalaria measures should be guided by a knowledge of the local epidemiology of malaria, because it may be expected that the more detailed this knowledge the more effective the measures and the less the amount of wasted effort. This has been recognized since the early days of malariology, when the great variability of epidemiologic conditions was being discovered: but it was also realized at the same time that to acquire detailed local knowledge required considerable effort and expense, as well as highly sophisticated technical personnel.

The Future Outlook

As already mentioned, some progress is still being made toward malaria eradication, and the goals of the Ten-Year Plan for the Americas are still considered realistic. Nevertheless, progress in problem areas is dependent on better utilization of available resources, which implies that considerable changes in approach will be needed in order to implement selective integrated control programs capable of achieving this more efficient resource use. The efficacy of such a program is not in doubt, but its feasibility in tropical rural areas has to be shown. PAHO is studying the possibilities for carrying out a demonstration project to study the feasibility of the approach and to provide some information on its cost-effectiveness.

Among other things, the planning and operation of this kind of program require a deeper epidemiologic knowledge than is generally available at present, and therefore a stronger staff of professional and technical personnel. Moreover, many unsolved problems are involved in the operation of an integrated control program. Therefore, personnel at all levels should be of a higher caliber than those required for application of a single technique, and such personnel may be difficult to obtain.

Very high priority should thus be given to the training and retraining of the professional staff, in order that the emphasis turn away from the managerial ability looked for almost exclusively in training staff for eradication programs, and should turn again to the scientific study of the biodynamics of malaria (*1*).

Since 1976, training courses have been or are in the process of being established in the Americas following the recommendations of the WHO Consultation Group on Training of Malaria Staff (*32*). These include one and in the future possibly more courses of instruction leading to a master's degree in public health with specialization in malaria and other parasitic diseases, studies on the feasibility of establishing courses leading to a master's degree in medical entomology with similar specialization, and orientation seminars on integrated mosquito control for engineers.

It seems inevitable that the present trend involving deterioration of the epidemiologic situation will continue in the so-called problem areas until effective control programs can be fully implemented, and even then very swift progress should not be expected.

Hopes for significant progress toward eradication in those areas now depend on the development of more effective control measures and, therefore, on the results of research. It is also true, however, that basic

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research of past years has greatly advanced our knowledge of the parasite, the immune response of the host, and the biology and ecology of the vector and its relation to man. This basic research has led to great progress in applied research addressed to development of better means for controlling malaria (*16, 17*).

With regard to the development of applied field research in the Americas, PAHO/WHO is engaged in several types of activities. Among other things, it is encouraging, coordinating, and collaborating with the Region's malaria programs to undertake epidemiologic and operational research seeking answers to their problems. The Organization is also setting up intercountry field research projects to study problems of interest to various countries—such as the study of multiple *A. albimanus* resistance in Central America and evaluation of possible control measures; studies on malaria immunization and new antimalaria drugs, using first animal models and eventually clinical trials, in Colombia and Panama; studies on the epidemiology of chloroquine resistance; and studies on the cytogenetics of the species of the subgenus *Nyssorhynchus* and possible genetic control measures applicable to them. In addition, PAHO/WHO is promoting basic and applied research by outside institutions in areas of special interest for the solution of field problems.

SUMMARY

In general, most countries of the Americas affected by malaria now have areas where eradication cannot be achieved by a time-limited program and where it is necessary to plan in terms of less ambitious goals. Accordingly, the malaria programs of the Americas are being reoriented toward flexible local strategies employing all available methods, where applicable, in the approach known as "integrated control." In general, this reorientation involves substantial changes in the methods used to define malarious areas, conduct epidemiologic surveillance, investigate local conditions, and take advantage of those conditions.

Overall, it is expected that for some years indoor residual spraying with DDT will continue to be the method of widest applicability, but it will be necessary to develop methods for its selective use. Naturally, should there be DDT resistance, it is necessary to ascertain the local operational implications of this resistance. It should also be noted that where resistance occurs, the choice of replacement insecticides is quite limited. All the substitutes are considerably more expensive than DDT and resistance to all of them has tended to develop rather quickly in the few areas where they have been used, with the strategy of total coverage, as possible solutions to the problem of DDT resistance.

Other available control measures include ultra-low-volume application of insecticides, classical source-reduction methods, larvicidal operations, introduction of larvivorous fish, health education, and use of antimalaria drugs. In general, it is clear that any selective use of antimalaria measures should be guided by a knowledge of the local epidemiology of malaria.

Looking to the future, it is evident that the planning and implementing of integrated control programs will require a deeper epidemiologic knowledge than is generally available at present, and therefore a stronger staff of professional and technical personnel. Very high priority should thus be given to training and retraining of the professional staff. In addition, hopes for significant progress toward eradication in the so-called problem areas now depend on the development of more effective control measures and, therefore, upon research. More basic research is needed, but perhaps more important is increased applied field research, so that full use may be made of the results of recent research for the development of better means of malaria control.

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WORLD CONGRESS ON ENVIRONMENTAL SANITATION IN DEVELOPMENT PLANNING

The Ministry of Health and Welfare of Mexico and the World Health Organization will sponsor a Congress in Mexico City from 12-16 November 1979, for the purpose of presenting scientific and technical information on the interaction between health and the development process, as well as exchanging experiences related to the multidisciplinary organizational, conceptual, and methodologic issues in social and economic development and environmental health.

The information to be provided will assist professionals in both the health and development sectors to incorporate environmental health objectives in national development planning, with emphasis on practical national experience that could be applied elsewhere.

The Congress is open to all countries and should be of special interest to professionals engaged in economic analysis and funding of development projects, to those responsible for the design and implementation of development projects, and to scientists and educators concerned with research and training in this area.

Additional information may be obtained from: Congreso Mundial sobre Saneamiento Ambiental en la Planificación del Desarrollo, Secretaría General, Avenida Chapultepec No. 284, 13° Piso, México 7, D.F.

11.647

EARLY SEASON APPLICATION OF *ROMANOMERMIS CULICIVORAX* PROVIDES CONTINUOUS PARTIAL CONTROL OF RICE FIELD MOSQUITOES

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ABSTRACT.

An early season application of postparasites of the mosquito parasitic nematode. *Romanomermis culicivorax* demonstrated that the nematodes could mature to adults, mate and lay eggs in the rice field environment. Preparasites hatching from these eggs provided continuous partial control of larval *Anopheles freeborni* and *Culex tarsalis* throughout the rice growing season. Control of *An. freeborni* was comparable to that obtained in the past by direct applications of preparasites and exceeded that previously achieved for *Cx. tarsalis*. Infection was observed up to 20 feet from the original point of application.

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Westerdahl, et. al., 1979.

11.647

AERIAL APPLICATION OF *ROMANOMERMIS CULICIVORAX* (MERMITHIDAE:NEMATODA) TO CONTROL *ANOPHELES* AND *CULEX* MOSQUITOES IN SOUTHWEST FLORIDA

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ABSTRACT. An experimental release of preparasites of the mosquito nematode *Romanomermis culicivorax* Ross and Smith (=*Reesimermis nielseni* Tsai and Grundmann, auct., partim.) was made in 3 ponds via helicopter at the rate of ca. 4,629/m² of water surface to control natural populations of *Anopheles quadrimaculatus* Say, *An. crucians* Wiedemann, and *Culex erraticus* (Dyar and Knab) larvae. Mean levels of parasitism of 52.4, 66.7 and 43.8% of the 1st-4th instar *Anopheles* spp. and 39.2, 40.0, and 53.3% of 1st-4th instar larvae of *Cx. erraticus* from 3 ponds, respectively were obtained.

The aerial spray tests indicated that a helicopter equipped with a Simplex low profile spray system with TeeJet® spray nozzles can be an effective means of disseminating the preparasitic stage of *R. culicivorax* in mosquito control operations.

Control of natural populations of mosquito larvae with a pathogenic agent applied by air has not been demonstrated. However, Levy et al. (1977) reported encouraging results in tests using a helicopter spray system to deliver the mosquito nematode *Romanomermis culicivorax* Ross and Smith. Tests were conducted at ground level with a Bell 47G helicopter equipped with a Simplex low profile aerial spray system having a boom fitted with TeeJet® nozzles at 25 psi and no adverse effects on preparasite infectivity or nematode development were detected. These tests suggested the potential use of this aerial spray system for the dissemination of *R. culicivorax* in mosquito control operations.

The susceptibility of larvae of *Anopheles quadrimaculatus* Say, *An. crucians* Wiedemann and *Culex erraticus* (Dyar and Knab) to field parasitism by preparasites of *R. culicivorax* applied by conventional compressed air hand sprayer has been demonstrated by Petersen and Willis (1972; 1974). We are now reporting on the aerial application of preparasites of *R. culicivorax* via helicopter to control field populations of larvae.

METHODS AND MATERIALS

Three semipermanent ponds (30.5 × 15.2 × 0.5-1.0 m deep) constructed in 1976 at the Lee County Mosquito Control District were used as the sites for the tests (Fig. 1). The ponds contained rain and standing ground water (0.3-0.6 m deep) and vegetation scattered throughout but concentrated primarily around the edges. Pre-treatment sampling showed large populations of larvae and pupae of *An.*

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quadrimaculatus and *An. crucians* (1–30 per dip with a pint dipper) and lower populations of *Cx. erraticus* (0–5 per dip). Numerous insect predators (water scorpions, back swimmers, water scavenger beetles, predacious diving beetles, dragonflies, water striders, whirligig beetles, *Gambusia* spp. and tadpoles) were present in each pond.

Pre-treatment analyses of the ponds indicated that the water was low in salinity (1.0–7.5 mg/l Cl^-) and conductivity (117–163 μmhos/cm), and therefore was suitable for *R. culicivorax*. The water temperature of the ponds was 30° C at the time of aerial application.

Twenty to 24 hr prior to application 12 cultures containing eggs of *R. culicivorax* were flooded with ca. 800 ml of water purified by reverse osmosis (RO) filtration to induce egg hatching. Volumetric determinations of the preparasite population in the water from the combined cultures indicated that ca. 11,250,000 infective stage nematodes were available for the tests.

Bioassays to determine infectivity of unsprayed and sprayed preparasites in unfiltered well water and water from the 3 ponds were conducted in 400 ml glass beakers containing 300 ml of the test waters and 20 1st instar larvae of *Cx. quinquefasciatus* per container (3 replications/water type). In addition, one 18 × 30 cm enameled pan was filled with ca. 1,000 ml of RO water and placed at the south end of each of ponds 1 and 3 to catch preparasites sprayed during the aerial application (Fig 1.a). Preparasites sprayed through the helicopter spray system at ground level before and after the aerial spray tests were also bioassayed in RO water. Volumetric determination of the number of preparasites sprayed into the enameled pans in ponds 1 and 3 was determined to be ca. 0.25 per ml or about 250 preparasites per 540 cm^2 pan (4 replications); preparasites collected from the helicopter before and after the aerial application averaged ca. 15 and 31 ml, respectively. Preparasites in the pre-test sample were sprayed at ground level almost immediately upon additions of the preparasites to the helicopter spray tanks and apparently were not thoroughly mixed in the ca. 296 liters of well water in the tanks prior to spraying. However, preparasites taken in post-spray samples were thoroughly mixed from typical helicopter agitation during aerial application. Preparasites from the helicopter were bioassayed in 400 ml glass beakers containing 300 ml RO water and 20 hosts (3 replications). Preparasites sprayed into the enameled pans during the aerial tests were transferred to 1,000 ml glass beakers containing RO water for bioassay (1 trial of 20 and 100 hosts for ponds 1 and 3, respectively).

A 10:1 preparasite to host ratio was used in the bioassays and mosquito hosts were fed ground rabbit chow daily and maintained at ca. 20° C (ambient). Percentage parasitism of *Cx. quinquefasciatus* larvae was used as the criterion to determine the effects of the pond water and spray application of infectivity of preparasitic *R. culicivorax*. Infectivity was determined with a compound microscope and by emergence of postparasites. Larvae determined to be negative for nematode parasitism with the compound microscope were confirmed by dissection.

A Bell 47G helicopter equipped with a Simplex low profile aerial spray system fitted with 12 TeeJet® spray nozzles (FloodJet® Tip No. TK20) was used for the aerial application of preparasites (Levy et al. 1977). Helicopter spray tanks were filled with ca. 296 liters of unfiltered well water (conductivity = 720 μmhos/cm @ 25°C) while the rotor blades were operational. Then 7.6 liters of RO water containing preparasites were added directly into the spray tanks. The spray system was calibrated to spray 9.5 liters/min/nozzle or 114.0 liters/min at 25 psi. Preparasitic nematodes were ca. 30 hr old when sprayed.

Preparasites were applied from the helicopter at the rate of ca. 2,925,315 per pond at 32 kmph and at 2.5–3.0 m above the ground. The ponds were sprayed consecutively in a series of north to south

and south to north spray passes. A west to east wind of ca. 10 kmph was recorded during the aerial application. About 25 liters of water containing preparasites (ca. 31 preparasites/ml) could not be sprayed and remained in the tanks and dump section of the Simplex system after the ponds were treated (Levy et al. 1977).

A total dosage rate of ca. 4,629 preparasites per m^2 per pond was determined from the average number of preparasites per ml of water recovered in enameled pans during aerial application (i.e. 0.25 preparasites per ml). A substantial number of preparasites were lost because of drift during the aerial application. Based on the preparasite dosage and water surface area per pond, each pond was considered a replicate even though the number of mosquito larvae per pond varied.

Each pond was sampled 24 and 48 hr post-treatment and the percentage parasitism by larval instar was determined (as previously described), to evaluate the effectiveness of the aerial application of preparasitic *R. culicivorax* against *Anopheles* spp. and *Cx. erraticus*.

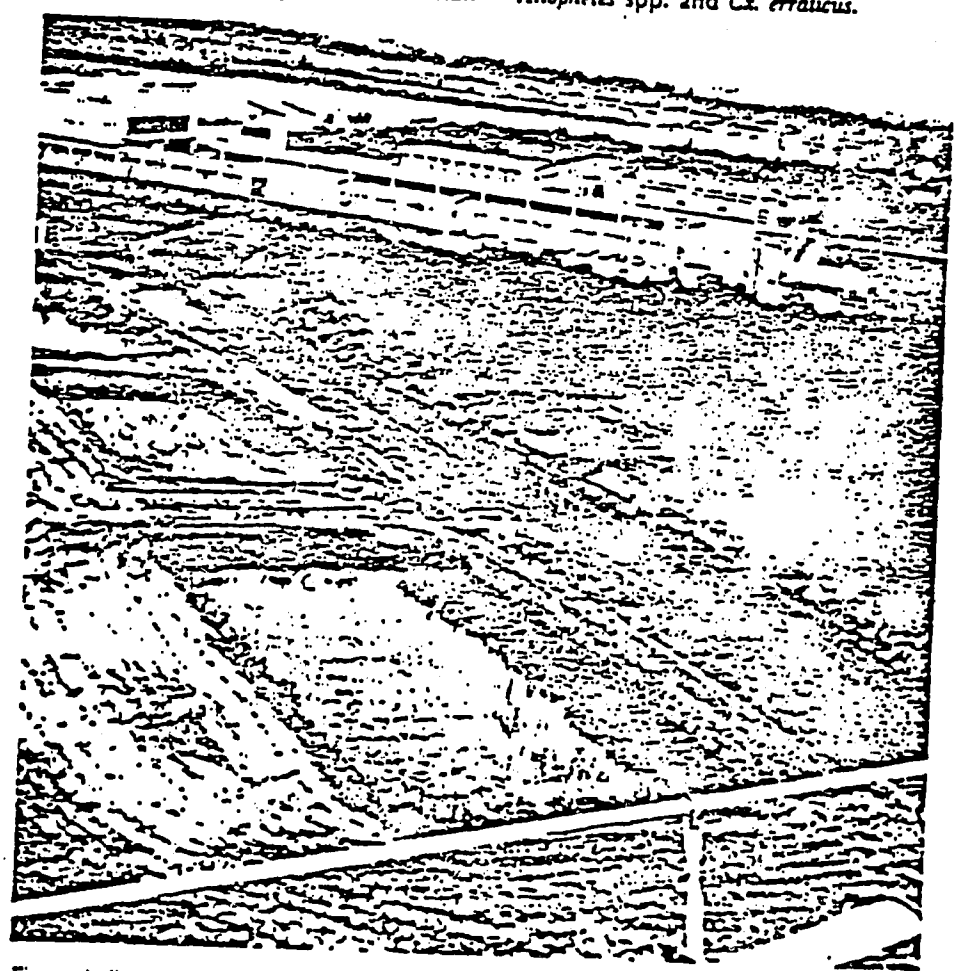

Figure 1. Experimental sites (Ponds 1–3) used for aerial application of *R. culicivorax* showing placement of enameled pans (a) used to catch preparasites at south end of ponds 1 and 3.

RESULTS AND DISCUSSION

Data collected from bioassay of unsprayed and helicopter sprayed preparasites of *R. culicivorax* in water from the 3 sources indicated that there were no significant differences in infectivity to *Cx. quinquefasciatus* larvae when preparasites were sprayed through the Simplex system compared with unsprayed preparasites in RO water (Levy et al. 1977). However, a 10–12% reduction in infectivity was observed when unsprayed preparasites were exposed to mosquito larvae in unfiltered well water (Table 1). In addition, all postparasites obtained from exposure of hosts to the sprayed and unsprayed preparasites in the various water types showed no observable morphological and behavioral differences when compared to postparasites recovered from the RO controls (Table 1).

The bioassay data indicated that little or no inhibitory effects on the infectivity and development of *R. culicivorax* could be expected as a result of the helicopter spray system or preparasite exposure to water in the 3 ponds. However, possible inhibition of the infectivity of preparasites exposed for 24 hr to unfiltered well water used in the spray tanks was observed. No effects were apparent in nematodes held in tanks for 30–45 min and reared in RO water, a condition similar to ponds.

Aerial application of preparasites of *R. culicivorax* to control mixed populations of larvae of *An. quadrimaculatus* and *An. crucians* in the 3 ponds resulted in mean parasitism ranging from 43.8–66.7% (Table 2). The mean parasitism of all 402 *Anopheles* spp. larvae sampled 24 and 48 hr post-treatment from the 3 ponds was 53.7% (100, 50.3, 59.5, and 43.4% parasitism of 2-1st, 117–2nd, 196–3rd, and 87–4th instar larvae, respectively).

Encapsulation and melanization of the nematode was observed in several 3rd and 4th instar *Anopheles* spp. larvae and indicated a certain degree of physiological resistance to this nematode (Petersen and Willis 1974). It should be noted that the majority of the *Anopheles* spp. larvae examined 24 and 48 hr post-treatment were infected with more than one *R. culicivorax*. Multiple parasitism may have resulted in some early host death prior to sampling. In addition, 100% postparasite emergence was observed from 25 *Anopheles* spp. larvae parasitized in the ponds by *R. culicivorax* and indicated the potential for nematode recycling in this type of habitat.

The parasitism of larvae of *Cx. erraticus* resulting from aerial application of preparasites of *R. culicivorax* is presented in Table 3. A mean parasitism of 41.9% was observed in the 86 *Cx. erraticus* larvae examined from the 3 ponds, i.e. 50, 35.9,

Table 1. Bioassays of infectivity of preparasitic *Romanomermis culicivorax* to 1st-instar larvae of *Culex quinquefasciatus* as a function of water quality and spray application.

Water Type Tested	Preparasites Tested	Percentage parasitism (No. larvae sampled)	Percentage postparasite emergence from infected 4th instar larvae (No. larvae sampled)
RO	Unsprayed	88.3 (60)	100 (29)
Well	Unsprayed	78.3 (60)	100 (30)
Ponds 1–3	Unsprayed	90.0 (60)	100 (30)
RO	Sprayed[a]	93.3 (15)	—
RO	Sprayed[b]	86.6 (15)	—
RO	Sprayed[c]	85.0 (120)	100 (60)

[a] Preparasites in well water sprayed from helicopter at ground level into buckets prior aerial test and added to beakers containing RO water.

[b] Preparasites in well water sprayed from helicopter at ground level into buckets after aerial test and added to beakers containing RO water.

[c] Preparasites in well water sprayed from helicopter into enameled pans containing RO water during aerial application.

Table 2. Parasitism of *Anopheles* spp. larvae resulting from aerial application of preparasites of *Romanomermis culicivorax* at a dosage rate of 4,629 nematodes/m².

Pond No.	Hr Post-Treatment	Percentage parasitism by instar (No. larvae sampled) First	Second	Third	Fourth	Combined Instar
1	24	100(1)	43.5(23)	48.4(31)	30.0(10)	44.6(65)
	48	—(0)	—(0)	79.0(19)	—(0)	79.0(19)
	Combined	100(1)	43.5(23)	60.0(50)	30.0(10)	52.4(84)
2	24	100(1)	73.0(28)	61.9(42)	73.0(4)	68.0(75)
	48	—(0)	33.3(3)	70.6(17)	50.0(4)	62.5(24)
	Combined	100(1)	71.0(31)	64.4(59)	62.5(8)	66.7(99)
3	24	—(0)	44.5(36)	60.4(53)	38.5(52)	48.2(141)
	48	—(0)	23.9(27)	42.9(34)	33.3(17)	35.9(78)
	Combined	—(0)	36.5(63)	54.0(87)	37.7(69)	43.8(219)

55.4, and 0% parasitism of the 4–1st, 48–2nd, 31–3rd, and 3–4th instar larvae, respectively. Little multiple parasitism was noted in larvae of this species. Two 4th instar *Cx. erraticus* larvae were found to have encapsulated and melanized *R. culicivorax* parasites, thus indicating possible physiological resistance to *R. culicivorax* or limited resistance due to weakened or damaged parasites dying after entering the host.

Although the preparasite dosage per m² appears high when considering the total percentage parasitism, one must consider the type of parasite application as well as the physical features of the test sites in relation to the orientation of parasites and hosts. Preparasites were evenly distributed over the 464 m² of water surface of each pond; however, the *Anopheles* spp. and *Cx. erraticus* larvae were unevenly distributed around the vegetative edges of each pond. Since preparasites are believed to swim only a few feet from their point of introduction (unpublished data), numerous preparasites sprayed in the central portions of each pond probably did not encounter hosts. In addition, preparasites were ca. 30 hr old when sprayed and 10–15% of the nematodes were assumed to have lost their infectivity (Petersen 1975). Some predation of infected mosquito hosts can also be expected to have occurred.

It is important to determine the most efficient and effective means of disseminating *R. culicivorax* preparasites (i.e. ground or aerial spraying) in the various types of fresh water mosquito habitats. In the test sites utilized in this study it is expected that the dosage per m² could have been reduced by as much as 50% if

Table 3. Parasitism of *Culex erraticus* larvae resulting from aerial applications of preparasites of *Romanomermis culicivorax* at a dosage rate of 4,629 nematodes/m².

Pond No.	Hr Post-Treatment	Percentage parasitism by instar (No. larvae sampled) First	Second	Third	Fourth	Combined instars
1	24	0(1)	39.1(23)	66.7(9)	—(0)	45.5(33)
	48	—(0)	36.4(11)	25.0(4)	0(3)	27.8(18)
	Combined	0(1)	38.2(34)	33.3(13)	0(3)	39.2(51)
2	24	—(0)	—(0)	50.0(2)	—(0)	50.0(2)
	48	—(0)	33.3(3)	—(0)	—(0)	33.3(3)
	Combined	—(0)	33.3(3)	50.0(2)	—(0)	40.0(5)
3	24	66.7(3)	57.1(7)	66.7(15)	—(0)	64.0(25)
	48	—(0)	0(4)	0(1)	—(0)	0(5)
	Combined	66.7(3)	36.3(11)	62.5(16)	—(0)	53.3(30)

the preparasites were applied with a conventional compressed air sprayer around the vegetative edges of each pond where sampling indicated the mosquito larvae were concentrated. Further, problems associated with drift during aerial spraying would have been greatly reduced or even eliminated. However, the potential for recycling that this nematode has exhibited in certain habitats (Petersen and Willis 1975) may justify the higher dosage rates when applying preparasites of *R. culicivorax* by helicopter over large areas.

Examination of samples of *Anopheles* spp. and *Cx. erraticus* larvae obtained 8 weeks post-treatment revealed no parasite activity and therefore indicated that natural recycling of *R. culicivorax* had not occurred in the test sites during this period. Eight weeks post-treatment, pond 2 was dry and the water in ponds 1 and 3 had receded from the vegetative perimeter where the infected larvae were originally sampled: larvae at this time were more evenly distributed in the central portions of the 2 ponds. It is expected that postparasite emergence from the *Anopheles* and *Culex* hosts and subsequent nematode egg laying occurred in the soil around the vegetative portions of each pond. Eleven soil samples per site obtained with a standard soil core from these areas and flooded with RO water for 24 hr indicated no preparasite activity at this time.

Test results suggest that a helicopter having a Simplex low profile aerial spray system equipped with TeeJet® spray nozzles can be an effective means of disseminating preparasites of *R. culicivorax* against natural populations of *An. quadrimaculatus*, *An. crucians* and *Cx. erraticus* larvae, as well as other species of mosquitoes in certain fresh water habitats.

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INTRODUCTION

The use of fish for mosquito control is not a new concept. The technique was first seriously investigated in the early 1900's after it was discovered that malaria was transmitted by mosquitoes. A continuing interest in larvivorous fish suffered with the advent of DDT and subsequent fast-acting residual insecticides. Recently, however, renewed attention is being given to fish as biological control agents. Two reasons for this change in attitude are the greater public concern over unintentional effects of pesticide contamination on the environment and the fact that chemical control with insecticides has become more costly, shorter-lasting and, owing to the evolution of resistant mosquito strains, less effective (Bay, 1967a).

In an annotated bibliography, Gerberich and Laird (1968) list 686 papers dealing with the use of fish for mosquito control. While over 200 different fish species are mentioned, 279, or 41%, of the references cited deal partly or entirely with only one species: the mosquito fish, Gambusia affinis. The southeast Asian Panchax group and the common guppy, Lebistes reticulatus, are the closest runners-up, accounting for 8.6% and 6.5% of the literature, respectively. Bay (1967b) states that Gambusia affinis has become almost synonymous with biological control of mosquitoes and is, in fact, one of the most widely disseminated of any biocontrol agent. From an immediate and practical standpoint, mosquito fish offer great potential as an adjunct to chemicals for mosquito control. It is on this species, therefore, that we focus our attention.

The development of a mosquito fish program should, from the onset, be a cooperative effort between medical department and public works personnel and, when available, with individuals assigned fish and wildlife duties for the activity. For maximum impact, the program night also be coordinated with local state and county health departments, particularly those involved in mosquito control. Assistance with the program can be obtained from Navy entomologists at a Navy Environmental and Preventive Medicine Unit, a Navy Disease Vector Ecology and Control Center or a Field Division of the Naval Facilities Engineering Command. Since most states have regulations concerning the importation of certain fish species, it is mandatory that the state Fish and Game Commission or their military representative be contacted when interstate shipment of Gambusia is contemplated. Additional advice may also be obtained from the U. S. Fish and Wildlife Service and most state universities.

HISTORY

Baird and Girard first described Gambusia affinis in 1854. However, it was not until the early 1900's that their usefulness as mosquito predators become well established. During that period, the first long distance transplantation of mosquito fish was made. At the request of the Hawaii Agriculture Experiment Station, about 150 Gambusia were brought from Seabrook, Texas to Honolulu in 1905 by Mr. Alvin Seale. The fish flourished and by 1907 they had already established a record of being extremely effective mosquito predators (Rockefeller Foundation, 1924; Krumholz, 1948). In 1913, Seale (1917) transported 24 Gambusia in a glass jar in his stateroom from Hawaii to the Philippine Islands where they also thrived and reproduced in large numbers. From the Philippines, they were eventually introduced into areas of Indochina, Japan and China. In 1921, at the request of the International Red Cross, stocks of Gambusia were sent from Augusta, Georgia to Spain by the U. S. Bureau of Fisheries (Rockefeller Foundation, 1924; Krumholz, 1948). Gerberich and Laird (1968) consider this event to be significant as it eventually became one of the earliest and most widespread uses of a biological control agent in the medical entomology field. Italy imported Gambusia from Spain in 1922 and from this Italian stock, distribution was soon extended to countries throughout Europe including Yugoslavia and the USSR (Transcaucasia Region) in 1924, Algeria and Corsica in 1926, and Greece in 1928. From Corsica, mosquito fish were imported to Egypt and then to Cyprus, Syria and the Sudan. Finally, through the support of the Rockefeller Foundation, Gambusia were established in Mexico, Central and South America to combat container-breeding mosquitoes in a drive against yellow fever (Gerberich and Laird, 1968). During yellow fever control efforts in Key West, Florida, Gambusia were used successfully in place of oil for the control of Aedes aegypti in cisterns (LeVan, 1941).

Gambusia affinis also received widespread distribution and use in malaria control. In the United States, Hildebrand (1921) reported that the use of mosquito fish during 1920 malaria control campaigns was responsible for producing more permanent results in many southern states at a

reduced cost. It is also of interest that during this time the Health Department in Richmond, Virginia, stocked all of its fountains, reservoirs and lakes with Gambusia and established hatcheries to upply mosquito fish to any community in the state that requested them (Rockefeller Foundation, 1921 and 1924). In other regions, the New Guinea Medical Service provided local distribution of Gambusia in 1930 which later were reported to be the most successful antimalarial measure taken in the Territory (Ford, 1949). The Canary Islands imported Gambusia from Spain in 1943 to control an outbreak of malaria (Gerberich and Laird, 1968), and during World War II, U. S. Army Malaria Control Units utilized Gambusia in the Pacific to control anopheline breeding in wells and cisterns (Krumholz, 1948; Simmons, 1949). More recently, Tabibzadeh, et al., (1970), reported the extensive use of Gambusia in Iran for malaria control. [redacted] (Hoy and Reed, 1970; Hoy et al., 1971).

BIOLOGY

Taxonomy

Gambusia affinis (Baird and Girard) is one of some 150 species of fish in the family Poeciliidae. Members of this family are all from the New World with the majority found in Central America and the West Indies (Rosen, 1972). The etymology of the name Gambusia comes from "Gambusino", a provincial Cuban word which denotes "nothing". Thus, it is said, "to fish Gambusinos is to catch nothing". The species name, affinis, means "neighboring" or "related". Because of its well known appetite for mosquito larvae, the common name usually applied to the species is "mosquito fish". However, it may also be referred to in the literature as a "top minnow", due to its habit of seeking food at the surface.

A relatively flat head, small body and protrusible mouth are characteristic of mosquito fish. At birth, and for some time afterward, the anal fin of both sexes is similar. However, sexual dimorphism is later exhibited as the fin becomes small and rounded in the female while that of the male is modified into an elongated, rod-shaped copulatory organ, the gonopodium (Figure 1). The gonopodium is normally carried backward and parallel to the body, but it can be quickly moved at any angle, forwards or sideways. Adult females range from 1 to 2 1/2 inches in length while the mature males average about 1 1/4 inches. According to Krumholz (1948), female Gambusia attain greater lengths than males since they continue to grow until death. Males, however, grow very little after the gonopodium is completely formed. In both sexes, the body is pale grey with a blue metallic sheen. The belly is silver and the back, or dorsum, is brown or olive green. In many cases, there is a dark, transverse bar across the eye. Female Gambusia and guppies are similar in appearance, but Gambusia can be distinguished by the rows of tiny black dots on the dorsal and caudal fins (Figure 1); a characteristic found lacking in guppies. Another good field characteristic is that in female Gambusia, the first ray of the dorsal fin is posterior to that of the anal fin, while in the guppy they are almost in direct line with one another.

Two subspecies of Gambusia affinis occur that are essentially identical in color, form, size and habits: Gambusia affinis affinis and Gambusia affinis holbrooki. The two can be separated by the fact that G. a. holbrooki has eight rays in the dorsal fin while G. a. affinis has only seven. A velvet black spotting or blotching may be present in either subspecies. Such individuals are not different species, but are merely showing a melanistic or pigmented expression such as occurs in "marbled mollies", a fish well known to tropical fish hobbyists. This phenomenon is rarely seen in females but may occasionally occur in the males.

Distribution

Because of its use for mosquito control, Gambusia affinis probably has the widest range of any fresh water fish. It is found in nearly all of the warmer regions of the world and has been successfully established in areas where relatively severe cold weather occurs, such as Utah (Rees, 1934 and 1945), Michigan (Krumholz, 1944), Alberta, Canada (Mail, 1954) and the USSR (Krumholz, 1944). In the U. S., Innes (1971) divides the distribution of Gambusia affinis between the two subspecies. The range of the eastern subspecies, G. affinis holbrooki, is given as Delaware to Florida and Alabama, whereas the western subspecies, G. affinis affinis, ranges from Alabama to Illinois and south to the Texas coastal region. In Alabama, the two subspecies reportedly meet and their distinctive features are lost. It is likely that this

phenomenon occurs elsewhere as Gambusia is found in Arizona, California, Colorado, Kansas, Massachusetts, Nevada, New Jersey, New York, New Mexico, Ohio, Washington, Wisconsin, and probably many other states.

Habitat

In areas where it is native or has become well established, Gambusia affinis is found in all sluggish or standing lowland waters which are accessible to it through natural channels. It thrives in a wide variety of water types including fresh or brackish, clear or muddy, shallow or deep. Gambusia is seldom found in swift-flowing streams or water that is polluted with chemical wastes acid in nature, but it can tolerate water moderately polluted with sewage. When larger fish are not present, Gambusia may be found in deep water. However, it shows a distinct preference for shallow water where it is protected from predaceous fish and where food and vegetation are more abundant. Kuntz (1913), in fact, observed large numbers of mosquito fish in water less than 1 inch in depth.

Although essentially a warm water fish, Gambusia can tolerate a wide range of temperatures. Hildebrand (1925) reported mosquito fish withstanding water temperatures of 102°F. in nature. Conversely, relatively cold water strains have been developed and have been reported to successfully overwinter under ice in certain areas of Utah (Bay, 1967a), Michigan and Illinois (Krumholz, 1944), and Manitoba, Canada (Smith, 1960). It should be noted that during winter, the fish hibernate in the lower water depths and are rarely seen until the spring when water temperatures become increasingly warmer.

It is well known that Gambusia can inhabit areas seriously deficient in dissolved oxygen for short periods of time without apparent stress. This ability is attributed by Lewis (1970) to a morphological adaptation of the fish to oxygen depletion. Specifically, their dorsally oriented mouths and flattened heads enable them to effectively utilize the oxygen-rich water at the air-water interface without dramatically altering their usual swimming posture. Gambusia are, therefore, suited for occupying habitats subject to periodic oxygen depletion such as in sewage lagoons.

Feeding Behavior

Gambusia feed on a number of aquatic organisms including mosquitoes. They are, in fact, voracious feeders that consume a wide variety of phytoplankton, zooplankton, as well as some of the larger aquatic insects (Mulla and Isaak, 1961). Hildebrand (1921) found that while insects constitute a large part of the diet, a majority of the Gambusia studied also consumed some plant tissue consisting mostly of algae. In determining what organisms will be taken as food by Gambusia it would appear that availability is more important than choice. Studies in Alabama by Hess and Tarzwell (1942) demonstrated, for instance, that when mosquito populations increased, the number of Gambusia eating them, as well as the number eaten per fish, also increased. By analysis of stomach contents, it was also found that a peak in feeding activity occurred soon after daylight with a decline to a minimum in the later afternoon and then an increase to a second, but lesser peak, in the evening. Hubbs (1971) found similar peaks to occur at 1100 and 1900 hours in Texas. Gambusia searches for food at or near the water surface, but will search the bottom if food is scarce elsewhere.

Mosquito fish are highly attracted by prey movement. Hildebrand (1921) reports their range of vision to be only 4 to 5 inches, and the movement of larger prey is more quickly noticed than small prey. Anopheles larvae will, in fact, remain motionless when fish are nearby. According to Hildebrand (1919), such a protective instinct is probably highly developed in mosquito larvae since "inactivity" is the only protection they have against their fish predators. The fact that Gambusia show increasing preference for later instars and even greater predilection for pupae has been reported by a number of investigators (Hess and Tarzwell, 1942; Mulla and Isaak, 1961; Harrington and Harrington, 1961).

The voracious appetite Gambusia have for mosquitoes has been reported on several occasions. Hildebrand (1921) observed one large female to eat 225 larvae and pupae within a 1 hour period. In another instance, a pair of half grown Gambusia consumed over 5,000 larvae in 11 weeks (Seale, 1917). All sizes and ages of Gambusia readily feed on mosquito larvae, and even fry only a few hours old will attack young instars. Gambusia are also cannibalistic and will prey on their own young if given the opportunity.

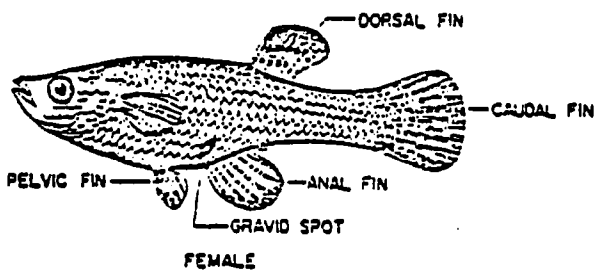
Reproduction

Oviparous or egg-laying fish generally produce thousands or millions of unprotected eggs, but relatively few survive. Gambusia which are ovoviviparous (livebearers), give birth to smaller

numbers, but the newborn are in a more advanced stage of development and better adapted to begin the struggle for existence than most fish hatched from eggs.

After mating, a female Gambusia has the ability to store the sperm and deliver a number of successive broods without further contact with a male. Mating is strictly promiscuous and pairs are not formed as in many egg-layers. The fertilized eggs hatch within the body cavity of the female and the young lie folded with head and tail meeting. They are delivered in this form at birth; either one or two at a time. Almost immediately, they straighten out and swim to the nearest refuge available. At birth, they are approximately 3/8 inch in length, and the ratio of males to females is essentially 1:1. As the fish grow older, this ratio gradually favors the females since they are hardier and live longer than the males (Krumholz, 1948).

A gravid or sexually mature female is easily recognized by the distended abdomen and a large, black triangular area in front of the anal fin known as the "gravid spot" (Figure 1). This characteristic is due to black pigment in the peritoneum (a thin membrane which lines the abdominal wall and various organs) which shows through the distended abdominal wall of the pregnant female. The pigmented area is also found in immature females, but it reaches its maximum size just before the female gives birth.



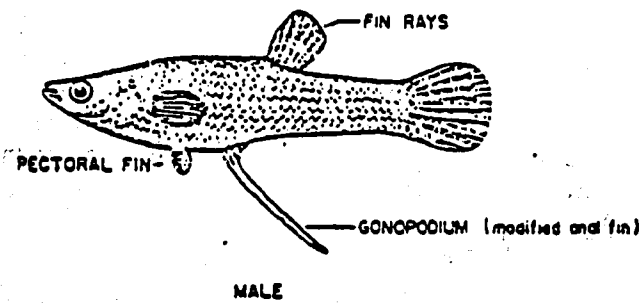


Figure 1. Gambusia affinis.

The average gestation period in Gambusia is 23 to 24 days (Krumholz, 1948), but varies with temperature and possibly with other factors. Parturition, or the time required for a single brood to be born, will take about 30 to 60 minutes (Krumholz, 1948). On the average, three to four broods will be produced and, depending on the size of the female, the number of fry per brood may average from 40 (Hildebrand, 1921) to 104 (Hoy and Reed, 1970), although broods of 300 or more may occasionally be encountered. Overlapping of broods does not occur and only a single brood of developing embryos are present in the ovary at any one time. Therefore, the female will have broods throughout the summer at intervals of 3 to 6 weeks, the length of the breeding season varying with the latitude and climate (Hildebrand, 1921). According to Hildebrand (1921), the breeding season in the southeastern U. S. begins in May and ends in September or October, while in Florida (Key West), it occurs all year round, although gravid females are less numerous during the winter months. Hubbs (1971) reports that female Gambusia are reproductive from March through September in Texas. In Illinois, Krumholz (1948) found that females present at the onset of summer were virgins born the preceding fall, which became gravid for the first time at an age of 8 to 10 months. However, female offspring of the first, and possibly second, generations became gravid at an age of 4 to 6 weeks. Hubbs (1971) made similar observations in Texas.

According to Krumholz (1948), mosquito fish may live for 4 to 5 years in aquaria, but they seldom survive more than 2 years in the wild. As a general rule, both males and females were found to die the same summer in which they reached maturity. However, the maximum life span was found in females which did not become mature until their second summer. These fish liberated their series of broods and died at an age of 15 months.

EFFECTIVENESS

The effectiveness of Gambusia as a mosquito predator has been well documented over a period of 70 years. Characteristics which have contributed to this effectiveness include the following: (1) flattened heads and protrusible mouths which enable the fish to readily prey on surface feeding larvae and pupae; (2) small size which enables them to inhabit shallow waters and penetrate dense vegetation growth where larvae and pupae hide; (3)

ovoviviparous reproduction which does not require specialized substrates for oviposition as in many egg-layers; (4) high fecundity, short life cycle and voracious appetite for aquatic insects; (5) broad tolerances for temperature changes, salinity, organic pollution, overcrowding and poor food supply; (6) hardiness, adaptability and subsequent ease of handling during transporting; and (7) relative lack of susceptibility to disease.

Gambusia is a hardy species and yet their effectiveness in any given situation will depend on: (1) the amount and type of vegetation and debris present; (2) the density of the fish population; and (3) the existence of natural enemies such as predaceous fish. In situations where dense vegetation or debris provide adequate protection for larvae and pupae, good control may not be obtained. Exceptions to this will occur when an overabundance of fish causes an active search for prey, even in heavily vegetated areas (Hildebrand, 1921). According to Hildebrand (1925), the degree of control obtained will be dependent upon the density of the fish population. When population numbers are small, a comparatively larger number of mosquito larvae will escape than when mosquito fish are in abundance, and the competition for food correspondingly greater. This is particularly true when adequate hiding places exist for larvae and pupae. The existence of natural enemies which prey on Gambusia will tend to keep the population at a level which precludes good control. Gambusia is more effective when introduced into areas where its predators are naturally absent or have been eliminated prior to stocking. These predators may include birds, frogs and larger aquatic insects. In most cases, however, they will be larger carnivorous fish such as pike, bass and sunfish.

Because the effectiveness of Gambusia is obviously dependent on the three factors discussed above, it may be necessary to disturb or change one or all of these to obtain adequate control. This is very evident when Gambusia and mosquito larvae are found living together in the same areas. As discussed by Barney and Anson (1921), it is necessary to modify the environment of such an aquatic situation in order to magnify the effectiveness of Gambusia as a larval predator. This can be accomplished by removing the vegetation, increasing the number of fish by stocking, providing places of refuge, or eliminating natural fish predators.

In Michigan, Krumholz (1948) found in experiments over two summers that Gambusia were about 81% and 95% effective in controlling anopheline breeding in ponds. In Georgia ponds and swamps, Hildebrand (1925) found mosquito fish to be responsible for a 50% and 80% average reduction in anopheline and culicine populations, respectively. The most effective control of both species was in artificial ponds while control was much less successful in several heavily vegetated swamps. Similar observations have been made by other authors, and it should be realized that absolute or 100% control cannot be expected in every situation. The fish may also be more effective under some circumstances than others.

The mosquito-like chironomid midge, while non-biting, is often an intolerable pest in certain areas. Like mosquitoes, the immature stages are aquatic and it might be expected that Gambusia would also be effective in their control. Bay and Anderson (1966) concluded from studies in California, however, that mosquito fish had little or no value in the control of chironomids.

HUSBANDRY

Mosquito fish "husbandry" has become an accepted term for the manipulation of fish for mosquito control (Fowler, 1972). In this guide, husbandry will include methods for collecting, transporting and stocking mosquito fish along with information on integrated control techniques.

Collecting

In order to establish a mosquito fish program for biological control, a system for mass collection is essential. There are basically two major styles of collecting: active and passive. In active collecting, the fish are actually chased or surrounded in a physical manner, while in passive collecting, a trap is used in which the fish capture themselves. The type of equipment used to accomplish collecting may include aquatic or dip nets, minnow seine nets and a number of different fish traps. In order to select the best method for each individual situation or locality, each of the methods and their advantages and disadvantages are discussed below. A review of the Biology section will indicate those areas to investigate for sources of mosquito fish.

The aquatic net, or dip net, (Figure 2) consists of a small mesh bag on a long wooden handle. It is an inexpensive piece of equipment which is used in active collecting by one man. While an aquatic net might be useful in a small scale operation, its use is

Figure 2. Aquatic net or dip net

time consuming, inefficient and inadequate when large numbers of fish are required. In addition, the fish may be seriously stressed as they are scooped from the water.

The minnow seine, or drag net; is suitable for use in most locations as another form of active collecting. These vary in size from small scoops to large drag nets of 6 x 50 feet (Figure 3). The ideal mesh size is 3/16 inch and the addition of 1 1/2 inch x 6 foot dowelling, attached at each end of the seine, will facilitate handling (Fowler, 1972). In shallow water where large populations of Gambusia are present, this method has a definite advantage as large numbers can be easily surrounded and captured. However, there are certain disadvantages: seining may require the assistance of several individuals; it is usually time consuming; and in deep water, such as a reservoir, a boat is required to operate the nets. Stress is also an important factor. When the seines are dragged over muddy bottoms, the fish frequently become fouled with mud which clings to the fins and scales. If not completely washed off, death will usually follow. If the net is dragged too long,

Figure 3. Fifty foot minnow seine or drag net

particularly in shallow water, the fish may be injured, leading to death or a fungus infection. Finally, seining is indiscriminate and will capture all species of fish. Sorting will be required before stocking can be accomplished, and this additional handling results in longer exposure to air and subsequent high mortality.

Active methods of collecting have inherent disadvantages and the use of fish traps for passive collection is usually recommended. The wire minnow trap is a popular bait fish trap constructed of small mesh hardware cloth (four or eight mesh) in the shape of a barrel or cylinder, with funnels at either end (Figure 4). The trap is baited inside with dry bread, crackers, or dog food and then positioned in shallow water with both funnel ends submerged. A cord or chain is attached to the trap to anchor it to the bank. Where small populations exist, best results will probably be obtained when the trap is set during the peaks in feeding activity. In areas where populations are heavy, large numbers may be captured throughout the day and within a few minutes after the trap has been positioned.

Where large numbers of Gambusia are required in a program, the "box-style" traps are recommended. The box trap is basically an oversized minnow trap. Caton and Sjogren (1969) described a box trap consisting of a wood frame box covered with 1/8 inch hardware cloth and a hinged top for fish removal. The trap utilized one

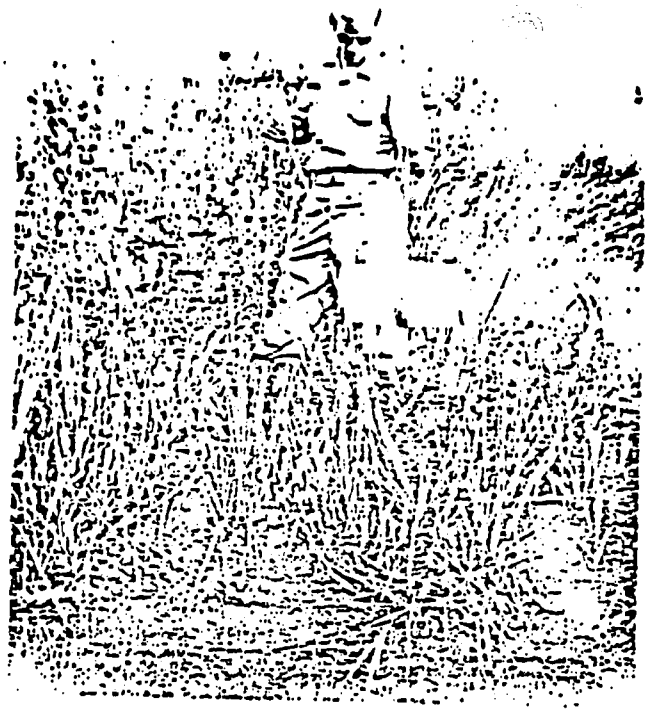


Figure 4. Wire minnow trap

funnel entrance and a lateral weir arrangement to channel the fish into the interior. Stains (1970) developed a similar box trap for use in sewage lagoons. Constructed of redwood two by fours, it was rectangular in shape with dimensions of 3' x 3' x 10'. Two hardware cloth funnels were utilized along with lateral weirs to channel the fish into the trap from either direction.

The permanent box trap is well suited for large activities requiring an extensive mosquito program and having access to a permanent source for collection. However, to fulfill the need for a less permanent and more portable collection system, a modified box trap (Figure 5) was designed which was lightweight, collapsible and easily assembled in the field by one man (Ehrhardt and Sholdt, 1972). This portable trap is described in detail in Appendix A in anticipation that it will be of use to activities not requiring permanent traps and having limited storage facilities.

Because Gambusia is a shoaling species, best results are achieved when the trap is set parallel to, and about 2 to 4 feet from, the shoreline (Figure 6). Obviously, the water level must at least be above the funnel entrances. Conversely, it should not be too far above the entrance holes, as Gambusia normally swim near the surface and will not enter the holes if the trap is too deeply

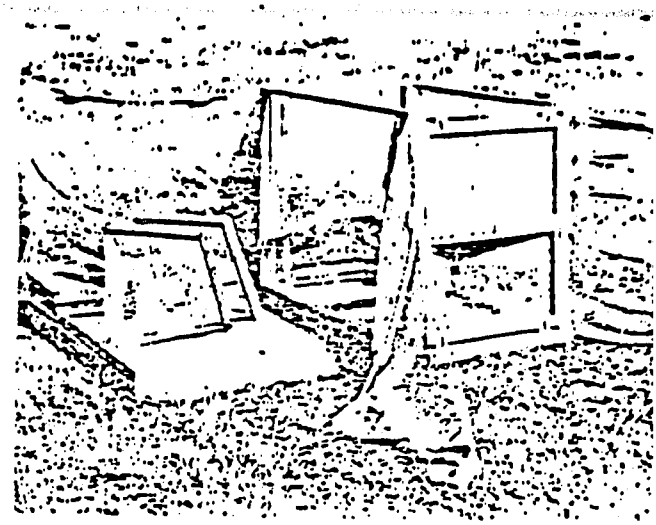


Figure 5. Portable box trap, assembled and disassembled

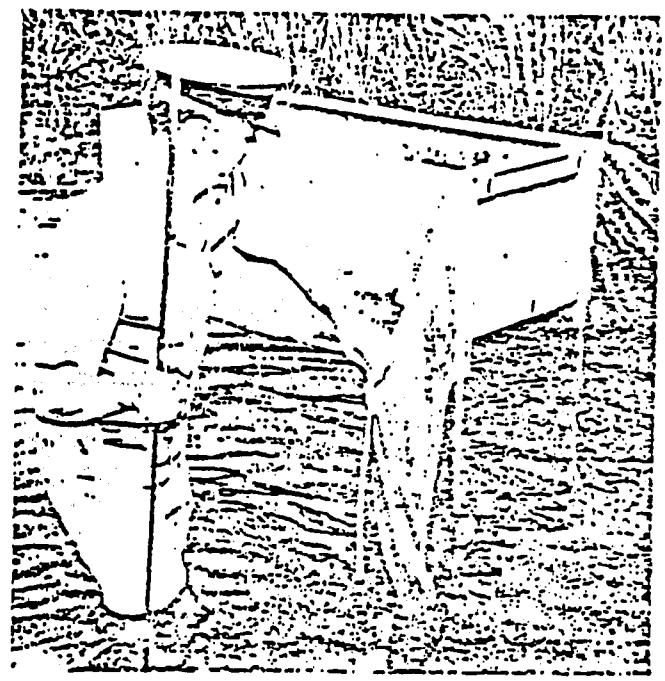


Figure 6. Positioning of portable box trap

submerged. The best time for collection is in the early morning and late evening periods when their feeding activity is peaked. In heavy populations, the numbers captured with this box style trap is phenomenal. Stains (1970) roughly estimated that as many as 100,000 fish were collected in a single day using his trap.

Transporting

To collect the necessary quantity of fish required for effective control, one of several trapping methods can be selected. Similarly, after capture, the fish may be transported in a number of opened or sealed containers such as garbage cans and plastic bags, repectively. Sweetman (1936) recommended using 10 gallon lard tins for transporting 30 to 40 larger-sized fish. In Iran, Tabibzadeh, et al., (1970) transported about 300 fish in 30 to 40 liter polyethylene bags half-filled with water and then pumped full with oxygen and sealed. Nakagawa and Ikeda (1969) successfully transported 2,000 fish in 30 gallon polyethylene storage containers. Aeration was provided using airstones and compressed oxygen. Russell (1970) reported that 10,000 to 15,000 fish could be transported for short periods in 15 gallon garbage cans with lids, provided the water was well aerated. Ice was used to cool the water during extended holding periods in hot weather. When small numbers of up to 200 fish are to be transported for short distances, a commercial minnow bucket or 3 gallon plastic pail is usually adequate. Due to the exceptional jumping ability of Gambusia a cover or screen may be required on open containers to reduce the loss of fish while in transit.

Regardless of the type of container used, oxygen in some form should be supplied to the fish. As pointed out by Lewis (1970), when Gambusia become overcrowded, mutual interference occurs at the surface and each fish periodically loses contact with this oxygen source. Under these circumstances, the fish become less efficient in using surface oxygen. Therefore, high mortality results very quickly as the critical level of overcrowding is reached. This is particularly true during hot days when the fishes' requirement for oxygen is higher, but the amount of oxygen available in the water for respiration has decreased due to the increased water temperature. In open containers, adequate aeration can be achieved using diffusers or airstones made of porous stone. The source of air can be any type of air compressor, electric fuel pump or compressed air cylinder. Tygon tubing is used to connect the diffusers with the source of air. When a stream of fine bubbles is released through the airstone, direct oxygenation occurs, and the water is circulated bringing the poorly oxygenated water in the deeper layers to the surface where oxygen is available at the air-water interface. In deep containers, such circulation is particularly critical. Airstones found in tropical fish stores are sufficient for small containers while the type used in fish hatcheries are necessary for very large containers. The air can be metered using a surgical clamp or an aquarium air valve. Optimum aeration is a heavy stream of fine bubbles. Over-bubbling should be avoided because it creates heavy turbulence and little oxygen is dissolved.

A compact system designed for aeration of fish bait has been used successfully for Gambusia transportation. The system, as shown in Figure 7, consists of a small air cylinder, metering valve, air stone and tubing. The cylinder can be charged with air from a service station tire pump and when filled to capacity will deliver air to a container for 5 to 7 hours.

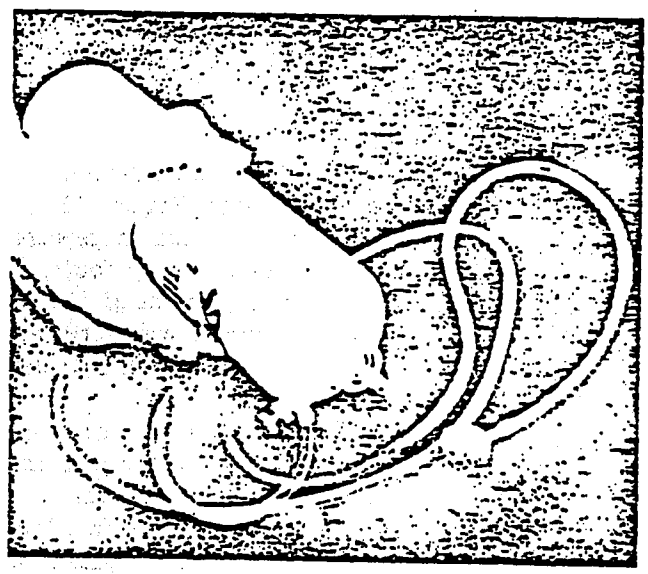


Figure 7. Compact aeration system used for keeping bait fish

The use of plastic bags as sealed containers for the transportation of fish has been known to tropical fish importers for several years. In addition to being inexpensive and suitable for transporting fish long distances, plastic bags have advantages over metal containers in that they: (1) do not rust; (2) prevent injury to fish bumping into container walls; (3) protect against temperature changes; (4) are lightweight but durable; (5) are portable; and (6) require little storage room. Also, the bags readily facilitate water temperature equalization at the stocking site by floating them in the water before releasing the fish (Miller, 1956).

The fish are placed in 5 to 20 gallon, heavy duty plastic bags contained in styrofoam boxes (Figure 8), which provide good insulation against

temperature fluctuations. Each bag is filled about 1/4 to 1/3 full of water and a maximum of about 200 fish per gallon are added. After deflating the bag, pure oxygen is added from a compressed

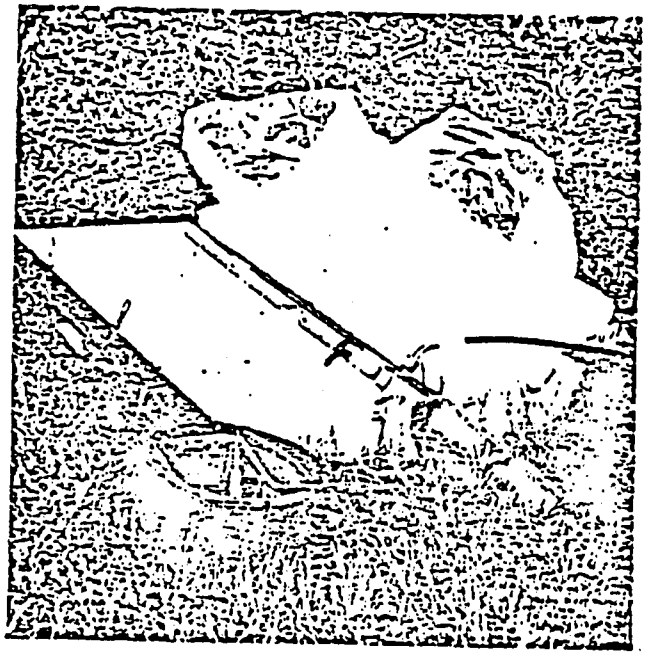


Figure 8. Closed container equipment for transporting mosquito fish

oxygen cylinder (Figure 9), and the top of the bag twisted, bent over and fastened with a rubber band. When the lid of the box is in place, the fish are ready for transporting to the stocking point. For added protection, it may be desirable to use a cardboard box or plywood case to hold the styrofoam boxes during transit.

The plastic bags and styrofoam boxes can often be obtained from local tropical fish dealers at minimum or no cost. A small compressed oxygen cylinder (FSN 6505-132-5181) is usually available from a dispensary. It is important that pure oxygen be used in the plastic bags instead of atmospheric air. Nemoto (1957) found in studies with two different fish species that survival time was increased by 20 to 70 hours when air was replaced with pure oxygen in sealed containers. The maximum holding time using the plastic bag technique will usually be 2 to 3 days; however, the bags must remain fully inflated. Fowler (1972) reported that Gambusia were air shipped from California to Montana using the above technique. The time in transit was about three days with negligible mortality.

Regardless of the method of transportation, the fish should not be artificially fed on the day prior to, and during, actual transportation. Temporary

Figure 9. Adding oxygen to plastic bags

starvation lowers metabolic discharges (Nemoto, 1957) and reduces the amount of regurgitated food that can contribute to pollution. The fish also appear to become more resistant to temperature fluctuations and handling injuries (Norris, et al., 1960). Another critical factor to consider during transportation is water temperature on hot days. In open containers, the addition of block ice may be useful. However, the amount of ice used should be controlled so that the temperature remains within 4 to 5 degrees of the water from which the fish were taken. Sudden and drastic changes in temperature cause undue stress on the fish producing mortality or predisposing them to disease.

Besides temperature, other factors which limit the maximum numbers of fish that can be transported include: oxygen consumption, carbon dioxide production, excretion of nitrogenous wastes and excess activity (Norris, et al., 1960). To control these factors, chemical agents capable of inducing anesthesia or deep sedation in fishes have been studied and used by Fish and Game

Commissions, tropical fish importers, and commercial bait haulers. These agents, termed general anesthetics, all act to varying degrees as central nervous system depressants (Bell, 1964). According to McFarland (1960), fish anesthetics decrease the fishes' reactions to external stimuli during transportation. Subsequently, hyperactivity decreases causing a reduction in oxygen consumption, and a reduction in the accumulation of carbon dioxide and metabolic waste products. As a result, significantly more fish can be transported than without the use of anesthetics.

Some studies have been made on the use of anesthetics for transporting Gambusia in California (Sjogren, 1971), however, published data is scarce. The limiting factors discussed above are probably less critical for Gambusia, an extremely hardy fish, and it is unlikely that most programs will require the use of fish anesthetics if proper aeration requirements are met. It should be noted, however, that the potential for fish anesthetics for transporting large numbers of Gambusia for extended periods of time is promising. Those wishing to further investigate the subject should review the paper by Bell (1964) which lists the properties and characteristics of several fish anesthetics in a useful chart form, in addition to the references cited above. Certain general anesthetics are extremely hazardous compounds, and their use should not be attempted before consulting with a Fish and Game Commission or other cognizant source.

Stocking

In selecting a suitable stocking site, several factors should be considered. To begin with, the sites where Gambusia often fail include those areas which are too cold, too plant infested, too extensive or too temporary for them to reach control levels or where protection from natural enemies is not adequate (Bay, 1967a). Next, in areas where extremes in summer and winter temperatures occur, ponds with a minimum depth of 5 feet will be required for the fish to survive (Krumholz, 1948; Tabibzadeh, et al., 1970). Thirdly, Krumholz (1948) states that Gambusia are capable of maintaining a population in salt water and, in fact, they have been shown in the laboratory to withstand salinity over twice that of sea water (Ahuja, 1964). But, Harrington and Harrington (1961) consider them to be overvalued in their usefulness for the control of salt marsh mosquitoes, and there is little information available to the contrary. Their use in salt marsh areas may, therefore, be limited. Finally, in North Carolina, ponds have been observed to support large populations of both game fish and Gambusia with no apparent adverse effects. However, there have been reports of mosquito fish threatening the existence of game fish by feeding on their young fry (Myers, 1965). It is recommended, therefore, that those areas designated for sport fishing not be stocked if a possible decline in game fish will result in adverse command reaction.

Areas in which mosquito fish generally provide adequate control include: irrigation systems, drainage ditches, sloughs, ponds, cisterns, shallow wells, watering troughs, seepage areas, borrow pits, sewage oxidation ponds or lagoons, water hazards on golf courses, undrained swimming pools, and other areas of standing water which allow for mosquito breeding. Gambusia are effective in ornamental fish pools, but are best used by themselves due to their aggressive behavior against other fish species such as goldfish (Myers, 1965). Personnel with state Fish and Game Commissions or the U. S. Fish and Wildlife Service are probably best qualified to assess the ecological situation and specify areas which can safely be stocked with Gambusia It is highly recommended, therefore, that these agencies be contacted before transplanting Gambusia to any new site within the activity or the local area. Such precautions will preclude any unpleasant repercussions from arising later.

While not normally required, artificial feeding may be desirable to achieve maximum potential in some areas. In such instances, the food of choice is "trout chow" such as fish hatcheries use. The amount of trout chow necessary for good growth will be dependent upon water temperature, the availability of natural food, and the size and total numbers of fish to be accomodated. Prior to stocking, the elimination of predaceous fish and other natural enemies of Gambusia may on rare occasions be necessary. Obviously, the killing of fish for any reason can have serious repercussions if accomplished improperly or without good justification. Therefore, coordination of such a project must again be made through the command, the activities' fish and wildlife representative, and appropriate medical department and public works personnel. Recommendations and approval for an appropriate chemical should be obtained from the cognizant Naval Facilities Engineering Command Applied Biologist.

After a site has been selected, approved, and properly prepared, the number of fish to be stocked will be governed by the surface area of water and the amount of protection available to the mosquito population. Overstocking is usually not a critical factor. Therefore, when fish are readily available, the higher the number stocked, the quicker the degree of control. Each situation will vary and as a guideline, the rates used by other workers are reviewed. Hoy, et al., (1971) found that good mosquito control was achieved in California rice fields with stocking rates of 300 mature females per acre when applied early in the season. In Louisiana, Craven and Steelman (1968) achieved 96% control of mosquito larvae populations in flooded rice fields stocked at rates of one half fish per square foot of water surface. Tabibzadeh, et al., (1970) found that fifteen females and one male per square meter of water surface gave good control in extensive breeding areas of Iran, while for small water collections with no vegetation, two females and one male per square meter were adequate. Before releasing the fish, the temperature of the water in the container and that of the stocking site should be equalized, particularly if a difference of more than 4 to 5 degrees F. exists. This can be accomplished by floating the container in the water or gradually adding water from the stocking site to that of the container.

Nakagawa and Ikeda (1969) reported on two methods utilizing helicopters for stocking fish in inaccessible areas. In one instance, storage tubs were lashed to the skids of a helicopter and the fish scooped out by hand and dispersed as the aircraft hovered from site to site. In another case, the fish were placed in a spray tank from which the spray system had been removed. The fish were dispelled from the aircraft by opening the main valve on the tank. In California, 1 gallon plastic bags were filled with water, approximately 200 fish, and a rock, and then released over stocking areas from a helicopter at heights of 20 to 30 feet. The weighted bags sank to the bottom where they opened and released the fish (Wolfe, 1970).

The amount of time required for a population to reach a level sufficient to provide effective control will depend on the number stocked initially. When minimum numbers are planted (i. e., 100 to 300 females per acre), a period of 30 to 60 days may be required before the population reaches an effective level. It is, therefore, advisable to stock as early in the season as possible such as in April or May. After stocking, the populations may reproduce at phenomenal rates. Fowler (1964) reported that within 30 days, 4,000 to 8,000 fish were obtained from rice fields initially stocked with 200 mature females. Russell (1970) estimated that a total of 1.5 million fish were removed over a 3 year period from one pond initially stocked with 2,000 Gambusia.

Integrated Control

The management of pest populations through the use of integrated control techniques has received increased attention in the past few years. The term "integrated control" is usually defined as the use of chemical, biological, and physical control measures either in sequence or simultaneously against a pest population. In a mosquito fish program, the central theme consists of using chemicals, namely larvicides and herbicides, to augment the biological control efforts of the fish; thereby assisting in the achievement of adequate mosquito control. The larvicides are applied early to control new mosquito broods, and then they may be discontinued when the fish multiply and become fully effective (Bay, 1967b). Herbicides, on the other hand, serve to control dense vegetation where mosquitoes see refuge from the predatory Gambusia.

Obviously, the choice of insecticide is critical since a selective material is required which will kill the mosquitoes but spare the fish population. At field dosages, Lewallen (1959) found Thimet, Guthion, malathion and parathion to produce high mortalities in mosquito fish. Similarly, chlordane, endrin, heptachlor, Thiodan, toxaphene, aldrin, dieldrin and isodrin were found to be hazardous to Gambusia (Mulla, 1963). Compounds found to be relatively safe against mosquito fish at mosquito larviciding rates include: Abate (Hamon, 1970); fenthion (Mulla and Isaak, 1961; Patterson and von Windeguth, 1964; Nakagawa and Ikeda, 1969); naled (Lewallen, 1959; Mulla, et al., 1963); Dursban (Ferguson, et al., 1966); methyl parathion (Mulla, et al., 1963); and FLIT MLO (Welsh, 1972). Diesel fuel will probably not kill fish at low rates but it may have some repellent action. Hildebrand (1921) suggests that diesel fuel is disagreeable to the fish and may destroy some of their food. Also of interest is the reported resistance or tolerance of mosquito fish to a number of the organochlorine insecticides (Boyd and Ferguson, 1964a, 1964b; Hamon, 1970).

Hildebrand (1921, 1925) felt aquatic plants with slightly submerged leaves or with a dense network

of roots near the water surface offered the best protection to immature mosquitoes from fish. Algal mats were also considered to provide good refuge when water found in portions of the mat was sufficient to harbor mosquitoes. Anopheles larvae have been found in the thin film of water that covers partially submerged leaves. In such instances, they cannot be detected or reached by larvivorous fish (Hildebrand, 1919; Miller, et al., 1970).

Whenever possible, it is recommended that the vegetation be removed mechanically. If, however, a herbicide is required then a number of factors (Sims, 1972) must be considered: (1) if the plants are growing in the shallow edges or margins, or on the bank above the water line; (2) if the water is free-flowing as in a canal or static as in a pond; (3) if the vegetation is a grass or a broadleaf and if it is submerged or immersed; (4) if the herbicide will be compatible with the aquatic animal life; and (5) if the treated water will be used for drinking by livestock, wildlife or humans. General guidelines for aquatic weed control are contained in Agricultural Handbook No. 332 (U.S. Department of Agriculture, 1969) and NAVFAC Manual MO-314 (U.S. Department of Defense, 1970). Final approval and assistance should be obtained from the cognizant Applied Biologist, Naval Facilities Engineering Command.

If compatible, the appropriate herbicide and insecticide may be used in combination to selectively control vegetation and mosquito larvae at the same time. It should be emphasized that the use of larvicides and herbicides be initiated only when and where necessary. Chemicals will not be required if the fish are keeping the mosquito population under control or adequate vegetation is unavailable for the mosquitoes to find refuge.

Other methods of improving or facilitating integrated control would be to construct drains leading to ponds stocked with fish and to thin or remove vegetation with herbivorous or omnivorous fish, such as carp. In Hawaii, Gambusia were used in combination with an algae-feeding fish, Tilapia mossambica. As the Tilapia multiplied, their feeding cleared surface algae enabling Gambusia to successfully attack mosquito larvae (Nakagawa and Hirst, 1959). This type of biotic activity has been termed "bio-synergistic" by Gerberich and Laird (1968).

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APPENDIX A
CONSTRUCTION OF A PORTABLE BOX TRAP

The portable box trap was patterned after the one described by Stains (1970). It was constructed of 3/4" X 1 5/8" Cypress slats covered with eight mesh or four mesh hardware cloth. The eight mesh (1/8 inch) hardware cloth was generally used for collecting large numbers of males and females. In cases where only the larger, mature females were desired, a trap covered with four mesh (1/4 inch) hardware cloth was used, as the smaller males and immature females easily passed through the screen. Approximately four man hours were required to complete the trap at a total cost of about twenty-five dollars.

The trap was constructed in a series of steps (Figure 10) to insure a proper fit of all pieces. In step A, the two trap sides were completed with outside measurements of 35 1/4" X 55 1/2". All corners were joined using 2 1/2" finishing nails and secured with polyvinyl resin white glue. The hardware cloth was trimmed to the proper dimensions and attached with a hand-operated stapler and 1/2" staples.

The two trap ends, step B, measured 25 1/2" X 35 1/4". Double wood slats were utilized at the top and bottom of the trap ends to allow space for the turn buttons to function. The center slat was carefully measured in order that the funnel and flat inserts could be freely interchanged. Finally, small strips of hardware cloth were stapled in place over the double slats.

The bottom of the trap was completed in step C, after minor adjustments were made to insure a uniform fit of the side and end pieces. After the hardware cloth was secure, the trap sides and ends were placed in position upon the bottom piece. By taping the parts together with masking tape, the hook and eye latches could be accurately secured. Fourteen latches were used; two in each corner and six along the bottom. To hold the funnel and flat inserts in place, a total of ten turn buttons were fastened to both sides of each trap end as shown in Figure 10, B.

In step D, four rectangular frames were constructed with outside measurements of 14 7/8" X 23 7/8". Two of the frames were fitted with hardware cloth to make the flat inserts. For each funnel insert, two triangles of hardware cloth measuring 13 7/8" X 15" and two measuring 12" X 23" were cut (Figure 10, E and F). The base lines of the four triangles, which corresponded to the inside measurement of the rectangular frames, were stapled in place. Wax covered string was used to draw the sides together into a pyramid. The string was tied at the base of each corner and then woven upward through the hardware cloth using a chain stitch.

Diverging lateral weirs made of minnow seine nets were used to direct the fish into the trap. Two 4' X 10' seine nets were cut in half for each end of the trap and fastened in place with 1/2" staples. Unlike hardware cloth weirs, the seine nets readily conformed to the contoured beds of collecting areas, were easily installed, and reduced the bulk and weight of the trap.

The trap worked equally well in shallow and deep water. Approximately fifteen minutes were required for assembly. In shallow water, the funnel inserts were placed in the lower trap openings. After collection was completed, they were carefully exchanged with the flat inserts. By quickly installing the flat insert behind the funnel as the latter was pulled up through the interior, the fish could be removed with no interference from the protruding cones. In deep water, the funnel inserts were used in the upper openings while the flat inserts closed off the lower. After the trap was pulled close to shore, removal of the captured fish was easily accomplished using a large tropical fish net.

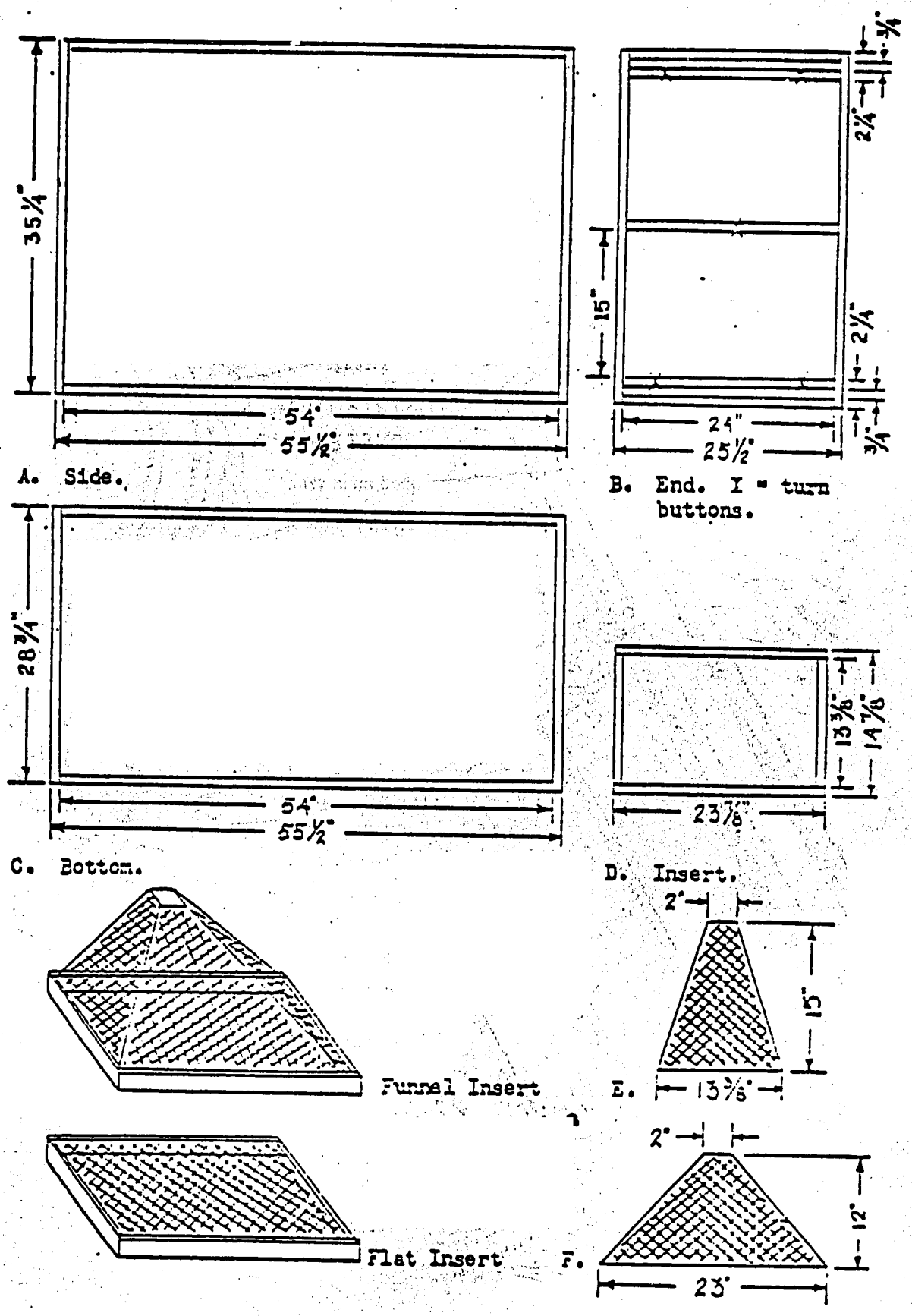

Figure 10. Specifications for construction of a portable box trap

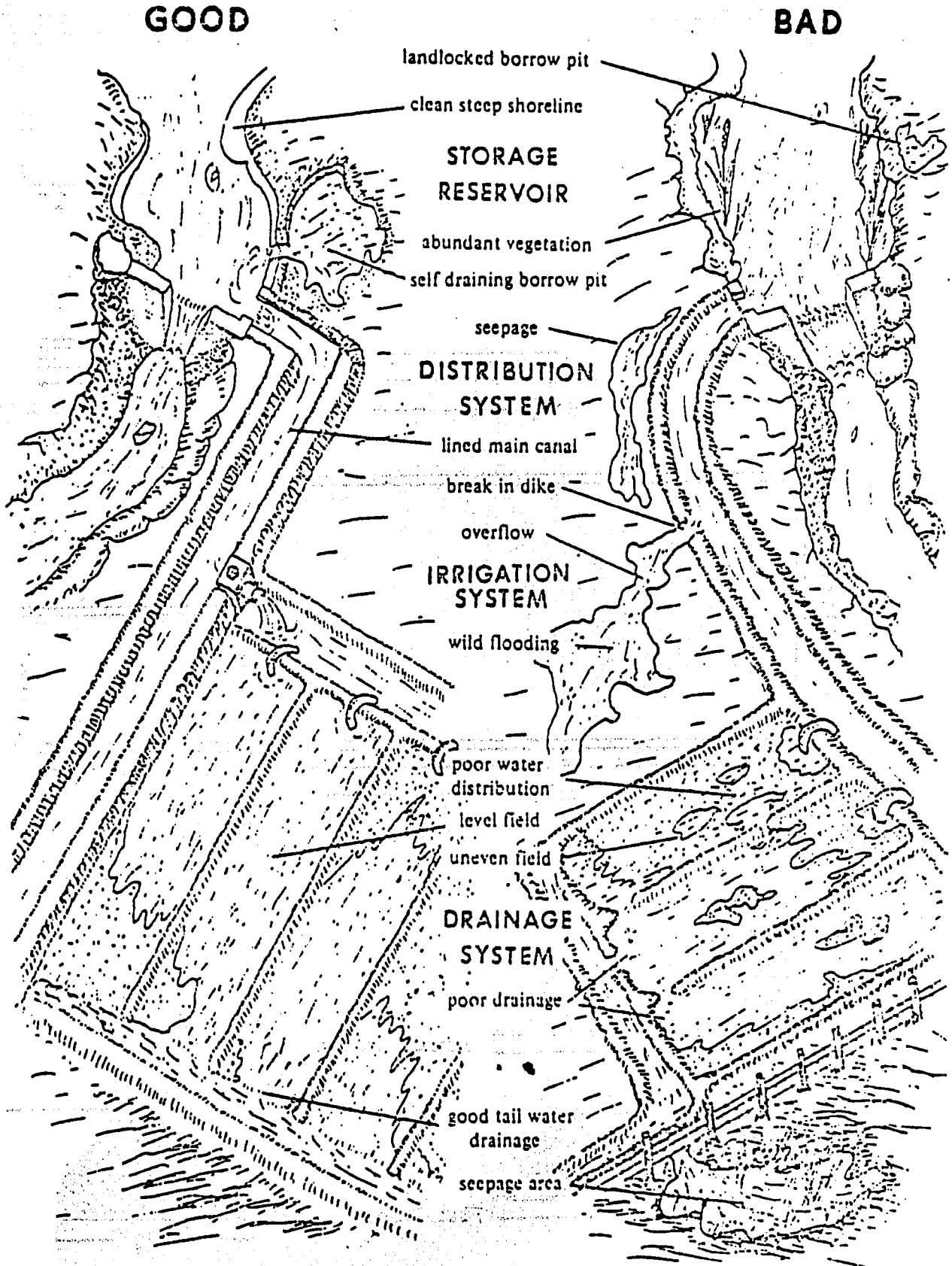

Figure 9.3 Good and bad features often found in irrigation systems.

Mulhern, 1975.

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FACTORS IN THE ELIMINATION OF THE IMMATURE STAGES OF *ANOPHELES QUADRIMACULATUS* SAY IN A WATER LEVEL FLUCTUATION CYCLE [1]

BY

EDITH M. DARROW [2]

(Received for publication May 26th, 1949)

INTRODUCTION

The development of water level management as a measure for the control of *Anopheles quadrimaculatus* breeding in impounded water has resulted in more economical and effective control of this malaria vector in the Southeastern United States. It has been demonstrated by Hess and Hall (1943) and Rozeboom and Hess (1944) that there is a positive correlation between the amount of intersection line at the water surface and the extent of *Anopheles quadrimaculatus* production. Water level management for malaria control is primarily concerned with the reduction or elimination of this intersection line at appropriate times and intervals. The usual schedule of water level management on main river reservoirs includes 4 phases (Hess and Kiker, 1944): (1) an early spring surcharge, during which the water is raised to flood elevation and then lowered to the maximum summer pool level in order to strand winter accumulations of drift; (2) constant pool phase, with the water maintained at the maximum summer elevation to discourage germination or sprouting of vegetation in the marginal zone of fluctuation; (3) cyclical fluctuation, which begins with the breeding season of *A. quadrimaculatus* and is sufficient to control the early summer breeding; and (4) seasonal recession, in which, in addition to weekly fluctuation cycles, there is a gradual drawdown to keep the water out of the advancing band of marginal vegetation. The first two phases of water level management are measures for limiting intersection values in the impoundments, but cyclical fluctuation and seasonal recession may destroy the larvae either by drawing them out of the vegetation into the open water where they are decimated by a lack of food and protection, or by stranding them on shore where death results from exposure to desiccation and other factors. Hinman (1938) found that as the water was lowered in an experimental pool, concentrations of larvae occurred in small depressions with subsequent death of the larvae. However, he felt that the drawing of the larvae out of the vegetated margin into the open water where predators could attack them played a more important part in their destruction. So far, there have been no detailed studies to determine the fate of larvae in a water-level drawdown. Sometimes a fluctuation cycle proves to

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Darrow, 1949.

be ineffective, and large numbers of mature larvae appear almost immediately after a dewatered shore is again inundated. This suggested the possibility that under certain conditions the larvae can survive on the mud for the duration of a fluctuation cycle.

In the formulation of a more successful program of water level management, a knowledge of the length of the time the eggs, larvae and pupae of the mosquito can survive on the shore under various climatic conditions should prove of great value. From observations that have already been made, it appears that the immature forms of anopheline mosquitoes can stand a surprising amount of desiccation. Anopheline eggs, which are the most resistant of the stages to desiccation, have been found by various investigators to remain viable for 2 to 3 days on a dry substratum under laboratory conditions. On a moist substratum the eggs can withstand desiccation much longer. Mayne (1926) reported that eggs of *A. quadrimaculatus* exposed on drying mud hatched after as long as 16 days and those of *A. crucians* exposed in a similar manner hatched after 21 days. An even longer survival was reported by Stone and Reynolds (1939). They found that mud from seepage areas in the Panama Canal Zone which had been without standing water for at least 1 month produced larvae of *A. punctimacula*, *A. albimanus*, and *A. aquasalis* when placed in water. Deane and Causey (1943) found that a small proportion of the eggs of *A. gambiae* could survive up to 18 days in drying mud.

Inasmuch as anopheline larvae are much less resistant to desiccation than are the eggs, there have been fewer observations on their survival out of water. Stephens and Christophers (1904) quote Grassi and Casagrande as having found that anopheline larvae can resist desiccation at 20 C for 2 days, at 35 C for 1 day, and 40 C for only 2 minutes. Larvae of anophelines stranded on moist mud, they said, will live as long as 4 days, but in the tropics as soon as the mud loses its glistening surface they die.

Nickolsky (1925) observed that *A. maculipennis* larvae in pools around Moscow could live on moist soil for 3 days when the weather was cloudy and for 1 day when it was sunny. Imagos emerged from the pupae even when the latter were stranded on the mud.

Hill and Cambournac (1941), while studying the effect of intermittent irrigation used for mosquito control in rice cultivation, found that larvae on soil with a 20 per cent moisture content could survive for only 2 hours out of doors in the shade but for 12 hours in the laboratory. They suggested that it was necessary only to reduce the moisture in the rice fields to the point where the surface film was lost, as indicated by a lack of luster, since the mud began to lose its luster at 20 per cent soil moisture content.

In the laboratory Kalandadze and Sagatelova (1945), in a series of experiments on *A. maculipennis maculipennis* and *A. claviger*, using substrata of known moisture content for the exposure of the larvae, found that the third- and fourth-stage larvae survived longer than did the first two stages. The 3 oldest stages of *A. claviger* survived from 3 to 7 days on substrata of 33.3 per cent moisture content at room temperatures and 59 to 75 per cent relative humidity. On a dry substratum they survived only 35 minutes.

The purpose of the present study was to determine the importance of certain factors governing the effectiveness of cyclical fluctuation of water level for the control of the immature stages of

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be ineffective, and large numbers of mature larvae appear almost immediately after a dewatered shore is again inundated. This suggested the possibility that under certain conditions the larvae can survive on the mud for the duration of a fluctuation cycle.

In the formulation of a more successful program of water level management, a knowledge of the length of the time the eggs, larvae and pupae of the mosquito can survive on the shore under various climatic conditions should prove of great value. From observations that have already been made, it appears that the immature forms of anopheline mosquitoes can stand a surprising amount of desiccation. Anopheline eggs, which are the most resistant of the stages to desiccation, have been found by various investigators to remain viable for 2 to 3 days on a dry substratum under laboratory conditions. On a moist substratum the eggs can withstand desiccation much longer. Mayne (1926) reported that eggs of *A. quadrimaculatus* exposed on drying mud hatched after as long as 16 days and those of *A. crucians* exposed in a similar manner hatched after 21 days. An even longer survival was reported by Stone and Reynolds (1939). They found that mud from seepage areas in the Panama Canal Zone which had been without standing water for at least 1 month produced larvae of *A. punctimacula*, *A. albimanus*, and *A. aquasalis* when placed in water. Deane and Causey (1943) found that a small proportion of the eggs of *A. gambiae* could survive up to 13 days in drying sand.

Inasmuch as anopheline larvae are much less resistant to desiccation than are the eggs, there have been fewer observations on their survival out of water. Stephens and Christophers (1904) quote Celli and Casagrande as having found that anopheline larvae can resist desiccation at 20 C for 2 days, at 35 C for 1 day, and 40 C for only 2 minutes. Larvae of anophelines stranded on moist mud, they said, will live as long as 4 days, but in the tropics as soon as the mud loses its glistening surface they die.

Nickolsky (1925) observed that *A. maculipennis* larvae in pools around Moscow could live on moist soil for 3 days when the weather was cloudy and for 1 day when it was sunny. Imagos emerged from the pupae even when the latter were stranded on the mud.

Hill and Cambournac (1941), while studying the effect of intermittent irrigation used for mosquito control in rice cultivation, found that larvae on soil with a 20 per cent moisture content could survive for only 2 hours out of doors in the shade but for 12 hours in the laboratory. They suggested that it was necessary only to reduce the moisture in the rice fields to the point where the surface film was lost, as indicated by a lack of luster, since the mud began to lose its luster at 20 per cent soil moisture content.

In the laboratory Kalandadze and Sagatelova (1945), in a series of experiments on *A. maculipennis maculipennis* and *A. claviger*, using substrata of known moisture content for the exposure of the larvae, found that the third- and fourth-stage larvae survived longer than did the first two stages. The 3 oldest stages of *A. claviger* survived from 3 to 7 days on substrata of 33.3 per cent moisture content at room temperatures and 50 to 75 per cent relative humidity. On a dry substratum they survived only 35 minutes.

The purpose of the present study was to determine the importance of certain factors governing the effectiveness of cyclical fluctuation of water level for the control of the immature stages of

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A. quadrimaculatus in impounded water. Investigations in the natural habitat of the mosquito in the Tennessee Valley Authority impoundments were designed to reveal the movement of the larval populations as the water level recedes and to determine the extent and causes of mortality of larvae and pupae stranded on shore by the receding waters.

Field observations were supplemented with laboratory experiments on the survival of larvae and pupae under controlled conditions of temperature, humidity and soil moisture.

Studies on the eggs of this mosquito were of a more preliminary nature and were designed to determine, under controlled conditions, the factors disposing the eggs to resist desiccation.

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DISCUSSION

In the schedule of water level management for the control of *Anopheles quadrimaculatus* in impounded waters, the third phase, i.e., cyclical fluctuation, owes its success to several larva- and pupa-destroying mechanisms. From the results of the observations on the movements of larvae during a drawdown, it is evident that at the experimental rates of drawdown, 75 per cent or more of the larvae are flushed out of marginal breeding areas with a moderate cover of a leafy emergent plant, into open water where they are destroyed, presumably by fish and other predators. However, abundant vegetation and flotage, which is essential to heavy breeding, may cause a higher percentage of the larvae and pupae to be stranded than was observed in the present study. For example, in some preliminary studies about 92 per cent of the larvae were stranded in an experimental area with high intersection line, as compared with only about 3 per cent where the intersection line was eliminated. Another factor affecting strandage is the rapidity of water recession; when the experimental drawdown was the slowest, approximately 0.5 feet in 24 hours, about a fourth of the larvae were stranded. On Lake Wilson, one of the smallest reservoirs and hence one with a comparatively rapid drawdown, the recession phase of a fluctuation cycle amounts to only about 0.3 feet in 24 hours, which is slower than any of the experimental rates. Thus, it may be assumed that in the more favorable breeding places in the Tennessee Valley impoundments a somewhat higher percentage of the larvae and pupae are stranded than was observed in the experimental pool. Because this stranded fraction may represent large numbers of potential adult mosquitoes, it is important to understand the ecological factors affecting their survival.

TABLE 16

Comparison of successful emergence from pupae continuously stranded and from those replaced in water after 24 and 36 hours of stranding

	Exposure period (hours)			
	In water		On soil	
	24	36	36	48
Number of pupae	166	76	130	292
Per cent successful of those attempting emergence	74.6	75.0	60.7	60.6

The most obvious factors to be considered are the climatic ones of temperature and humidity and the amount of water in the substratum upon which the aquatic stages are resting. Under natural conditions larvae and pupae can survive overnight strandage, but during the day an 8-hour exposure killed over 90 per cent of the larvae and over 70 per cent of the pupae. Under optimum conditions, in cool, humid weather, 95 per cent of the stranded larvae were dead after 48 hours. Even under laboratory conditions, with a soil moisture of from 22 to 27 per cent and a relative humidity of 100 per cent, the fourth-stage larvae were almost all dead at the end of 40 to 72 hours. Although pupae were better able to survive desiccation, and might give rise to adults while rest-

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ing on mud, strandage also affected them adversely, so that about a fourth of those returned to water after 24 to 36 hours of desiccation were unable to produce adults.

In the laboratory higher soil moistures permitted significantly longer larval survival, but in the field the effect of this factor could not be measured. The consistency of the soil appeared to be of greater significance than its actual moisture content, which is not surprising, for in a mushy substratum the larvae can sink down into the soil so that a larger area of its body is in contact with the wet soil and evaporation from the larva would be less. In a saturated atmosphere both in the laboratory and in the field, larvae which had crawled away from the moist mud onto the tin of the exposure apparatus were almost immediately desiccated, while their companions in the moist mud lived sometimes for days. Davies (1929) found that Collembola could not survive exposure on a dry substratum even in a saturated atmosphere, but when the substratum was moistened, they survived until starvation killed them. Mosquito larvae apparently are also able to absorb moisture from the soil to a certain extent to replace that lost to the atmosphere.

Temperature affected survival in the laboratory perhaps through increasing the evaporation rate, but in the field soil temperatures could not be correlated with survival of larvae and pupae.

Of all the climatic factors studied in the field, positive correlation was obtained only with evaporation, which is especially significant in that it integrates effects of temperature, radiation, relative humidity and air movements, as well as the exposure time. To arrive at an estimate based upon evaporation of the time required for effective kill of larvae and pupae stranded along the shores of the impoundments, evaporations recorded by the Livingston spherical atmometer in different kinds of weather in the Wilson Dam area were tabulated. The average evaporation for 8-hour midday periods during July, August and September in the experimental area and the expected mortality for larvae and pupae as derived from figures 4 and 5, are summarized in table 17.

TABLE 17

Evaporation, temperature and expected mortality of larvae and pupae during 8-hour mid-day period, July through September

Weather	Average evaporation (ml)	Average temperature (C)	Expected mortality (per cent)	
			Larvae	Pupae
Clear	23.5	30.3	78	62
Clear and windy	30.0	29.9	92	82
Intermittent showers	15.5	29.5	52	34

From this table it appears that during clear weather, stranding for one full day is sufficient to kill nearly all of the larvae and a substantial number of pupae. In rainy weather it would probably require 2 days for complete kill. It is during this type of weather that the insect predators might play a supplemental role in mosquito control. In the author's experiments species of Dolichopodidae and Saldidae reduced the larvae by as much as 21 per cent and the pupae by 38 per cent within an 8-hour period in the middle of the day. The predator effect would be greatly enhanced on well established shores by the presence of ants, which appear to be much more thorough in the destruction of stranded larvae than were flying insects. Although no observations were made of the removal of anopheline eggs by ants, it seems probable that these insects would be par-

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ticularly valuable in the destruction of eggs, which are able to survive a complete cycle of dewatering on a moist substratum.

The laboratory studies showed that eggs of *A. quadrimaculatus* must complete from two- to three-tenths of the total embryonation period before they acquire the ability to resist desiccation. On damp mud or in a saturated atmosphere, the embryo will continue to develop, but as long as the eggs are not in free water some of them, at least, will not hatch. Such eggs have been observed to remain viable without hatching up to 6 days in a saturated atmosphere, but how much longer than this they would remain alive is not known. Neither is it known how long eggs could survive stranding in cracks in the ground, where a high humidity in the microclimate might permit embryonation, but lack of free water would prevent hatching. Such problems offer a most interesting field for future investigations. At present there is increasing emphasis upon eradication of disease-bearing insects. Eradication of some species may be possible only after there is a complete understanding of the factors enabling the insect to survive in a marginal zone or during the off season. In the light of the observations by Stone and Reynolds (1939), the possibility of off-season survival in the egg stage by the anopheline mosquitoes should be investigated to the fullest extent.

Summary and conclusions

The object of this study was to determine the mechanisms by which control of *Anopheles quadrimaculatus* is effected through water level fluctuation in impoundments.

In an experimental pool at a drawdown rate somewhat faster than is usual in the main reservoir system of the Tennessee Valley Authority, 75 to 100 per cent of the larvae migrated with the receding waters. The numbers stranded appeared to be inversely proportional to the rate of water level recession and directly proportional to the intersection values in the dewatered area. All stages were equally susceptible to stranding.

During an 8-hour period in the middle of the day, the mortalities of the stranded larvae and pupae were increased 21 and 38 per cent, respectively, by predators. These predators were identified as belonging to 3 species of Dolichopodidae, one species of Saldidae and perhaps also a carabid beetle. They were not active at night.

The climatic effects on stranded fourth-stage larvae and pupae were mainly a function of water loss. During the night when the relative humidity remained at about 100 per cent, larval mortality amounted to about 11 per cent, while there was no mortality of the pupae.

In the daytime the mortality rate of stranded larvae and pupae protected from predators was correlated most closely with the evaporation rate. Observed mortalities in per cent were plotted opposite the evaporation as measured by a Livingston atmometer on arithmetic probability paper so that an estimate of the evaporation necessary for effective kill of the larvae and pupae could be derived. Using the 90 per cent mortality point for an indication of effective kill, this proved to be 28.5 ml for the larvae and 33.4 ml for the pupae. Soil moisture differences had no demonstrable effect on the mortality of the larvae for similar amounts of evaporation. However, trampled soil which was of a soupy consistency enabled larvae to survive more evaporation than did firm soil.

Combined effects of exposure to both predators and climate for 8-hour periods

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on clear summer days resulted in the death of about 92 per cent of the larvae and 75 per cent of the pupae. Under the most favorable conditions observed for larval survival, aside from rainy weather, all of the fourth-stage larvae died at the end of 48 hours of exposure.

In controlled laboratory studies first- and fourth-stage larvae and pupae were exposed on soil to saturated air. Because of the negligible evaporation in the exposure-chamber, significant differences in larval mortalities could be attributed to differences in moisture content of the soil. Loam soil with a moisture content of 24.7 per cent resulted in the death of half of the fourth-stage larvae in 37.2 hours, whereas it took only 27.8 hours for 50 per cent of them to die at 22.2 per cent soil moisture.

The real effect of soil in prolonging life of the larvae is due to the availability of the moisture present. This varies with the type as well as moisture content of the soil; hence sandy loam with a soil moisture of 15 per cent in these experiments acted similarly in regard to larval mortality to loam soil with a moisture content averaging 24.7 per cent. An estimated half of the fourth-stage larvae were dead in 37.2 hours on the loam soil and in 29.6 hours on the sandy loam.

Saturated air was more lethal at 31 C than that at 25 C, presumably because the higher temperature increased diffusion of the water vapor and resulted in an increase in evaporation.

First-stage larvae were more susceptible to desiccation than were the fourth-stage larvae. At 25 C in a saturated atmosphere the first-stage larvae were all dead after a day and a half of exposure on loam. Fourth-stage larvae exposed under comparable conditions survived from 2 to 3 days, depending upon the soil moisture.

Pupae exposed on soil to saturated air in the laboratory survived up to the time for eclosion about as well as controls kept in water. Survival, however, did not indicate the full damage done to the pupae because the proportion able to transform successfully into adults was only 55 to 72 per cent of the controls. The length of the exposure time beyond 12 hours did not have a demonstrable effect on this proportion. Pupae which were forced to transform on the mud were, on the average, only 80 per cent as successful as those exposed for the same length of time on mud and then transferred to water. Moisture content of the soil on which they were exposed was not observed to make a difference in the proportion able to transform successfully.

Eggs had to be kept moist for between 0.2 and 0.3 of the total embryonation period in order for them to acquire a degree of resistance to desiccation. Eggs incubated for this period survived a maximum of 54 hours at a temperature of 25 C and 65 per cent relative humidity. When the eggs were desiccated under these conditions for intervals less than this, it was found that they had ceased development for the length of time they were out of water. When the relative humidity was 100 per cent, the eggs continued in their development although out of water and on a dry substratum.

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MOSQUITO CONTROL IN RESERVOIRS BY WATER LEVEL MANAGEMENT

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Water management, properly employed, is the most potent single measure that can be applied toward controlling mosquito production from a manmade impoundage. Water management for mosquito control means the manipulation of reservoir water levels in such a manner that the microhabitat normally favorable for mosquito production is either destroyed or the mosquitoes are prevented from developing beyond their larval stage. Before attempting to apply the principles of water management for the control of a particular species of mosquito in a particular reservoir, however, it is first necessary that the primary purpose of the reservoir and the water management problems associated with carrying out this primary purpose be clearly understood.

Dams are constructed and reservoirs impounded for any one of a wide variety of reasons. Such reasons might include domestic or industrial water supplies, flood control, irrigation, hydroelectric power, recreation or navigation, to mention only a few. Some reservoirs are termed multi-purpose in that they are constructed and operated to serve two or more principal interests. The particular purpose or purposes to be served by a reservoir dictate within certain limits the general type of water management schedule required to carry out its primary mission.

Water supply or irrigation reservoirs are constructed to store water during periods of high streamflow to assure an ample supply for use throughout the year as needed. The operation of such reservoirs is aimed, in general, toward maintaining the reservoir water level at or as near full pool elevation as possible consistent, of course, with supplying the demand for water. It is usually desirable, with respect to recreational reservoirs also, that near full pool elevations prevail especially during seasons when recreational activities are at their peak. Spilling of excess water is of no particular concern after the full pool elevations have been reached. Spilling at elevations below full pool, however, may at times be most undesirable.

On the other hand, a flood control reservoir requires an opposite type of operation. In order to carry out its designed mission, there must be ample storage space available when needed to collect and hold, at least temporarily, flood flows in excess of that which can be safely passed downstream. Following such a flood control operation, the reservoir must be lowered sufficiently through regulated discharge to place it in readiness again to temporarily store and regulate the next flood. Thus, the operation of a flood control reservoir is toward maintaining a minimum pool elevation rather than a full pool.

Operation of a reservoir designed to serve navigation interests demands that a certain channel depth be maintained at all times. In addition, sufficient water must also be available to operate the navigation locks. Navigation requirements, therefore, necessarily fix the minimum reservoir drawdown elevation. Operation of the reservoir at elevations above this minimum is not of too much concern to navigation as long as excessive flow velocities and too rapid rise and fall in stage are avoided. These latter considerations demand careful regulation of reservoir discharges to prevent swift currents and

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unduly large water level fluctuations downstream from the dam or in certain critical stretches of the reservoir.

Reservoir water management for hydroelectric power generation may at times be more complicated than those operations previously discussed. Theoretically, one acre-foot of water through one foot of head is capable of producing 1.025 kilowatt hours of electricity. Thus power generation depends upon both the volume of water and the head available. The most efficient operation also demands that all available water be passed through the turbines and not over the spillway. Water over the spillway is lost to power generation and also will tend to reduce the effective head at the dam by raising the elevation of the tailwater level. Depending upon many factors too numerous and too complicated to mention here, it may be beneficial from a power standpoint to operate at times over a wider range of reservoir water elevations than is normally planned. In the face of an expected increase in flows, for instance, it may be advantageous to draw heavily on a reservoir through turbine use. The possible loss in efficiency from such an operation which might result from a steadily decreasing head may be more than overcome by the ability to store the impending flows for future use rather than being forced to waste water over the spillway.

Consistent with other influencing factors such as climatic conditions, water temperatures and shoreline topography, the type of reservoir water management followed in any given reservoir will determine the prevalence and type or types of mosquitoes occurring in that reservoir. Mosquito production in a reservoir may be considered, therefore, as an undesirable byproduct obtained in the process of operating the water levels of a reservoir to accomplish its primary purpose. But, just as certain water management practices can create biological conditions which favor mosquito propagation, the water itself can also be employed in most instances to limit or completely eliminate such mosquito-producing situations. Water management "tools" such as weekly cyclical fluctuation and seasonal recession have been successfully employed on reservoir mosquito control programs for a number of years. Their effectiveness and economy have been well documented. From the foregoing brief discussion of the several types of reservoirs, however, it should be apparent that each individual reservoir presents specific problems with respect to the application of water management for mosquito control. The degree of effectiveness which can be achieved through such a program on a particular reservoir will depend chiefly on the following factors:

(1) How well the specific water management requirements for mosquito control can be identified in advance.

(2) How closely these requirements can be incorporated into an over-all reservoir water management schedule without materially interfering with or adversely affecting operations necessary in carrying out the primary purpose of the reservoir.

(3) The development of a system of communication whereby the day-to-day water management needs for mosquito control can be made known and can be weighed along with other considerations by those responsible for making decisions concerning management of the reservoir water levels.

Much has been said, written, and published during the past 20 years concerning the use made of water management by the Tennessee Valley Authority in controlling the production of *Anopheles quadrimaculatus* mosquitoes on its system of reservoirs. The manner in which the salient water management features of *A. quadrimaculatus* control are integrated into the over-all TVA reservoir water management plan, however, may not be so widely known or understood.

The TVA plan for the development of the natural resources of the Tennessee River Valley called for the construction of a series of dams to impound the main river from a point near Paducah, Kentucky, to Knoxville, Tennessee, a distance

of some 650 river miles. In addition, reservoirs were also to be constructed on the principal tributary streams. The plan was unique in many respects. The concept of operating a multi-purpose reservoir was relatively new in the early thirties and there was no precedent for operating so large a system of such reservoirs, nine of which would form a continuous chain in the main Tennessee River. The reservoirs were to serve the primary purposes of flood control, navigation, and power. Superimposed upon the water management problems presented by these primary considerations was the need to control malaria transmission in the areas contiguous to these reservoirs. This meant the use of water management to control the production of *A. quadrimaculatus* mosquitoes in the reservoirs.

Eight years elapsed between the impounding of Wheeler, the first main river reservoir constructed by TVA, and Kentucky Reservoir, the last link in the main river chain. These years were spent in identifying the water management needs of each program interest and attempting to reconcile requirements which at first appeared to be somewhat conflicting at times. Recommendations by each program interest were studied by what is now the TVA Division of Water Control Planning in the light of over-all needs and benefits, always giving priority to the main statutory objectives. Temporary schedules of water management operations were set up as each reservoir was completed and impounded. It was often necessary during this period, however, to make departures in the planned management to facilitate construction of the dams. Periodic contacts made in the beginning of TVA reservoir operations were expanded so that by 1942 direct lines of communication between the malaria control staff and the water control planning staff had been established administratively within TVA. This permitted the day-by-day needs of malaria control to be considered along with the needs of the primary programs. Also, the effect on malaria control of any proposed temporary departures from scheduled operations could be discussed. In February 1945, from the experience gained during these construction years, TVA officially adopted the Multiple-Purpose Reservoir Operations water management charts which are still in use today. Each reservoir has its own individual characteristics which are reflected in its operation chart. The chart for Pickwick Reservoir, however, contains the main features which are typical of the water management schedules followed by TVA on its main river reservoirs.

The minimum flat pool level was set by navigation requirements to assure at least a nine-foot navigable channel at all times for river transportation. From studies of rainfall, runoff, flood frequencies and histories, it was determined that major basin wide floods in the Tennessee Valley occur chiefly in the winter and early spring months. Major floods on the main river have been limited almost exclusively to the months of December through April with the highest frequency in March. To provide flood storage space during this potential flood period, Pickwick Reservoir is held from 4 to 6 feet below normal full pool elevation from December to mid-March. Storage space below normal full pool in the other main river reservoirs ranges from a minimum of 2 feet in Guntersville to a maximum of 7.5 feet in Chickamauga. The generation of power is not materially affected by this operation since the effective head at all dams but Kentucky remains approximately the same except during actual flood control operations. The head at Kentucky Dam is 5 feet less since it is lowermost in the reservoir chain. This low water during the winter is helpful to malaria control in that it attenuates the growth of submerged aquatics in the reservoirs. The exact date to begin filling the reservoirs in the spring is determined by present and prospective streamflow conditions with the aid of current weather forecasts and longer range weather outlooks. The ideal time to initiate filling is after the last danger of flood

has passed but before the normal spring rains have appreciably diminished. The decision to begin filling is arrived at by the Division of Water Control Planning primarily on the basis of flood control considerations but with the full knowledge of the adverse effects delayed filling might have on both power and malaria control. Power is interested, of course, in filling the reservoirs while sufficient water is still available so as to have the water for use during the low flow season later in the year. Malaria control desires a full pool before the growing season becomes well advanced to prevent growth of vegetation in the exposed portion of the reservoir basin.

Upon filling to full pool elevation, a flood surcharge is provided. This is a malaria control operation, although some minor benefits to navigation and power may result. The water is raised above full pool, by one foot or more where practicable, and returned rapidly to strand accumulations of drift and flotage upon the reservoir margin above full pool elevation. In effect, this is a mechanical cleaning operation.

The constant pool phase which follows is extremely beneficial to malaria control as a long-range marginal plant growth control operation. Since an ample supply of water is usually available at this time for power generation, this full pool phase of the water management schedule does not adversely affect the power program.

Mosquito production at first occurs sporadically. As production becomes more generalized, weekly fluctuation cycles of about one foot in depth are initiated simultaneously at the request of malaria control on all the main river reservoirs except Kentucky. It takes about ten days to two weeks to set this operation in full motion. It is accomplished through a complex system of shifting power loads so that as one reservoir is being drawn down, the one immediately downstream is filling. As long as no water is spilled in carrying out this operation, cyclical fluctuation does not adversely affect the power program since the average head available at each dam remains essentially the same.

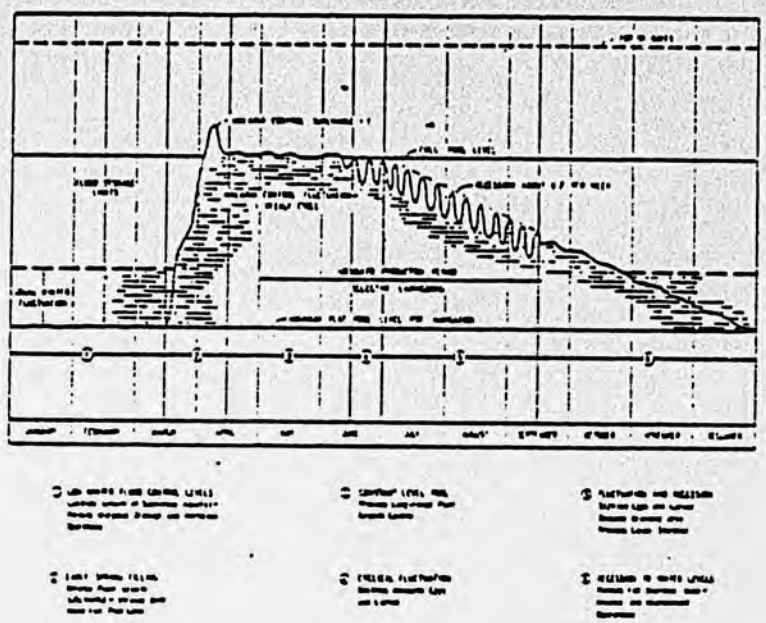
The next step in the TVA water management schedule is water level recession. Recession provides excellent immediate *A. quadrimaculatus* control by continually drawing the water below the marginal band of vegetation and thus maintaining a clean shoreline. Coming as it does in the latter part of the growing season, the chances for further invasion of significant plant growth into the reservoir are lessened. Recession benefits power by making water available for generation without substantial change in effective head during the late summer and fall dry season when river flows are normally lessening. Also, to reach the required flood control elevations by December, recession provides for the use of water previously stored, usually without the necessity of spilling. Malaria control takes advantage of the lower lake levels in the fall to perform shoreline improvement and maintenance operations. Last year the Malaria Control Branch began a joint study with the TVA Division of Power Operations to determine if any net gains to TVA operations could be realized through delayed recession. As a result, recession was delayed on Watts Bar and Fort Loudoun Reservoirs on an experimental basis for two months beyond the present guide curve schedules. The results looked good and the experiment is to be repeated this year. From studies on other reservoirs, however, possible over-all gains from delayed recession do not appear promising.

Last year also, TVA broadened the scope of its mosquito control program to include, in certain limited situations, the control of floodwater mosquitoes. Studies to date indicate that the over-all water level management schedules now in use by TVA are effective in controlling these mosquitoes. It is the occasional necessary deviation from normal operation that may be significant with respect to floodwater mosquito production. The need for special operations does occur from time to time

on any reservoir. Unusually heavy rainfall in June, a barge stranded on a mud bank outside of the channel, bridge construction or emergency repairs at a dam, or other special situations may all require temporary departures from guide curve operations. It is at these times that the close liaison which TVA provides between its water control and mosquito control staffs becomes of utmost importance.

An operations schedule which embodies the needs of all interests together with a system by means of which day-to-day needs may be considered is required to assure the most effective reservoir water management for mosquito control.

MALARIA CONTROL FEATURES OF WATER LEVEL MANAGEMENT ON TVA MAIN RIVER RESERVOIRS



Flatworm Control of Mosquito Larvae in Rice Fields

Abstract. *We describe some flatworms (some in the genus* Mesostoma*) that kill mosquito larvae and may account for the variability in the population densities of* Culex tarsalis *and* Anopheles freeborni *in rice fields. When mosquito larvae brush against these worms, the larvae immediately become paralyzed and die. When* C. tarsalis *larvae are placed inside floating cages that exclude flatworms (50-micrometer mesh), there is a fourfold increase in their survival. Rice fields that have abundant mosquito populations lack flatworms. Most such fields have only recently been turned over to rice production, suggesting that the flatworms have difficulty dispersing to new fields but, once established, are able to overwinter and control mosquitoes for the subsequent years of rice production.*

The densities of larvae of *Culex tarsalis* and *Anopheles freeborni* from one rice field to another in the Sacramento Valley (*1*) may differ by two or three orders of magnitude (*2*). This pattern of distribution is slightly bimodal. Most of the fields have few or no mosquitoes, but a few fields virtually teem with larvae (*3*). Early investigators thought that toxins produced by blue-green algae might account for these differences, but results from laboratory and field studies have been ambiguous (*4*). Previously, we found that the survival of larval *C. tarsalis* in screened cages in various rice fields was directly related to the abundance of mosquitoes in the fields at the time of the experiment (*3, 5*). We have now discovered that rice fields with low densities of mosquito larvae are heavily populated with microscopic flatworms (many under 1 mm in length) that kill the larvae by means of a slimy toxic secretion. These flatworms are able to penetrate the 200-μm mesh screening that we used in our early field cages. When we placed two flatworms in a 300-ml cup containing 20 second-instar larvae of *C. tarsalis* and observed their activity under a stereoscopic microscope, we noted that the larvae became paralyzed (rigid and motionless) as soon as they contacted one of the worms (Fig. 1). Later, the worms might recontact a paralyzed larva and feed on it, but the worms killed many more larvae than they ate. In fact, all the larvae were killed within 2 hours.

Extensive field sampling and laboratory identification revealed that as many as eight types (species?) of flatworms may be present in the rice fields. The predominant flatworm in these fields was found to be a *Mesostoma* species, a turbellarian in the order Rhabdocoela (*6*). Certain species in this order possess epidermal rhabdoids (rhabdites) that are slightly curved rods shorter than the height of the epidermis (*7*). These rods are secreted by gland cells and are discharged to the surface as a toxic mucous secretion that is repellent to predators (*8*). Although a few literature citations document the toxicity of some flatworm secretions, the extreme potency of the rice field species appears unprecedented (*7–9*).

We identified 14 rice fields in Sutter, Yuba, and Sacramento counties that differed in aquatic fauna, flora, and physical attributes, and placed four floating cages in each field (*10*). We stocked the cages with 25 third-instar larvae of *C. tarsalis* bred in the laboratory, and capped each cage with a tight organdy top. In two of the cages we placed 0.2 ml of Tetramin (a commercial fish food used successfully as larval food in the laboratory) every alternate day; the larvae in the other two cages in each field received no supplemental food and served as controls. Each day the dead and live larvae, pupae, and adults were counted, and the dead ones were removed.

After 1 week an average (over all cages in all fields) of 51 percent of the larval mosquitoes had died in the Tetra-

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died in the control cages: In some rice fields, more than 60 percent of the larvae died during the first day. In the laboratory, third-instar mosquitoes can survive without food for up to 3 days, so that food limitation in the field was not indicated. Nevertheless, using two fields in Yuba County that had particularly low larval survival rates, we tested the effect of altering various nutrient levels on larval survival (Table 1).

In each of these two fields we placed 12 experimental and 6 control cages, each cage containing 25 third-instar larvae of *C. tarsalis*. Once every alternate day, the following reagents were added to the experimental cages in each field: (i) 2.5 g of Envirotrol-F, a commercially cultured dry bacteria formulation (*11*); (ii) a water-soluble tablet containing assorted vitamins (*12*); (iii) 0.4 g of a plant nutrient formulation containing 2 percent total nitrogen, 9 percent phosphoric acid, and 13 percent soluble potash (*13*); and (iv) 1.0 g of calcium carbonate. The number of live and dead larvae, pupae, and adults were counted daily and the dead ones were removed. None of these treatments significantly affected larval survival: the average mortality in the experimental cages was between 86 and 94 percent, whereas in the control cages the average mortality was 85 percent.

We then assessed the influence of flatworms on mosquito mortality in the field. Since the flatworms are able to invade the 200-μm openings in the floating cages, we constructed new cages with windows of only 50-μm mesh. In each of six rice fields that previously had low larval survival rates (and contained flatworms), we placed three 50-μm mesh cages and six 200-μm mesh cages. As before, 25 third-instar larvae of *C. tarsalis* were introduced into each cage and every day for 1 week we counted the number of live and dead larvae, pupae, and adults and removed all the dead.

In every field we found that larval survival in the 50-μm mesh cages significantly increased (Table 2). Overall, about a fourfold increase in weekly survival was achieved. However, the survival rate in these 50-μm mesh cages (about 46 percent per week) was substantially lower than the rate we had measured in the 200-μm cages in some other rice fields (about 90 to 95 percent per week).

Could those mosquito-rich fields with high larval survival rates lack flatworms? To assess the presence or absence of flatworms, we placed three 200-μm mesh cages in each of 32 rice fields and placed 20 third-instar larvae of *C. tarsalis* in contents of the cages for flatworms. Simultaneously, we assessed mosquito numbers in each field by sweeping 30 times with an aquatic sweep net. Rice fields were categorized according to the number of mosquitoes collected in 30 sweeps: fields with five or fewer mosquitoes, fields with between 6 and 30 mosquitoes, and fields with more than 30 mosquitoes. All developmental stages were counted together. We found that no rice field ($N = 11$) in which we found flatworms in the baited floating cages yielded more than five mosquitoes per 30 sweeps. In the fields without flatworms we collected from seven fields less than six mosquitoes, from eight fields between 6 and 30 mosquitoes, and from six fields more than 30 mosquitoes per 30 sweeps (*14*). A χ^2 contingency analysis

Table 1. The effect of selected nutrients on the survival (percentage survival per week) of *C. tarsalis* larvae (25 larvae per cage) in floating cages. The compounds were placed twice daily in the cages in two rice fields with particularly low larval survival rates in earlier experiments.

Treatment	Percentage survival (mean)	Two standard errors	Number of cages
Bacteria culture	8	7.8	6
Vitamins	14	24.0	6
Calcium carbonate	6	13.8	6
Plant nutrients	10	9.0	6
Controls*	15	20.6	12

*No supplemental nutrients provided.

Table 2. Mean percentage survival per week for 25 larvae of *C. tarsalis* introduced into floating cages placed in various rice fields that contained flatworms. The cages had windows that were screened with either a 200-μm mesh organdy or a 50-μm mesh plastic fabric. Only the latter excluded the flatworms.

Field	Cages with 200-μm mesh ($N = 6$)		Cages with 50-μm mesh ($N = 3$)	
	Percentage survival (mean)	Two standard errors	Percentage survival (mean)	Two standard errors
A1	13	8.4	39	22.0
A3	13	11.4	35	16.7
B1	1	1.2	51	24.3
B2	24	11.8	51	22.8
B5	7	9.5	78	11.0
B9	14	4.6	24	18.2
Grand mean	12		46	

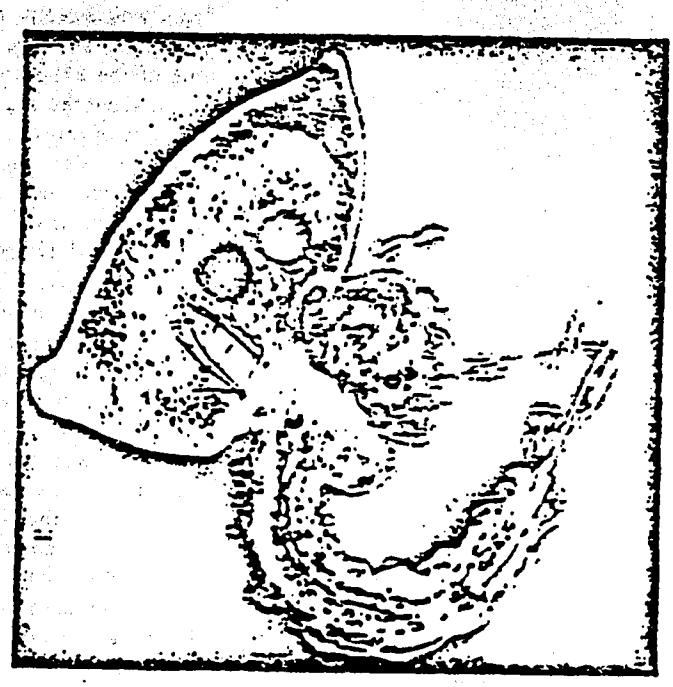

Fig. 1. Photograph (× 48) of a flatworm (*Mesostoma* sp.) from a California rice field killing a *Culex tarsalis* mosquito larva. (Photo by J. K. Clark, University of California, Davis.)

of these results revealed that the negative association between flatworms and mosquitoes could not be explained by chance alone [$\chi^2(2) = 13.3$, $P < .95$]. The seven rice fields that lacked flatworms but still yielded few (less than six) mosquitoes either had been stocked with the mosquito fish *Gambusia affinis* or were cool-water fields with a summer midday temperature usually below 21°C (*3*).

Our data show that rice fields with large numbers of flatworms produce few mosquitoes, even if the fields contain warm water and are not stocked with *Gambusia*, and that in the laboratory the flatworms are directly responsible for the death of larvae of *C. tarsalis*. Exclusion of the flatworms by the floating field cages causes a fourfold increase in larval survival but survival under these conditions is still lower than that in other fields that lack flatworms altogether. Perhaps in fields infested with flatworms the toxin is able to diffuse into the floating cages in sufficient quantities to adversely affect the mosquito larvae. An alternative explanation is that some other microorganism in flatworm-infested fields may also be detrimental to mosquitoes but may lack virulence under our laboratory conditions (*3*).

Also puzzling is the question of why some rice fields support flatworms and others do not. Most of the fields in this study that had inordinate numbers of mosquitoes and lacked flatworms were fields that had just been turned over to rice production. Typically, farmers in the Sacramento Valley will rotate rice to safflower or some other crop every 3 to 5 years. Perhaps flatworms have difficulty dispersing to new rice fields but once established they are able to overwinter and control mosquitoes for the successive years the field is in rice. Further study should reveal the validity of this hypothesis.

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References and Notes

1. In the Sacramento Valley of California, typically over 400,000 acres of land are devoted annually to rice production. These fields are usually flooded in early May. When the rice emerges, populations of larval *Culex tarsalis* develop in the warm water and usually reach a peak abundance in mid-June. By July or August the rice increasingly shades and cools the water and populations of *Anopheles freeborni* then predominate. Both *C. tarsalis* and *A. freeborni* are serious pests and pose a threat to public health as vectors of Western equine and St. Louis encephalitis and malaria, respectively (S. F. Bailey and P. A. Gieke, *Proc. Calif. Mosq. Control Assoc.* 6, 53 (1968)).
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8. S. J. Coward and J. W. Piedilato, *J. Biol. Psychol.* 15, 5 (1973).
9. R. A. Medved and E. F. Legner, *Environ. Entomol.* 3, 637 (1974).
10. The floating emergence cages were constructed from 1-gallon size plastic buckets with organdy screen windows (approximate mesh size 200 μm) on the sides and bottoms. Three fishing bobbers were placed near the top to keep the buckets afloat.
11. Available from Mardel Laboratories, Carol Stream, Ill.
12. Available from Aquarium Pharmaceuticals, Perkasie, Pa. Each tablet contains 120 mg of thiamine, 0.10 mg of riboflavin, 2.0 mg of niacin, 0.20 mg of pyridoxine, 1.50 mg of pantothenic acid, 0.003 mg of biotin, 0.20 mg of folic acid, 0.025 mg of aspartic acid, 0.025 mg of glutamic acid, 0.03 mg of choline, and 0.02 mg of inositol.
13. Aquarium plant food, Aquarium Pharmaceuticals, Perkasie, Pa.
14. As an independent check on flatworm occurrence, micro-rice field ecosystems were made by filling 1-gallon plastic aquariums with soil, algae, macrophytic plants, and unsieved water samples from each of the fields. A 300-ml plastic cup with 200-μm mesh windows was floated in each aquarium. Fifteen third-instar *C. tarsalis* were placed in each cup and the presence or absence of flatworms in the cup was noted after 1 week. In all but 2 of the 32 fields these two methods gave the same results concerning the presence or absence of flatworms. For the two fields for which the results differed, flatworms were found in the field but not in the laboratory aquariums.
15. We thank the following persons for their assistance: C. K. Fukashima, K. Lucnessa, M. F. Feldlaufer, R. P. Meyer, T. McKenzie, R. Dunn, M. Lee, K. G. Whitesell, and the Colusa Mosquito Abatement District. This work was supported by a special University of California Augmentation for Mosquito Control Research.

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WATER LEVEL MANAGEMENT FOR MALARIA CONTROL ON IMPOUNDED WATERS

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INTRODUCTION

Water level fluctuation for malaria control has been used on impounded waters in the Southeastern United States for over a quarter of a century. Stromquist (1935) and Hinman (1938) have presented a resume of the development of this measure and have discussed its early application on the reservoirs of the Tennessee Valley Authority. Later, Hinman (1941) has given a general discussion of the management of water for malaria control and has summarized the experiences with water level fluctuation on the Tennessee Valley reservoirs through the season of 1940. It is the purpose of the present paper to discuss the water level management practices in current use on the impoundments of the Tennessee Valley Authority, with particular reference to new developments which have taken place since 1940.

Normal seasonal changes in water elevations have repeatedly been observed to play an important part in controlling the production of *Anopheles quadrimaculatus* in limesink ponds and similar natural bodies of water (Hinman, 1938; Taylor, 1940). For this reason, the authors of the present paper classify water level management as a naturalistic control measure, considering it a deliberate extension and intensification of a form of natural control.

Water level management for malaria control is effective through the creation and maintenance of clean water conditions which are unsuitable for the production of malaria mosquitoes; thus, it is merely a practical application of the well-known maxim that a clean water surface will not produce *Anopheles quadrimaculatus*.

The recently established correlation between *Anopheles quadrimaculatus* production and the amount of intersection line* produced by littoral vegetation (Hess and Hall, 1943; Rozeboom and Hess, 1943) provides a means for the precise measurement of anopheline production potentials and thus advances the knowledge of the relationship of this production to physical conditions of the water

*Health and Safety Department, Tennessee Valley Authority, Wilson Dam, Alabama. Submitted October 29, 1943.

*This is the intersection of air, plant, and water.

urface. The knowledge of this relationship provides for a better understanding and more intelligent application of water level management for malaria control. Thus, it would appear that the effectiveness of a schedule of water level management in controlling anopheline production is mainly dependent upon the extent to which it reduces the amount of intersection line in the problem areas at appropriate intervals during the mosquito breeding season. Since plants or plant remains are almost entirely responsible for the creation of intersection line in natural breeding areas of *Anopheles quadrimaculatus*, it follows that a knowledge of the water relationships of littoral plants is of paramount importance in applying this method of mosquito control. The relation of plants to the production of malaria mosquitoes on the Authority's impounded waters is discussed in detail in a manuscript now being prepared for publication by the Biology Section of the Health and Safety Department.

Accessory Measures

The successful utilization of water level management for malaria control is not solely dependent upon the proper manipulation of water elevations, but depends also upon the carrying out of certain accessory measures. These accessory measures may be outlined as follows:

1. Proper Reservoir Preparation
   - A. Reservoir Clearance
   - B. Marginal Drainage
   - C. Final Reconditioning of the Zone of Fluctuation
2. Wintertime Impoundage
3. Shoreline Maintenance
   - A. Annual Shoreline Conditioning
   - B. Aquatic Growth Control
   - C. Marginal Grazing
   - D. Drainage Maintenance
4. Permanent Shoreline Improvement

All of the above accessory measures except permanent shoreline improvement have been adequately discussed by previous authors (Kiker and Stromquist, 1939; Hinman, 1941), and a preliminary account of the use of permanent shoreline improvement measures is given in a paper recently prepared by Bishop and Gartrell (1943). The remainder of the present paper will therefore be limited to a discussion of water level management for malaria control on the reservoirs of the Tennessee Valley Authority.

Water Level Management

There has been a tendency to think of water level management for malaria control merely as water level fluctuation; however, recent developments have shown that under certain conditions the maintenance of a relatively constant pool level may constitute an equally important phase of an effective schedule of water level control. The most suitable schedule of water level management for malaria control on a particular reservoir is dependent upon the type of reservoir and the way in which it is used. On the impoundages of the Tennessee Valley Authority, the following three types of schedules are in current use: (1) Seasonal recession alone; (2) Cyclical fluctuation alone; and, (3) A combination schedule for main river reservoirs. Since the latter type schedule involves the combined utilization of all the common phases of water level management, it will be discussed first.

Water Level Schedule on Main River Reservoirs

The application of water level management for malaria control on the nine main river reservoirs of the Tennessee Valley Authority is complicated by the multipurpose use of these projects, namely, for navigation, flood control, and the generation of hydroelectric power. The controlled discharge of water is frequently desirable to afford adequate depths for navigation below the lowermost dam, and the maintenance of a nine-foot navigation channel on all main river reservoirs limits the extent of seasonal recession; also, the obvious desirability of releasing water through the turbines to provide maximum generation of power places a practical limitation upon the scope of cyclical fluctuation. It has therefore been impracticable to undertake the application of either of these measures alone, and a combination schedule has had to be developed. In developing this schedule, an attempt has been made to fit it as closely as possible to the normal operation schedules of the reservoirs and to keep special requests to a minimum. General observations over a period of years on the relative effectiveness of various types of water control schedules and detailed investigations of the water level relationships of littoral plants have made it possible to gradually improve the program of water level management for malaria control on main river reservoirs. The currently recommended schedule involves the combined use of flood surcharge, maintenance of a relatively constant pool level, cyclical fluctuation, and seasonal recession. The main features of this schedule are illustrated in Figure 1. Each of the four phases of the schedule has its particular function, and these are discussed separately in the following paragraphs.

1. *Spring Flood Surcharge*

Sometime during the late winter or early spring, it is desirable to bring the water level up to the maximum elevation in the flood surcharge zone for a brief period, subsequently drawing it back down

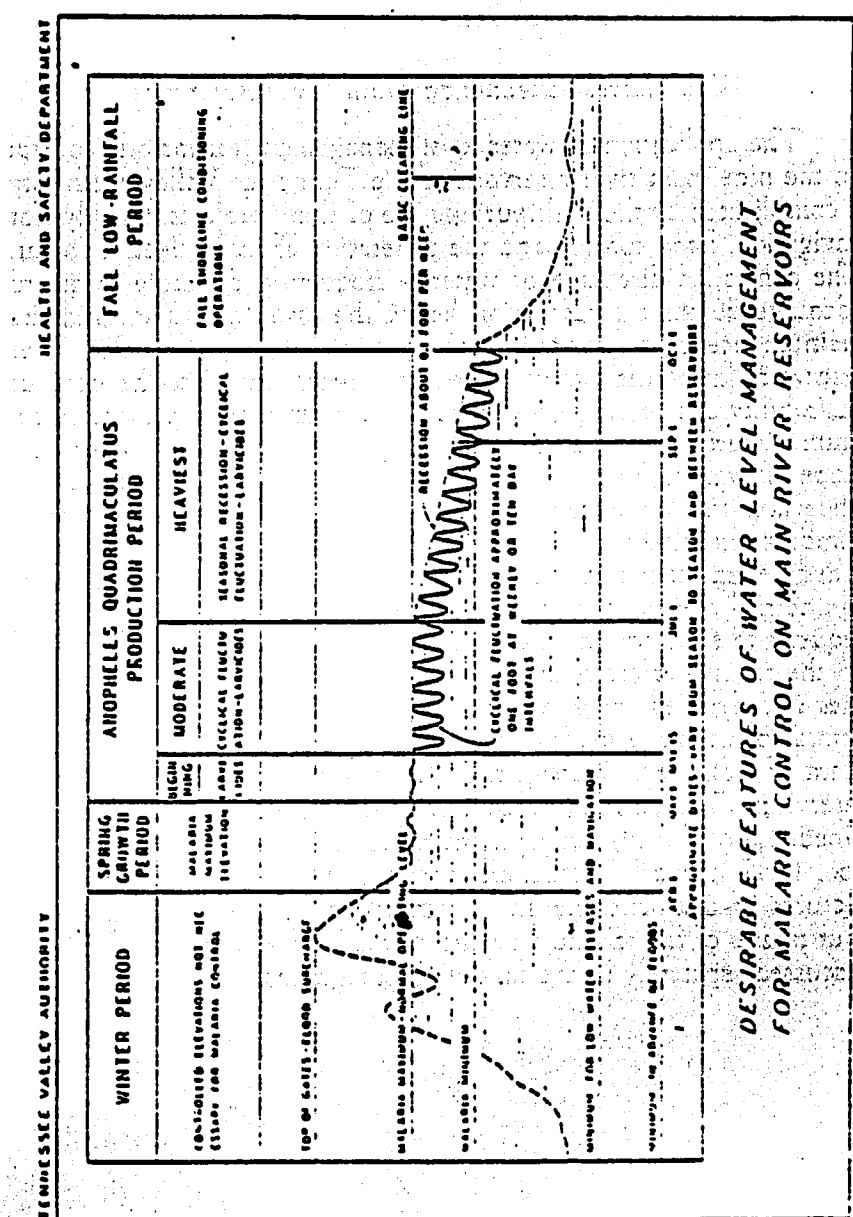

Fig. 1—*Desirable Features of Water Level Management for Malaria Control on Main River Reservoirs.*

to the normal maximum summertime elevation (See Fig. 1). This results in the strandage of the winter's accumulation of drift and flotage and thereby prevents this material from creating a mosquito breeding problem later in the season. Thus, the flood surcharge phase is purely a prophylactic measure which aids in preventing the development of suitable breeding conditions for *Anopheles quadrimaculatus*. The annual shoreline conditioning program increases the effectiveness of the use of the flood surcharge through the removal of coppice and stiff-stemmed annuals which would collect drift and flotage off shore and thus prevent its strandage.

The flood surcharge may be effectively used anytime during the late winter or spring when high precipitation and stream flows frequently result in the temporary storage of water in this zone: however, it is desirable that the water not be maintained in the flood surcharge zone for any considerable period after the beginning of the spring growth period, which is usually around April 1 in the lower Valley. The prolonged storage of water in the flood surcharge zone during the growing season results in the killing of timber in this uncleared zone, and this dead timber creates a continuing problem by adding to the accumulation of drift and flotage and through the falling of dead trees into the water. Also, after the beginning of the mosquito breeding season, which is usually between the 1st and 15th of May in the lower Valley, use of the uncleared flood surcharge zone for periods exceeding a week may result in uncontrolable production of *Anopheles quadrimaculatus*. Such objectionable conditions do not result from the use of the flood surcharge zone during the winter season since there is no production of mosquitoes during this period and the dormant trees are not killed by partial inundation.

2. *Maintenance of a Relatively Constant Pool Level During Spring Growth Period*

The second phase of the main river schedule is the maintaining of a relatively constant pool level at the normal maximum summertime elevation from the beginning of the early spring growth period until the beginning of moderate production of *Anopheles quadrimaculatus* (See Fig. 1). The importance of this measure has recently been more fully realized, and its incorporation into the water level management program marks a significant advancement. The function of this constant pool level phase is to prevent the invasion of marginal vegetation into the zone of fluctuation, thus providing for a clean shoreline when the water level is drawn down into the zone by cyclical fluctuation and seasonal recession later in the season; it also decreases the cost of the annual shoreline conditioning program.

by narrowing the band of marginal growth and limiting the production of coppice. In the Wilson Dam region, this phase usually begins about April 1 and ends between the middle of May and the first of June. In the upstream reservoirs, the beginning may be delayed a week or two, and the end may be delayed two to four weeks, moderate production of *Anopheles quadrimaculatus* frequently not beginning on these reservoirs until the first part of July. In order to extend this phase and thus realize the maximum growth retarding benefits, light production of *Anopheles quadrimaculatus* during the latter part of the period is controlled through the use of larvicides. At this time of season, vegetation covers are light and larvicides may be applied with a maximum of effectiveness.

A fuller realization of the potentialities of the constant pool phase of water level management has resulted from detailed studies of the water level relationships of the littoral plants normally associated with anopheline production. Observations indicate that seeds of the annual plants which grow in the zone of fluctuation will not germinate until they are dewatered and that woody species, such as willow and buttonball, will remain dormant until the buds are exposed to the air. The maintenance of a relatively constant pool level during the spring growth period therefore retards the normal phenology of the species in the same manner as a late season would, and species which normally initiate growth about April 1 may be held dormant until June 1 or later, depending upon the date when they are first dewatered. The delayed germination of these species results in greatly decreasing their total seasonal growth. For example, willow stumps which initiate growth the first part of April may produce coppice over nine feet tall by the end of the growing season, while those held dormant until July 1 will be less than half this tall. Similar contrasts hold for herbaceous species.

This information on the relation of seasonal water levels to the phenology of littoral plants indicates the importance of holding the water level at or above normal pool during the entire spring growth period. It now appears that even short periods of drawdown may result in the germination of seeds of herbaceous species and the sprouting of coppice which would otherwise be held dormant until later in the season: furthermore, once these plants lose their dormancy, they will continue to grow even though the water is brought back on them, particularly when portions of the stems and leaves project above the water surface. Thus, every effort should be made to hold the water as nearly as possible at a constant pool level, and, when variations are necessary, they should preferably be above normal maximum pool rather than below.

In addition to its function in the malaria control program, the

constant pool phase of water level management affords a real mutual interest with wildlife conservation. Maintaining the normal maximum pool level during the spring growth period provides ideal spawning grounds for bass and other centrarchid fishes and insures that the eggs will hatch and the young fish get well started before the water is drawn down. Thus, a malaria control measure is proving highly beneficial to the fisheries management program of the Authority.

3. *Cyclical Fluctuation*

The third phase of the main river schedule is the use of weekly cyclical fluctuations without seasonal recession (See Fig. 1). Some cyclical fluctuation occurs in the normal operation of the projects. For effective mosquito control, however, it is frequently necessary to augment the scope and regulate the frequency of the normal fluctuations. This is done by varying the loads or discharges at the dams at regular intervals. Such regulation appears to be more feasible on a chain of interconnected plants than would be the case where only one or two projects were involved. Cyclical fluctuation is initiated at the beginning of moderate production of *Anopheles quadrimaculatus* which, in the Wilson Dam region, is usually between the middle of May and the first of June; it ends with the beginning of heavy production of *Anopheles quadrimaculatus* which is usually around July 1. The function of this phase is to bring the water out of the marginal band of vegetation once a week, thus eliminating the intersection line and providing a clean shoreline which is nonproductive of *Anopheles quadrimaculatus*. Recent studies by Rozeboom and Hess (1943) indicate that it is through the creation of intersection line that plants provide adult *Anopheles quadrimaculatus* with optimum conditions for oviposition and supply the larvae with food and protection from natural enemies such as *Gambusia*; thus, the elimination of intersection line by means of the drawdown in cyclical fluctuation would appear to have a threefold effect against *Anopheles quadrimaculatus* and its microhabitat by creating unfavorable conditions for oviposition, interrupting the production of food organisms for the larvae, and exposing the larvae to the predation of their natural enemies. Furthermore, some larvae and eggs are undoubtedly stranded on shore and die from desiccation before the water returns. The return of the water to normal pool level following the drawdown completes the cycle of fluctuation, and this reflooding serves to delay the invasion of marginal vegetation. Thus, while the first two phases of the schedule of water level management on main river reservoirs are directed entirely at the mosquito habitat,

the third phase, cyclical fluctuation, is directed against both the habitat and the mosquitoes.

The moderate production of *Anopheles quadrimaculatus* which marks the beginning of the use of cyclical fluctuation is the result of the invasion of a narrow band of vegetation into the upper limit of the zone of fluctuation. If fairly constant pool levels have been maintained during the spring growth period, this band of vegetation will extend only into the water a few inches at the time cyclical fluctuation is initiated. Therefore, although it is generally agreed that the fluctuation cycle should have an amplitude of about one foot, cycles of considerably less extent may be effective early in the season. For this reason, it is the policy not to make a special request for a full foot cycle of fluctuation until field observations indicate that marginal vegetation has invaded far enough to make it advisable to use this full amount in order to insure adequate control of *Anopheles quadrimaculatus* production. In the use of cyclical fluctuation, it may not be necessary to completely withdraw the water from all marginal plants in order to obtain effective results. Most of the leafy emergent species produce leaves only at or above the water surface, and, if the water level drops down around the bare stems, most of the intersection line is eliminated. Obviously, cyclical fluctuation is relatively ineffective in floating-mat or floating-leaved types of vegetation since these types rise and fall with changing water levels and thus maintain their intersection values. Some plants of these types have appeared in TVA reservoirs and have necessitated the application of special control measures. Attempts to eradicate these species have, in general, proven impracticable; however, measures intended merely to restrict the species within manageable limits have prevented their spreading sufficiently to offer a serious problem.

4. *Combined Seasonal Recession and Cyclical Fluctuation*

The fourth phase of water level management for main river reservoirs is the combining of a seasonal recession of approximately one-tenth foot per week with the regular weekly cycle of fluctuation (See Fig. 1); thus, each week the water level is dropped one foot, but is brought back only nine-tenths of a foot. In the lower Valley, the need for initiating seasonal recession usually begins about July 1, when normal seasonal increases in the mosquito population and the continued invasion of marginal plants result in the beginning of heavy production of *Anopheles quadrimaculatus*. It is significant that this date coincides with the approximate normal beginning of drawing on the main river reservoirs for the development of power and to provide a water supply for navigation. This seasonal recession serves

to insure that at the low level of the weekly fluctuation cycle the water will be drawn far enough out of the advancing band of marginal vegetation to insure adequate control of *Anopheles quadrimaculatus* production. Although the rate of plant invasion obviously varies with a number of factors, a tenth of a foot recession per week is generally sufficient to keep well ahead of it. It is important that seasonal recession should not be in excess of the amount needed to insure adequate mosquito control since such excessive recession results in increased plant invasion, thus broadening the zone from which growth will have to be removed in the fall shoreline conditioning operations. The total amount of seasonal recession is, of course, greatly decreased by the incorporation of the constant pool level phase in the water level management schedule. In general, it appears that two feet of seasonal recession is ample which, combined with one foot available for cyclical fluctuation, would give an overall recession of three feet below the normal pool level. The importance of minimized recession is greatest during the first part of the recession period; sharper recession after about the first part of September is not seriously objectionable since most marginal plants put on very little growth after this date.

The need for specially controlled water levels for malaria control on the main river reservoirs ends about October 1, at which time the breeding of *Anopheles quadrimaculatus* is practically terminated. The use of stored water for the development of power may continue on through the normally dry fall period. Advantage is taken of this normally low water period to complete the marginal growth removal operations of the annual shoreline conditioning program. No special requests are made for controlled water elevations during the winter.

General Comments on the Main River Schedule

From the preceding discussion, it will be observed that the major recent improvements in the schedule of water level management for malaria control on main river reservoirs are the increased emphasis on the use and extended duration of a relatively constant pool level phase and the minimizing of seasonal recession. These phases of the schedule emphasize the importance of water level management in controlling the mosquito habitat in addition to its direct action against the mosquitoes. The effects of water level management tend to be cumulative; a proper seasonal schedule will kill many semi-aquatics and stumps and stubble of woody species and will thus result in a much improved shoreline condition for the following year; conversely, an improper schedule will result in an extension of these species and an increased problem during the following year.

An example of the benefits of the above type schedule is given in the data presented in Figure 2 for Guntersville and Wheeler Reservoirs. During the 1942 season, both of these reservoirs experienced drawdowns during the spring growth period, while in 1943 each had

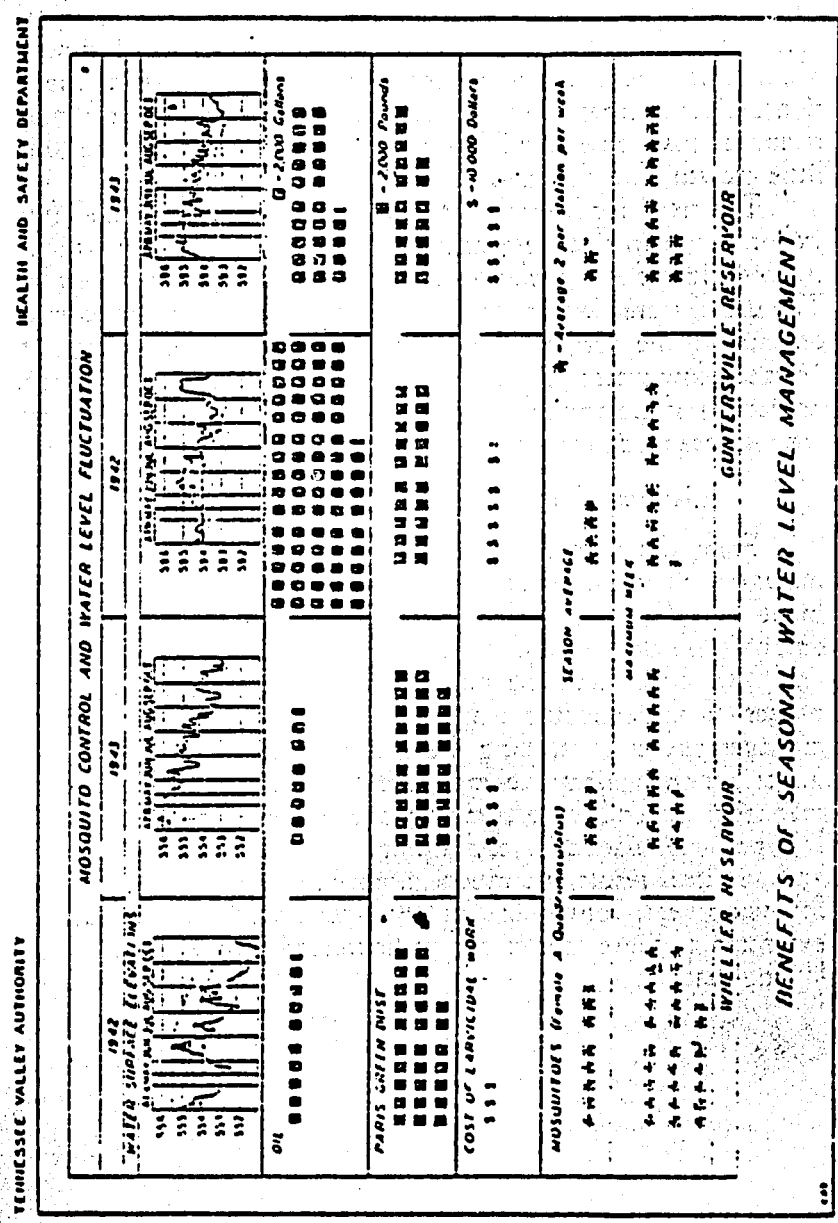

Fig. 11—*Benefits of Seasonal Water Level Management for Malaria Control as Illustrated by Wheeler and Guntersville Reservoirs During 1942 and 1943.*

a water level schedule which was a reasonable approximation of the desired schedule outlined above. The resulting increase in the effectiveness of mosquito control and decreased cost of larvicidal treatment and shoreline conditioning is evident from the data presented. The unfavorable water levels on the Guntersville and Wheeler Reservoirs during the 1942 season are typical of the emergencies which develop as a result of the extremes of river flow. Such emergencies are met in the most practicable manner to minimize the degree and length of *Anopheles quadrimaculatus* production. Larvicidal application is intensified and an effort is made to obtain the most favorable compromise on water level management: this might be increased scope of periodic fluctuation or a more rapid recession, depending upon the particular problems presented in the individual reservoir.

Seasonal Recession on Storage Reservoirs

Seasonal recession without cyclical fluctuation is used principally on the Authority's storage reservoirs. In addition to the nine main river reservoirs, the Authority has twelve completed storage reservoirs on the tributary streams and four others are to be completed at a future date. Although power generation is usually incorporated into these projects, their principal function is to store water during the spring rainy periods which is used to maintain stream flows to downstream reservoirs during the dry weather period which usually prevails during the summer and fall. The resulting drawdown during the summer and fall usually exceeds 20 feet and is frequently as much as 40 or 50 feet or more. Thus, the normal operation of the storage reservoirs usually provides satisfactory mosquito control because the summer drawdown keeps the shoreline well ahead of the encroaching band of marginal vegetation, resulting in a clean water surface relatively free from anopheline production. The generally steep shorelines and later season on the storage reservoirs also contribute to the ease of providing adequate mosquito control. As on the main river reservoirs, holding the water levels relatively constant during the spring growth period and delaying the beginning of seasonal recession increases the effectiveness of the water level recession in controlling *Anopheles quadrimaculatus* production: however, these measures are not as important as they are on the main river reservoirs because of the greatly increased range of seasonal recession.

The storage reservoirs serve a most useful purpose in the water level management program for malaria control in that they permit better regulation of downstream reservoirs where the most serious malaria problems exist. Earlier, some of the most serious, temporary situations developed as a result of either extreme high or low river flows, necessitating departure from recommended water level sched-

ules. The increase in the number of storage reservoirs has decreased the number and intensity of such unfavorable situations.

The chief difficulties experienced in utilizing seasonal recession for malaria control on storage reservoirs are caused by unseasonal rains which may necessitate bringing the water levels back up into the marginal vegetation during the mosquito breeding season. Such a situation is made even worse if a spring drought has made it impossible to fill the reservoir during the spring growth period, resulting in a heavy invasion of marginal vegetation which creates ideal breeding conditions for *Anopheles quadrimaculatus* when the water is brought into it later in the season. If the summer rise of water into the marginal vegetation is rapid and it is followed within a week or ten days by a rapid drawdown into a clean shoreline again, no serious production of *Anopheles quadrimaculatus* will result: for example, Hiwassee Reservoir was brought to the maximum elevation during the peak of the mosquito breeding season in July 1943, and rapidly lowered again without any discernible increase in anopheline production. However, if the water remains at a constant or gradually rising level in the marginal vegetation for an extended period, heavy production of *Anopheles quadrimaculatus* may result, calling for intensive application of larvicidal measures. The contrast between the effects of seasonal recession and seasonal progression of water levels on storage reservoirs is well illustrated in Figure 3.

Although seasonal recession has in the past been used mainly on the Authority's storage reservoirs, it appears that it will be necessary to use this type of water level management on the new Kentucky Reservoir, the last and largest of the Authority's main river reservoirs which is scheduled for impoundment during 1944. The immense size of this reservoir (185 miles long with a useful storage of over four and a half million acre feet and a normal surface area of 158,000 acres) will make it impossible to apply any regular schedule of cyclical fluctuation, and chief reliance will therefore have to be placed on the use of a seasonal recession of some five feet. Although this amount of recession is much less than that commonly employed on storage reservoirs, recent information indicates that it may be adequate, particularly if it follows a spring flood surcharge and a period of relatively constant normal maximum pool elevation during the spring growth period.

Cyclical Fluctuation Alone

As has previously been pointed out, the requirements of power and navigation generally limit the amplitude of cyclical fluctuation on the Authority's main river reservoirs and thus preclude the use of this measure alone in a water level management program. How-

ever, on Wilson Reservoir, which has the highest generating capacity and lowest water storage capacity of all the main river reservoirs, it has been found possible to obtain cyclical fluctuation of one and one-half to two feet in amplitude in connection with the normal

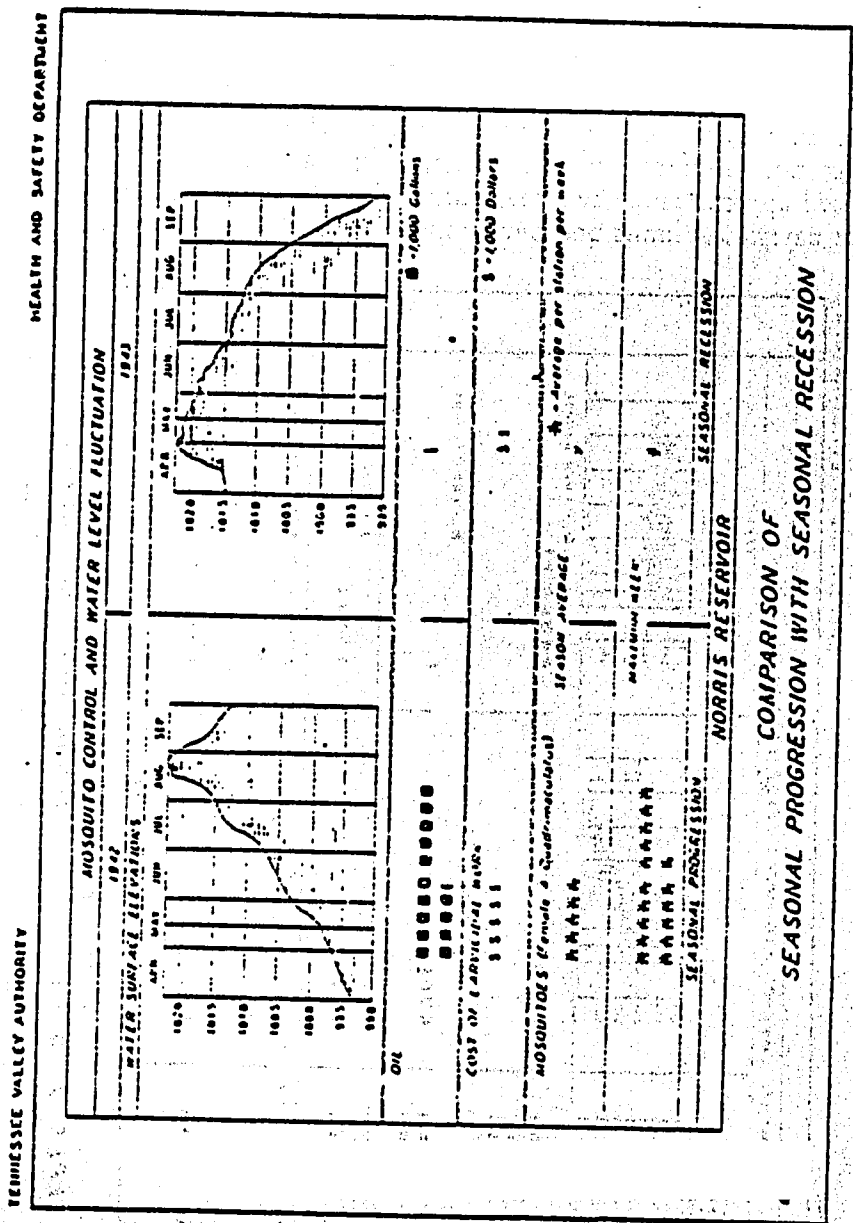

Fig. III—Comparison of Seasonal Progression with Seasonal Recession as Illustrated by Norris Reservoir During 1942 and 1943.

operation of the reservoir without spilling water. Such an operation does not interfere with navigation. The lake elevation has been raised approximately two feet during the past year, thus increasing the navigation limits and permitting more flexibility for water level

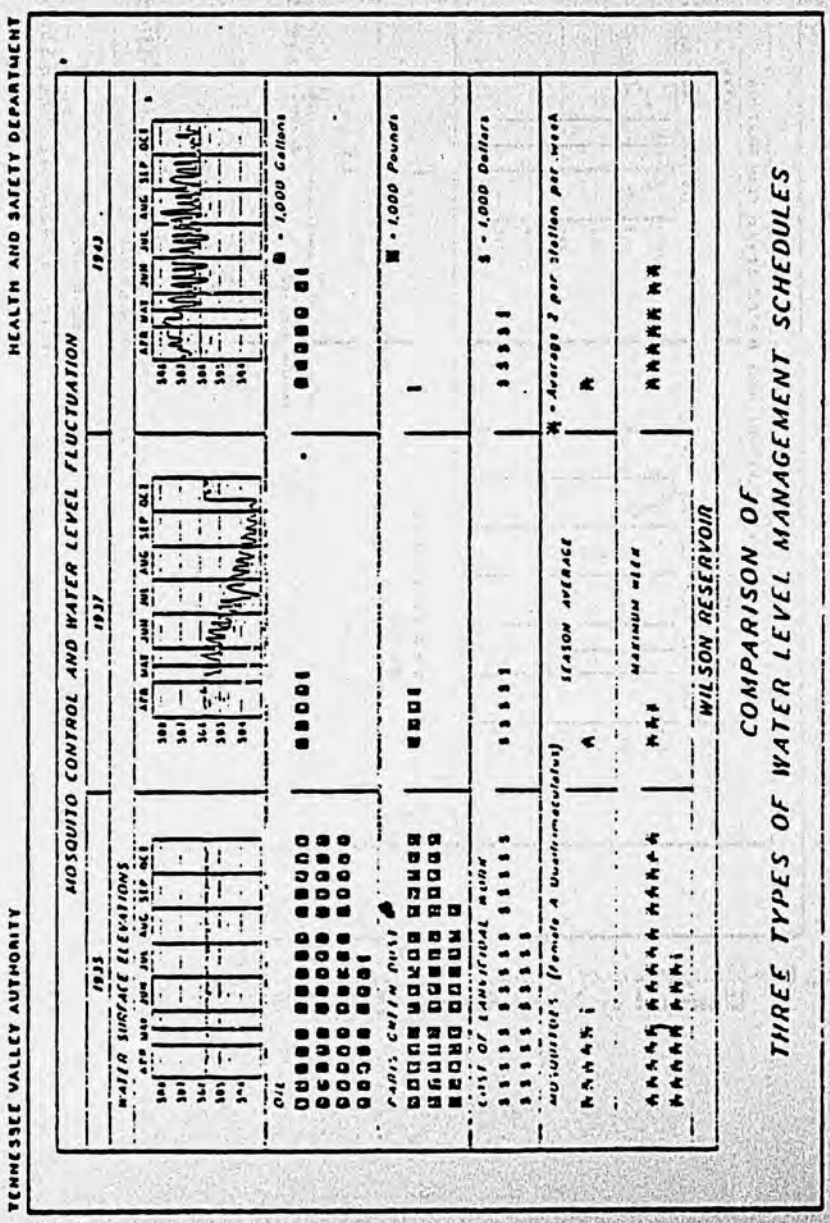

Fig. IV—*Comparison of Three Types of Water Level Management Schedules as Illustrated by Wilson Reservoir in 1935, 1937, and 1943.*

management. During the 1943 season this reservoir was operated on a schedule of cyclical fluctuation of one and one-half to two feet amplitude with satisfactory results, as illustrated in Figure 4. By way of comparison, this graph shows also the results of a schedule combining seasonal recession with periodic fluctuation during the season of 1937 and the very difficult, expensive, and ineffective control encountered during 1935 when it was necessary to maintain the lake at constant level to facilitate construction at Wheeler Dam.

Opportunity is taken here to express appreciation for the cooperation which has been exhibited by various departments in the Authority in executing a successful program of water level management for malaria control. Special appreciation is due those of the Water Control Planning Department who are in immediate charge of planning the regulation of the river system for the overall best interest of these multipurpose projects. Theirs is a Herculean task. Suffice it to say that during the past season of 1943 in the face of wartime power demands, accelerated construction activities, and a multitude of other lesser demands, there was provided the most successful overall program of water level management for malaria control which has yet been experienced on the reservoirs of the Tennessee Valley Authority.

SUMMARY

A review is given of the use of water level management for malaria control on the reservoirs of the Tennessee Valley Authority, particularly with reference to new developments which have taken place since 1940. Accessory measures for water level management are briefly outlined, including reservoir preparation, wintertime impoundage, shoreline maintenance, and permanent shoreline improvement; however, the main portion of the paper is devoted to a detailed discussion of actual water level management schedules.

It is pointed out that three types of water level management are in use on the Authority's reservoirs, namely, (1) Seasonal recession alone, (2) Cyclical fluctuation alone, and (3) A combined schedule for use on the main river reservoirs. Chief attention is given to this latter type since it is the one used on the reservoirs which offer the greatest problems in malaria control. It is stated that the most important new developments in water level management for malaria control on main river reservoirs are the incorporation of a relatively constant pool level phase and the limiting of seasonal recession to the amount necessary for current mosquito control. These new developments have resulted mainly from observations and investigations on the relation of water level management to the reservoirs consists of four phases, each with its own particular func-

tion, as follows: (1) spring flood surcharge, (2) maintenance of constant pool level during the spring growth period, (3) cyclical fluctuation, and (4) seasonal recession combined with cyclical fluctuation. The first two phases are purely prophylactic in that they limit or prevent the development of suitable breeding conditions for *Anopheles quadrimaculatus* later in the season by stranding flotage and limiting the invasion of marginal plants. The third and fourth phases are both prophylactic and curative in that they limit the invasion of marginal plants and also act directly against the mosquitoes by altering their microhabitat, exposing the larvae to natural enemies, and stranding eggs and larvae. The importance of the intersection line evaluation of the anopheline production potentials of marginal plants and flotage in relation to water level management for malaria control is pointed out.

A general discussion is given of the use of seasonal recession without cyclical fluctuation on the storage reservoirs. It is stated that present plans also call for the utilization of this type of a schedule on the new Kentucky Reservoir. The use of cyclical fluctuations of 1½ to 2 ft. in amplitude, without seasonal recession, has given satisfactory control on Wilson Reservoir during the 1943 season.

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COOPERATIVE EXTENSION

MOSQUITO RESEARCH HIGHLIGHTS

11.647

UNIVERSITY of CALIFORNIA - COOPERATIVE EXTENSION - AGRICULTURAL SCIENCES - PUBLIC HEALTH

October 1979

Preface

The editor of the UC Mosquito Research Highlights is privileged to present an interesting and informative article by Edward G. Platzer on the mermithid nematode, *Romanomermis culicivorax*, for mosquito control.

Dr. Platzer is Associate Professor of Nematology and Biology at UC Riverside and has held an appointment as Associate Nematologist in the University Experiment Station for the past eight years.

Although *R. culicivorax* and other species of nematodes appear to have potential for mosquito control when used in conjunction with other methods, yet, as Dr. Platzer is frank to say, there are still important gaps in the knowledge which remain to be investigated before one can know whether nematodes can be beneficially employed in mosquito control operations. Dr. Platzer's laboratory and field studies indicate that the nematode is amenable to mass production by individual local mosquito control agencies, using indigenous mosquito species as host. With appropriate training and minimal outlay in equipment, a mosquito control district could conceivably rear sufficient nematodes to supplement other control measures in an integrated mosquito management programs.

Dr. Platzer would welcome comments and questions on his article and is prepared to train mosquito control agency personnel in methods of mass production on request. His address is:

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Additional copies of this article are available on request.

Biological Control of Mosquitoes with a Mermithid Nematode

by

Edward G. Platzer

INTRODUCTION

In general, parasitic neamtodes are regarded as pestiferous organisms wreaking havoc with man and his domesticated plant and animal species. However, some members of this parasitic assemblage of nematodes are considered to be beneficial and highly desirable members of the environment. These are the entomophilic nematodes or parasitic nematodes which attack insects. Several taxonomically diverse groups of nematodes have insect

[illegible]

parasitic members. One such group of great importance is the superfamily Mermithoidea whose members are obligatory parasites of invertebrates (mainly insects). Within this nematode group, a substantial number of mermithid species have been discovered as parasites of mosquitoes. Dr. J. J. Petersen, a USDA scientist at Lake Charles, Louisiana, has discovered several of these species, developed mass rearing techniques and field testing procedures for mosquito parasitic mermithids. One mermithid found parasitizing Louisiana mosquitoes, *Romanomermis culicivorax*, has received the most attention as a potential biological control agent of larval mosquitoes because of the ease of mass-rearing, ready adaptability to current mosquito control technology, and a wide host range.

We initiated further studies on the feasibility of using *R. culicivorax* as a biocontrol agent in California for larval mosquitoes in 1973. Approximately one half of our research support was provided during the past six years by the Special State Funds for Mosquito Research. During this time, two doctoral dissertations (Drs. Becky Jo Brown, 1978, and Dr. Steven P. Schmidt, 1979) and two major undergraduate research projects (David S. Hughes, 1976, and Leslie L. MacKenzie-Graham, 1978) were completed in addition to other professional and technical reports.

RESEARCH OBJECTIVES

R. culicivorax has great potential for natural regulation of mosquito populations, and there is an urgent need for information on the ecology of this mermithid so it as well as other mermithids can be exploited for biological control with maximum efficiency. To attain this goal, we have studied the effects of physical and biological environmental factors on *R. culicivorax* during its life cycle. In addition, the mass-rearing procedures have received intensive study so optimal yields of highly active parasites will be available for field application.

THE PARASITE AND ITS LIFE HISTORY

The life cycle of *R. culicivorax* involves only one host, a larval mosquito. The preparasitic (infective) larvae attack the mosquito larvae and penetrate into the body cavity via the cuticle (Fig. 1). The parasitic stages are found in the head, thorax, and abdomen of the larval mosquito during the early stages of the infection and later move into the thorax of the host. Heavy infections of 25 or more larvae overwhelm the mosquito larva within two days. In light infections, the mosquito larvae die 6 to 8 days postinfection at 27 C when the parasitic larvae emerge from the host to complete their developmental cycle in the bottom sediment of the mosquito pool. Here the mermithids mate and the females produce eggs which are deposited in the bottom sediments. When maintained at 27 C, preparasitic larvae hatch from the eggs in approximately three weeks and swim actively in search of the hosts.

SMALL SCALE MASS-REARING

R. culicivorax is maintained in *Culex pipiens*. Our facility produces 50,000 mosquitoes per week for experimental use and, currently, we are infecting 43,000 mosquitoes per week. The mosquitoes are fed finely ground rabbit food and brewer's yeast. After emergence from the mosquito, postparasitic nematodes are allowed to mature in aluminum pans which contain clean, sterile sand covered to a depth of 1 to 2 cm with water. The cultures are stored at room temperature for approximately 3 weeks. Then the water is carefully decanted and the excess water is removed by blotting with paper towels. The cultures are stored at room temperature for 4 to 48 weeks before use.

Preparasitic larvae are obtained by flooding the cultures with dechlorinated tap water. Maximum hatch usually occurs within 12 hours and each culture yields approximately 2 million larvae. In general, we have found that an infection ratio of 2.2:1 (*R. culicivorax*:*C. pipiens*) gives the best yield of nematodes for maintenance of cultures (Table 1).

TABLE 1. Modified procedures for rearing *Romanomermis culicivorax* in *Culex pipiens* at 27 C.

Time (days)	Procedure
0 (Friday)	Dechlorinate tapwater in each rearing pan. Add 20 egg rafts (1,300 *Cx. pipiens* larvae) to each pan. Add 80 mg mosquito food to each pan. Add 2,800 preparasites (hatched within 8 hours) to each pan.
1 (Saturday)	Add 80 mg mosquito food to each pan.
2 (Sunday)	Ditto
3 (Monday)	Ditto Adjust pH of water in each pan to 4.0 to 4.5 with 40% acetic acid.
4 (Tuesday)	Add 80 mg mosquito food to each pan.
5 (Wednesday)	Ditto Adjust pH of water in each pan to 4.0 to 4.5 with 40% acetic acid.
6 (Thursday)	Add 80 mg mosquito food to each pan.
7 (Friday)	Collect infected mosquito larvae on 20 mesh sieve, wash off excess food, and place mosquito larvae in emergence containers. Initiate new rearing cycle (see Day 0).

MOSQUITO INFECTIVE STAGE

Most of our early studies were concentrated on the biology of the infective or preparasitic stage of *R. culicivorax* since this was the stage most readily dispersed under field conditions. The primary goals were to develop a thorough knowledge of the optimal and limiting temperature, water salinity and dissolved oxygen for this stage of the parasite.

Preparasites were found to infect *C. pipiens* between 12 and 33 C with the optimum range for infectivity between 21 and 33 C. Lower temperatures decreased the percent infective but increased the time thenematodes remained infective. The time required for host infection to occur increased as the preparasites aged at 15, 21, and 27 C.

An extensive study was undertaken on the effects of salts commonly found in fresh water on the infectivity of *R. culicivorax*. In general, at least 50 percent of the preparasites were able to infect mosquitoes at the median concentrations of these salts commonly found in fresh waters. However, less than half were infective at the maximum concentrations at which these salts were found in freshwater. Calcium, nitrates, nitrites, and phosphate were particularly toxic for the preparasites. However, it appeared the use of

R. culicivorax in most freshwater environments should be feasible unless the salt content is elevated.

The usual levels of oxygen in the aquatic environment should not hinder the use of this nematode. However, infectivity of preparasites is dependent on adequate oxygen levels and the low oxygen concentration in polluted waters (e.g. cemetery vases and sewage discharges) reduces the infectivity drastically.

PARASITIC STAGE

The parasitic stage is critical in the life cycle. During this phase, the parasite acquires all nutrients required for the developmental and reproductive activities of all other stages. The effect of a physical variable, temperature, on the development of the parasitic stage was investigated as well as the physiological and biochemical effects of parasitism on the host environment.

The median developmental time of the parasitic stage ranged from 28 to 6 days over a temperature range of 15 to 32 C. Optimal development occurred at 20 to 32 C, a range that corresponds closely to the optimum for the activity of the preparasites. Measurement of temperature effects allows prediction of the time for the complete life cycle under natural conditions through the use of heat unit calculations.

Major changes in the composition of the hemolymph in mosquito larvae occurred when infected with *R. culicivorax*. Protein content declined precipitously and the carbohydrates were significantly reduced. No change in free amino acids was detected. Parasitized larvae were unable to produce imaginal discs and thus could not pupate. This phenomenon appeared to be related to the nutritional stress placed upon the host by the nutritional and physiological demands of the parasitic stage in the hemocoel.

POST PARASITIC STAGE

During the postparasitic phase, the mermithid develops to the adult stage and produces the eggs for the next cycle. Our investigations have been directed primarily at the effects of temperature on developmental time and the physiology of the postparasites.

The temperature studies were performed in small outdoor ponds. When ambient temperatures averaged 27 C, the preparasitic nematodes emerged and infected sentinel mosquitoes 3 weeks after postparasites emerged from the mosquito host. Preparasites are not found in inoculated ponds when the average ambient temperature falls to 16 to 18 C. Preparasites resume activity when the average ambient temperature is equal to or greater than 20 C.

Physiological studies on the postparasites have demonstrated that this stage is either microaerobic or a facultative anaerobe. This observation is based on oxygen uptake, developmental and enzymological studies.

IMPROVED MASS REARING

Earlier attempts at improving the mass rearing and investigating the ecological physiology of the postparasitic stages of *R. culicivorax* failed because of a fungal hyperparasite, *Entomophthora muscae*, which invaded and killed the

postparasites. Techniques used for mass-rearing the nematode provided ideal conditions for dispersal and multiplication of the fungus; up to 90% of the postparasites of *R. culicivorax* were parasitized. The fungus was isolated as a pure culture and the effects of different temperature and pH regimes on the fungus were investigated as possible methods for prevention or control. A temperature suitable for mass rearing the nematodes but unsuitable for the fungus could not be found, as *C. anguillulae* had a broad temperature range. Adjustment of pH or a short period of chilling (0 C) were more promising control measures. Under acidic conditions (pH), motility and germination of the fungal infective stages and infection of postparasites were suppressed. The disease was controlled by rearing nematodes in water adjusted to a pH of 4.5 (Table 1).

After the development of this acidification procedure (Table 1), fungal infections decreased precipitously and investigations of mass rearing improvement and post-parasite physiology proceeded. Indeed, this procedure for fungal prophylaxis will enable other laboratories and commercial facilities to propagate *R. culicivorax* rapidly.

Five commercial substrates for sand provided by Nutrilite, vermiculite, and cellulose sponges were tested as substrates for storing postparasitic nematodes and eggs. Our usual medium-coarse blow sand proved to be superior. Further tests with a graded series of silica sand showed that medium-fine sand at a moisture content of 13.5% gave the best yield of preparasitic nematodes.

FIELD STUDIES

The feasibility of using *R. culicivorax* as a control agent in mosquito producing habitats in California was tested. Field tests were conducted in artificial and natural sites against four species of mosquitoes. Infective nematodes were disseminated at approximately 1000 per m^2 surface area. All species of mosquito larvae were infected and the percentage infection was dependent on the mosquito subfamily and test site. In mixed mosquito population anophelines were more susceptible to parasitism than culicines. Control of mosquito larvae was reduced in sites with dense vegetation or algal mats.

In 2 studies conducted in 30 m^2 ponds at Oasis, *Culex tarsalis* was found to be less susceptible to infection by *R. culicivorax* than was *Anopheles freeborni*. *Culiseta inornata* was found to be about as susceptible under field conditions as was *Cx. tarsalis*. Since many anopheline species, *Cx. tarsalis* and *Cs. inornata* are all highly susceptible in the laboratory, the reason for this difference in field susceptibility is not known. We suspect that the difference arises through a divergence in larval habits. Anopheline larvae tend to spend more time at the surface than larvae of culicines, therefore, it is reasonable that infection by preparasites is greatly enhanced since the preparasites are located mainly in surface waters.

In 2 rice field trials conducted during this study, *Anopheles freeborni* was highly susceptible to infection by *R. culicivorax*.

In Sutter County, 85% of sentinel anophelines were parasitized. In Colusa County, 43% of the anophelines were infected. The difference was due primarily to the density of the more mature rice in Colusa which prevented optimal distribution of the parasites.

Recent studies by Drs. B. J. Brown and R. Washino have indicated that inoculation of nascent rice fields with postparasites constitute a more effective way of introducing nematodes as biological control agents. In addition, the nematodes can overwinter in rice fields subjected to normal crop practices.

PREDATORS

Observations of high populations of aquatic arthropods in rice fields prompted a study on the potential of various aquatic arthropods as predators on two non-parasitic phases in the life history of the mermithid, the preparasitic or infective and postparasitic stages.

Preparasites were attacked by copepods, cladocerans, young gammarids, and ostracods. Green hydra did not attack the preparasites. Further studies with copepods in one liter of water demonstrated that 20 copepods significantly reduced infections of mosquitoes by the mermithid nematode and 53 copepods reduced the infection level by 50%. These findings indicate that high densities of copepods in mosquito infested waters may interfere with mosquito control by mermithid nematodes. However, it was observed that copepods were effective predators on all instars of mosquito larvae and suggested that these organisms play a substantial role in natural control of mosquitoes.

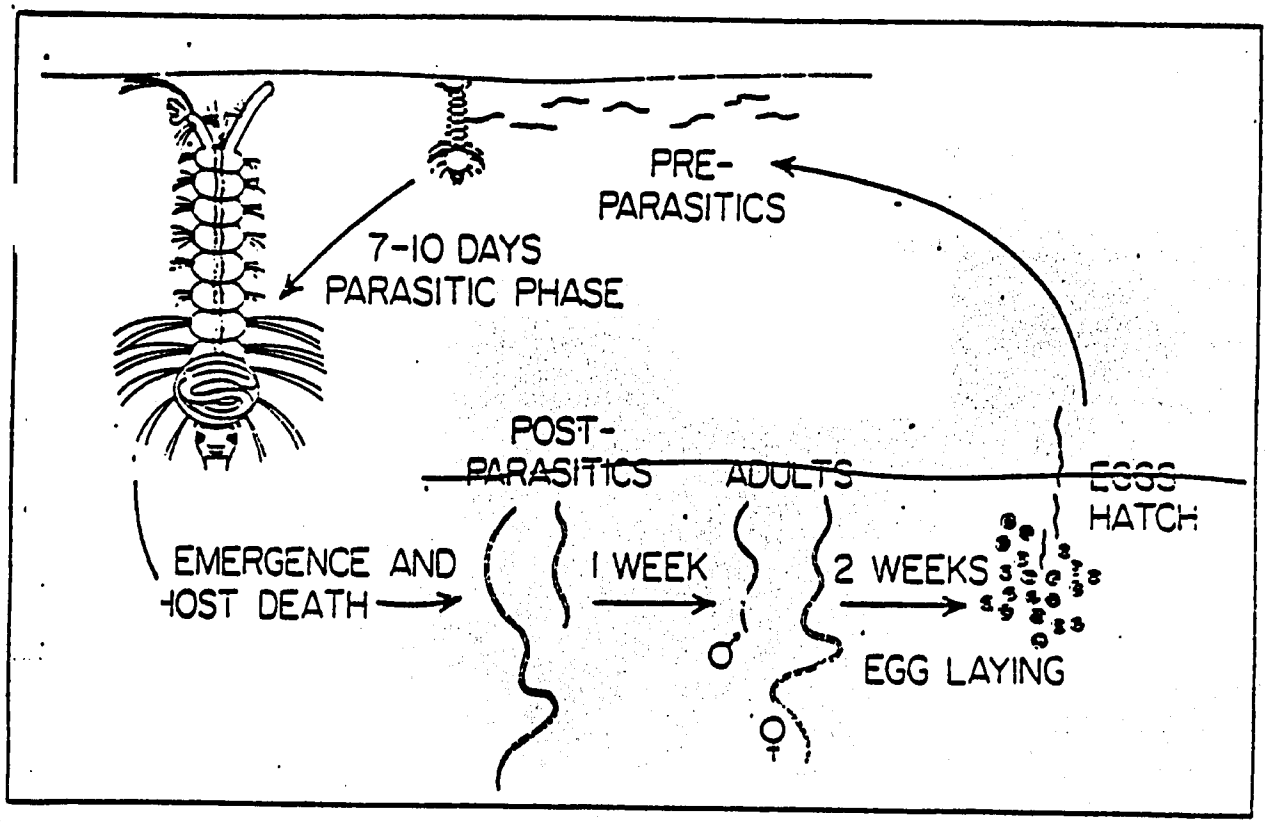
Postparasites of *R. culicivorax* were avidly consumed by diving beetles, gammarids dragonfly and damselfly naiads and small crayfish. Isopods attacked the postparasites only after starvation and planarians did not feed on the mermithid nematodes.

CONCLUSIONS AND RECOMMENDATIONS

R. culicivorax has good potential for anopheline control in California. Current investigations on the inoculation of rice fields with postparasites rather than preparasites has yielded encouraging results. The control of culicine species, on the other hand, does not appear suitable with this mermithid species and investigation of other mermithids for culicine control should be initiated.

The improvements in mass rearing will lead to a more economical production of nematodes for mass releases. Additional studies on the biological requirements of this species and others are mandatory if we are to obtain the potential biocontrol of mosquitoes offered by mermithid nematodes. Although substantial potential has been attributed to the capabilities of mermithids for vector control, much more time and investigation is required to realize this potential. Although there is immense pressure for the immediate control and suppression of vector organisms, it is just not possible to develop biological control organisms quickly without a substantial commitment of scientific man-years and funds. *R. culicivorax* has been under development for 10 years and with the current level of interest and funding it will probably require 10 more

years to obtain effective use of this or alternate organisms in biological control of ector populations. In addition, we need a strong commitment from agencies to train personnel (technicians to higher administrative levels) in the handling and use of biological control organisms.



Diagrammatic life cycle of Romanomermis culicivorax. *The eggs of the mermithid nematodes are found within the substrate of the mosquito pool. Upon hatching, the infective or preparasitic stages swim to the surface of the mosquito pool where early mosquito instars are penetrated. The mermithid nematodes develop rapidly in the body cavity of the mosquito larva and are ready to emerge and kill the mosquito host after 7 to 10 days. After emergence, the mermithids drop to the substrate and molt to the adult stages. Within 3 to 4 weeks, egg laying is initiated, and, after egg development, hordes of preparasitic larvae emerge in search of mosquito hosts.*

COLLABORATORS AND STAFF

The performance of these studies was greatly facilitated by the collaboration of Dr. R. Washino (UCD), Dr. M. S. Mulla (UCR), and Mr. E. E. Kauffman (Sutter-Yuba MAD). Dr. B. J. Brown (UCD), Dr. S. P. Schmidt (Oklahoma SU), Mr. J. Imbriani, Mr. J. Eby, Mr. M. Stirling, Mr. D. S. Hughes, and Ms. L. L. MacKenzie-Graham all made unique contributions to our understanding of the biology of this mermithid nematode.

Sincerely yours,

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