

## V. CURRENT ABATEMENT STRATEGIES AND THEIR IMPACTS

### A. Chemical Control


#### 1. Overview of Chemical Control

##### a. General Toxicity of Pesticides.

Pesticides are placed in one of four categories based on their acute toxicity (Table 13). Category I pesticides are extremely toxic while Category IV materials can range from mildly toxic to non-toxic.

Pesticides are also classified as either “General Use” or “Restricted Use” materials. General Use pesticides are available for use either by the general public or by licensed applicators. Restricted Use materials may only be applied by certified applicators or licensed applicators working directly under the supervision of a certified applicator. While Restricted Use materials are generally more toxic than General Use ones, toxicity is not the only issue. For example, resmethrin-based ULV products (Scourge) have recently been placed on the Restricted-use list, not so much because of toxicity but because the mode of application (ULV area-wide treatments) means that incorrect applications can have wide-ranging effects.

Table 13. Toxicity Category of Pesticides used in Mosquito Control

Category	Signal Word Required on the label	Categories of Acute Toxicity			Probable Oral Lethal Dose for 150 lb. man	Antidote Statement Other Cautions <sup>a</sup>
		LD <sub>50</sub>		LC <sub>50</sub>		
		Oral mg/kg	Dermal	Inhalation mg/l		
I Highly Toxic	DANGER  POISON	0 thru 50	0 thru 200	0 thru 0.2	A few drops to a teaspoon- ful	Skull and Crossbones “Call Physician Imme- diately” Antidote Statement
II Moderately Toxic	WARNING	from 50 thru 500	from 200 thru 2000	from 0.2 thru 2	>1 teaspoonful to one ounce	
III Slightly Toxic	CAUTION	from 500 thru 5000	from 2000 thru 20,000	from 2.0 thru 20	>1 ounce to one pint or one pound	
IV Relatively Non-toxic	CAUTION	>5000	>20,000	>20	Over one pint or one pound	

<sup>a</sup>All pesticide labels are required to include the statement, “Keep out of reach of Children.”

from: pesticide Applicator Trainging Core Manual: Northeastern Regional Pesticide Coordinators and Manual 2, Vectorborne Disease Control Homestudy Counrse 3013-G: Center for Disease Control

Newer pesticides have muddled the pesticide classification system. Bti and *Bacillus sphaericus* are biological organisms yet their mode of action is through the creation of a toxin that is activated in the insect's midgut. For the purposes of pesticide classification and this GEIR, therefore, they are considered pesticides, not biological control agents. Methoprene is also a case of a material (an insect growth regulator) that, while not directly toxic, so alters the life cycle of the insect that death results. Again, methoprene (Altosid) is classified as a pesticide but is far removed from the classic chlorinated hydrocarbon and organophosphate pesticides of the past.

b. Pesticides used for Mosquito Control In Massachusetts

Twenty-six different insecticide formulation distributed among fifteen product lines were used for mosquito control in Massachusetts between 1993 and 1995 (Table 14). Eight of these formulations used Bti as the active ingredient, five were methoprene-based, three were resmethrin-based, two each were pyrethrin-based or malathion-based, and there were one each of temephos, isoctadecanol, and mineral oil. Of these, Acrobe (Bti) and Vectobac AS (replaced by 12AS) are no longer produced. Arosurf-MSF (Isoctadecanol) was removed from the market but has just reappeared under the name Agnique MMF. Abate 4E was not used in either 1994 or 1995 and both the Malathion 10EC and the Resmethrin product (EPA rep # 4-339-53853) were used in small amounts only (Table 4 gives a break-down by Project of chemical use).

Of the insecticides used, all of the larvicides were classed as Category IV materials by EPA. Bonide Mosquito Larvicide, available but not used, is border line between Category III and IV. VectoLex CG, a new *Bacillus sphaericus* product, is Category IV. All adulticides are in Category III.

Many pesticides have dual actions. They are important in controlling injurious pests, but they may also present a hazard to species not considered to be pests in the environment. As a result, the concepts of "target" and "non-target" organisms have arisen. For example, in many freshwater systems, control measures may be taken against undesirable target organisms such as mosquito larvae or unwanted algae. Non-target organisms are those whose destruction is not intended but which may be affected. These non-target organisms may play key roles in aquatic ecosystems.

The distinction between target and non-target species is not absolute, because the same group may be non-target organisms in one area of the country but target organisms, under certain circumstances, in another area. For example, larvae of caddisflies (Trichoptera) and naiads of mayflies (Ephemeroptera) are important food sources for

Table 14. Chemicals used in Massachusetts mosquito control, 1993 through 1995

Trade Name	EPA Registration Number	Active Ingredient(s)	% Active Ingredient	Toxicity Class	Other Warning Statements
<b><u>LARVICIDES</u></b>					
Abate 4E	241-132	Temephos	43	IV	
Acrobe <sup>a</sup>	62637-1-241	Bti <sup>b</sup>		IV	
Arosurf-MSF <sup>c</sup>	42943-8	Isooctadecanol	100	III	
Altosid					
Briquets	2724-375-64833	Methoprene	7.9	IV	
XR Briquets	2724-421-64833	Methoprene	1.8	IV	
Pellets	2724-448-64833	Methoprene	4.0	IV	
Bactimos					
Briquets	43382-3	Bti	10	IV	
Granules	37100-43-2217	Bti	0.2	IV	
Pellets	37100-42-2217	Bti	0.4	IV	
GB-1111	8898-16	Petroleum Hydrocarbons			
GB-1356	8898-16				
Teknar HP-D	2724-365-64833	Bti	0.8	IV	
Vectobac					
AS	275-52	Bti		IV	
12AS	275-66	Bti	1.2	IV	
Granular	275-50	Bti	0.2	IV	
<b><u>ADULTICIDES</u></b>					
Malathion 8EC	34704-119	Malathion	8	III	
Permanone 10EC	4816-688	Permethrin	10	III	
Permanone 31-66	4816-740	Permethrin	31	III	
Resmethrin	4-339-53853	Resmethrin		III	
Scourge 4+12	432-716	Resmethrin	4	III	} { RESTRICTED USE } { CLASSIFICATION }   Due to acute fish toxicity }   Retail sale to and use only by   Certified Applicators or   persons under their direct   supervision and only for those   uses covered by the Certified   Applicators Certificate
		PBO	12		
Scourge 18+54	432-667	Resmethrin	18	III	
		PBO	54		
<b><u>MATERIALS REGISTERED BUT NOT USED - LARVICIDES</u></b>					
Altosid					
Liquid	2724-392-64833	Methoprene	5	IV	
Liquid Con	2724-446-64833	Methoprene	20	IV	
Bonide Mosquito					
Larvicide	4-195	Mineral Oil	98	III-IV	
VectoLex CG	275-77	<i>B. sphaericus</i>	50 <sup>d</sup>	IV	
<b><u>MATERIALS REGISTERED BUT NOT USED - ADULTICIDE</u></b>					
Fyfanon ULV	4787-8	Malathion	95	III	

<sup>a</sup>No longer marketed<sup>b</sup>*Bacillus thuringiensis* var. *israelensis*<sup>c</sup>Now marketed as Agnique MMF

trout and other valuable freshwater fish. In certain areas, these species of insects can occur in such large numbers that they become nuisance pests, and their immature stages are the target of planned control operations with pesticides.

The ideal situation in most control operations is to be able to destroy the undesirable species at pesticide concentrations that will have minimal adverse effects on the rest of the biota. However, some degree of contamination and hazard is assumed with nearly all pesticide use. The hazard to aquatic organisms and other wildlife species depends on the chemical and physical properties of the pesticide, type of formulation, rate and method of application, and characteristics of the receiving ecosystem system (Nimmo 1985).

Looking at the change in pesticide use by MCPs over the past decade (Table 3), all Category II insecticides have been phased out and Methoxychlor, the only organochlorine compound on the list in the early eighties, has likewise been dropped. Using malathion and permethrin for larviciding has also been discontinued and Bti and methoprene have taken over from Mineral Oil (Flit-MLO) as the dominant larvicides. For adulticiding, permethrin and resmethrin have essentially replaced malathion. These changes indicate that MCPs have responded to the desire of the public at large (including the staff of the MCPs incidentally) for materials with the lowest risks. The other conclusion to be drawn is that, until the discovery of new materials, both adulticiding and larviciding are presently be conducted with the materials that have the least overall risk. Advances in reducing the risk of chemical use must therefore come from improved targeting and increased use of water management and/or biological control techniques.

c. General Properties of Registered Mosquito Control Insecticides in Massachusetts, 1996.

Physical Properties. Using both water solubility and KoW, the following insecticides are classified as water insoluble or practically water insoluble; Bti, isooctadecanol, methoprene, petroleum oil/Flit MLO, pyrethrin I, resmethrin and temephos. Malathion is the most water soluble insecticide used (145 ppm). Nevertheless, it still has a relatively high partition coefficient (KoW 779) and because of its rapid environmental and metabolic degradation is not expected to bioaccumulate in any appreciable fashion as discussed below.

Insecticides which have little or no volatility are; Bti, *B. sphaericus*, malathion, methoprene, and temephos. Isooctadecanol and mineral oil are slightly volatile. Although pyrethrin I and resmethrin have appreciable vapor pressures, they are most likely to be bound to suspended organic matter or soil particulate in natural systems. If volatilization occurs, they are rapidly photodegraded.

Four of the insecticides have vapor densities less than 1.0 (relative index to air) and so could concentrate near the surface, resulting in increased exposure via inhalation. However due to the low vapor pressure of isooctadecanol and methoprene, these compounds are not likely to be available for concentration. The pyrethroid insecticides, as discussed above, are rapidly degraded by photolysis if they become available which is not likely from environmental surfaces.

For specifics on a given insecticide, the reader should refer to the sample labels and Material Safety Data Sheet (MSDS) for that material, located in Appendix C.

Analytical Methodology (from: The Pesticide Manual 7th ed. Worthing & Walker, British Crop Protection Council. Lavenham, UK. 1983. For more detailed methods consult The Agrochemical Handbook, 2nd ed. 1983).

1. Bti and *B. sphaericus* are measured in international toxicity units/mg product (i.u.) relative to that of an appropriate standard product against *Trichoplusia ni* or *Aedes aegypti* in standard bioassays. Assays based on the number of spores are not satisfactory.
2. Methoprene is analyzed by GLC or HPLC w/UV detection.
3. Isooctadecanol is analyzed by GLC.
4. Pyrethrin I is analyzed by GLC.
5. Resmethrin is analyzed by GLC.
6. Temephos is analyzed by GLC.
7. Malathion is analyzed by GLC.
8. Petroleum Oil is analyzed by GLC.

Transport, Persistence and Degradation in Soil, Air and Water. None of the insecticides used for mosquito control in Massachusetts are included on the 1987 EPA lists (I + II) of the 51 priority pesticide leachers.

Synergists and Inerts in Pesticide Products.

Synergists are compounds added to a pesticide that increase the efficacy of that pesticide. Relatively few combinations of insecticides and synergists lend themselves to practical use, either because the degree of improved performance is small or because too much of the expensive synergist is required, or both (Casida 1970). By the same token, no example of increased toxicity to man or useful animals under practical conditions has been reported (Hayes 1982).

In mosquito control, the synergistic effect of various compounds on pyrethrum and synthetic pyrethroids is well known. piperonyl butoxide (PBO) is the sole synergist used in Massachusetts. It acts as a substrate for the microsomal enzyme-NADPH<sub>2</sub> system, which also metabolizes many drugs and insecticides. By serving as an alternative substrate for the detoxification enzyme of the insect, PBO prolongs the persistence of the insecticide so that a lower initial dose is effective (Casida et al. 1966, Kamienski & Casida 1970, Casida 1970).

“Inert” materials which are used as formulation aids in the insecticides applied by towns/BOHs and Projects for mosquito control in Massachusetts are categorized as follows: Powders, granular carriers, solvents and special effects materials.

Dustable powders generally contain 1-10% a.i. mixed with powdered minerals as carriers and diluents. For wettable powders, the same diluents and carriers can be used as for dusts. However for dispersible powders, a finer particle-size spectrum is necessary, the proportion above 40  $\mu$ m not exceeding a few percent. No powdered materials are currently in use in mosquito control in Massachusetts.

Granular formulations are made by either impregnation (soaking or coating) of granular carriers or by granulation of powdery mixtures of active ingredient and formulation aids. These two types differ in the active ingredient concentrations attainable. For both types, an active ingredient content of 20% is the maximum technically feasible. Included in the grouping are Bactimos briquets, granules and pellets (Bti), Vectobac granules (Bti) and Altosid briquets, XR briquets, and pellets (methoprene).

Liquid formulations such as emulsifiable concentrates, soluble concentrates and ULV use various organic solvents as diluents. Aliphatic hydrocarbons are poor solvents for most active ingredients and are therefore normally only used for very dilute formulations. Aromatic hydrocarbons are used frequently, the fractions C<sub>6</sub> to C<sub>12</sub> being favored (e.g., from technical xylene mixtures to substituted naphthalenes). Apart from the toxicity of benzene, their high flammability prohibits the use of lower aromatics. On the other hand, phytotoxicity frequently increases with increasing molecular weight. Ketones are excellent solvents for many organic compounds. Most of them are at least partially water soluble, so they are mainly suitable for formulating water-soluble and liquid-active ingredients. When they are used for the formulation of emulsifiable concentrates of solid, water-insoluble active ingredient, the active ingredient often crystallizes out during the preparation of the spray mix. This is caused by the solvent passing into the aqueous phase whereupon the active ingredient is precipitated in the "oil droplets" of the emulsion. Here ketones can normally only be used together with other, water-insoluble solvents as cosolvents.

This is also valid for alcohols and glycols and their ethers and esters as well as for highly polar aprotic solvents such as dimethylsulfoxide and dimethylformamide. Chlorinated solvents are only used to a very slight extent.

Chlorobenzene is used occasionally; dichloromethane is used more frequently as a relatively non-toxic, highly volatile, and nonflammable solvent for hygiene and stored-product agents and for aerosols.

Emulsifiable concentrates used for mosquito control include the larvicide Abate 4E and the adulticides Malathion 10EC, Permanone 10EC, Permanone 31-66, Resmethrin, and the Scourge products.

With increasing public concern of off-site deposition of pesticides (e.g. aerial drift) and the movement of the pesticide industry away from organic compounds to biological insecticides, new formulation technology has emerged. Flowable concentrates not only reduce drift, but provide better coverage and greater adhesion to the substrate. Dried active ingredients are ground to a uniform particle size (usually 10  $\mu\text{m}$ ) as either a dry, wet ( $\text{H}_2\text{O}$ ) or oil suspension (which then is diluted with water for application). Included in this grouping are the Bti products Teknar flowable (Bti) and Vectobac AS and 12AS.

None of the direct nerve toxins (pyrethrin and resmethrin) have inert ingredients which appear on EPA/SAP List 1. Scourge (resmethrin) contains a List 2 compound (0.025% xylene) and 4-12% PBO and 12% Chevron 100 (a soybean oil diluent) as additional active ingredients.

None of the indirect nerve toxins (malathion and temephos) have inert ingredients which appear on the EPA/SAP List 1. Of the products which contain malathion, malathion 57% EC contains a List 2 compound, 31.5% and 36.55% xylene range aromatics, respectively. Temephos (Abate 4E) contains 39% Chevron 100 as an additional active ingredient.

None of the selective insect toxins (Bti and methoprene) contain any List 1 or 2 compounds. No other additional active ingredients are listed.

None of the physical toxins (isocadecanol (Arosurf MSF/Agnique MMF and petroleum oils) contain any of the List 1 or 2 compound. Petroleum hydrocarbons as a group are included on List 2 as potential toxic inert ingredients of pesticide products.

d. Pesticide Handling and Application

Pesticide storage varies considerably from project to project. Cape Cod uses only bagged Bti so no formulation work is necessary nor are there stringent storage requirements. Five projects store and formulate chemicals in an area within the main garage, generally separated by a wire cage (cabinets within a metal shed at Suffolk) from the main garage area. Ventilation fans operate either continuously or whenever the lights in the garage are turned on. Bristol, Central Mass and Essex all have separate buildings for pesticide storage and formulation. Central Mass mentioned that their structure was built in consultation with the local fire department and the State Pesticide Board. East Middlesex constructed its storage area from Department of Defense specifications.

All projects use pickup-truck mounted ULV sprayers for adulticiding. No thermal foggers remain in use. Backpack sprayers are used to apply liquid larvicides and, in some projects, adulticides. Granular materials are applied by hand or with cyclone-type spreaders. Briquet formulations are applied by hand. Additional information on the pesticides may be found on the sample labels and MSDS sheets in Appendix C.

Applicator certification is done by the Pesticide Bureau of the Department of Food and Agriculture. Mosquito control applicators are certified under the “Mosquitoes and Biting Flies” subcategory of “Public Health and Nuisance Control”. Aerial Applicators are licensed separately. Ongoing training is required and many mosquito-control personnel attend annual meetings of the Northeastern Mosquito Control Association for such training. Specifics for certification and other applicator issues are covered in CMR 333: Pesticide Board.

Most of the pesticides used are extremely safe, so handling instructions are minimal. For Bti, mineral oil or Golden Bear oil, and methoprene products, work areas should be ventilated and eye protection and impervious gloves worn. With these materials applicators should also wash thoroughly after handling or applying them. With malathion, permethrin and resmethrin products safety goggles, chemical resistant gloves and a respirator should be worn when formulating spray material. Eyes should be flushed immediately and skin washed with soap as quickly as possible if accidental contact occurs. Vomiting should not be induced for resmethrin and permethrin products but should be induced for malathion. In all cases where resmethrin, permethrin or malathion is ingested, a physician should be contacted immediately.



## 2. Larvicides

### a. Biologicals: Bti and *B. sphaericus*

#### i. *Bacillus thuringiensis* var. *israelensis*

**Mode of Action.** *Bacillus thuringiensis* (Bt) is a naturally occurring, gram positive, rod-shaped, spore-forming bacterium, which is pathogenic to the larvae of a number of insects species, especially Lepidoptera, when ingested by the larvae. Bti is also a pathogen to the larvae of some insects in the order Diptera (e.g., mosquitoes and midges). *Bacillus thuringiensis* var. *israelensis* Serotype H14 (Bti) is a biological insecticide produced during sporulation of this bacterium. The protein product of the H14 serotype (e.g., Bactimos, Vectobac and Teknar) is used to selectively control the larvae of mosquito and blackflies. It is a stomach poison which alters gut permeability to salts under alkaline conditions. This decreases feeding and development and eventually causes death by starvation (Hartley & Kidd 1983).

**Fate in the Environment.** The residual period for Bti has been estimated at 48 hr in water, as it gradually settles out or adheres to suspended organic matter (SCAMP 1987). As a natural part of the ecosystem, Bti degrades to complex but non-toxic organic compounds which are ultimately mineralized (Hartley & Kidd 1983).

**Effects on Non-target organisms.** There is no evidence of acute or chronic toxicity of the spore-crystal complex to amphibians (tree frog tadpoles, toad tadpoles, California newt), fish (mosquito fish, rainwater kill fish, two-spine stickleback) or birds. It is non-toxic to bees. Groups of organisms that have been reduced by Bti applications are from the suborder Nematocera of the order Diptera which includes species of the families Dixidae, Chironomidae, and Ceratopogonidae (Fisher & Rosner 1959, Garcia et al. 1980, Hartley & Kidd 1983, Worthing & Walker 1983).

A recently completed study in Minnesota found Bti reduced chironomids, tipulids, ceratopogonids and stratiomyids. There is reason to believe this may have negative impacts on nesting ducks and their ducklings, for which chironomids make up a significant part of their diet (SPRP 1996).

When a small stream was treated at 0.5 ppm/15 min. (13°C) with an aqueous suspension of unformulated Bellon primary powder of Bti, in contrast to the sharp reduction (89%) in black fly larvae in the 20-350 m area below the treatment point, Surber samples indicated increases in mayfly (35%), caddisfly (47%), stonefly (75%), chironomid (19%), and elmids (242%) populations. No adverse effect on any of these non-target populations was evident following stream treatment (Molloy & Jamnback 1981). In laboratory and field studies conducted with Bti to determine its effect upon *Ae taeniorhynchus* and non-target organisms in a salt marsh, Bti killed over 99% of the

mosquito larvae at concentration of 4.5 IU/ml and above. Out of 39 species collected prior to treatment, only a homipteran (true bugs), *Notonecta indica*, showed a significant decrease in population. However, this genus is known to fly from deteriorating habitats (Purcell 1981). Experimental testing has demonstrated no adverse effect against other aquatic insects including dragonflies, damselflies, mayflies, stoneflies, caddisflies, water beetles or true bugs. Other invertebrates such as *Daphnia*, cyclops, rotifers and crustaceans are also unaffected (SCAMP 1987).

Many acute toxicity/pathogenicity studies with various varieties of Bt have been conducted using several routes of administration in rats, rabbits, and guinea pigs. Among the various studies reviewed, the highest dose tested was  $6.7 \times 10^{11}$  spores per animal. There were no significant adverse effects associated with these studies (Castillo 1986). No acute toxicity was observed in rats gavaged with the maximum dosage of 2 billion spores of Bti H-14. No erythema or edema formation were observed after dermal exposure to 1.6 billion spores. All other parameters of the test animals were normal. There was no evidence that Bti H-14 multiplied on the abraded epidermis. No adverse effects were observed in rats given 80 million viable spores by instillation into the lungs. There was no histological evidence of multiplication of the organism in lung tissue. Instillation of 10 million Bti spores into the ocular cavity produced no eye irritation beyond 48 hr. No multiplication occurred in the ocular cavity (Castillo 1986). Acute, oral and dermal toxicity ( $LD_{50}$ ) of Bti H-14 (Vectobac) is in excess of 30,000 mg/kg.

No allergenic response to *B. thuringiensis* was elicited in guinea pigs by intracutaneous injection, inhalation, or topical application to the intact or abraded skin (Hayes 1982).

Each of 18 persons ingested 1 g of a commercial *B. thuringiensis* preparation containing approximately  $3 \times 10^9$  spores daily for 5 days on alternate days. In addition to ingestion, five of them inhaled 100 mg of the powder daily for 5 days. There were no complaints and no positive findings by physical and laboratory examination (Fisher & Rosner 1959). When Bt was applied by aircraft at a rate of 2 kg of preparation ( $3 \times 10^9$  organisms/g) per hectare ( $6 \times 10^{12}$  organisms/hectare) the concentration of viable organisms in the air over the field exceeded background by 42.5 times on the day of application and by 22.5 times 5 days later (Castillo 1986). No complaints were received from eight men exposed for 7 months to fermentation broth moist bacterial cake, effluent, and the final powder in the course of commercial manufacture of the pesticide (Fisher & Rosner 1959, Hayes 1982).

A dose of  $10^7$  (i.e., 10 million) Bti organisms killed the test animals when injected intracerebrally (IC). Death was probably due to the massive i.c. inoculum and not any infective process. Most of the animals died within 24 hours (Castillo 1986).

The EPA's review of the toxicological data on Bti determined that no data gaps exist in the toxicology data base and no major environmental mammalian safety concerns (except for certain endangered species of Lepidoptera) were identified (Castillo 1986). Toxicological data specially required for biochemical and microbial pesticide registration including: immunotoxicology studies, infectivity studies, intracerebral test, tissue culture tests and virulence enhancement studies (EPA 1984, Marquis 1986). Bti is effective only against dipteran larval (mosquitoes and black flies) and is safe to the environment (Worthing & Walker 1983).

Bti is non-phytotoxic and has shown no effects on seed germination or plant vigor (SCAMP 1987).

ii. *Bacillus sphaericus*

Mode of Action. . *Bacillus sphaericus* is a naturally occurring bacterium, which is pathogenic to the larvae of many genera of mosquitoes. There are a number of strains of *B. sphaericus*, that being most toxic to mosquitoes is *B. sphaericus* 2363. Like Bti, it produces a toxin that must be ingested and partially digested before it becomes activated. *Aedes* mosquitoes are generally less susceptible to *B. sphaericus*. It is currently marketed as a granular larvicide by Abbot Labs under the trade name VectoLex CG and is of interest because it works better than Bti in highly organic waters often favored by *Culex* species (Abbott Laboratories 1996). Also of interest is the fact that it is not toxic to other dipterans, including blackflies. Its limited range of toxicity, while a blessing in some respects, has slowed development as the market for *B. sphaericus* is likely to remain small (Federici 1985).

Fate in the Environment. *Bacillus sphaericus* has a field life of between two and four weeks. The spores settle out of the water column in as little as two days though they settle more slowly than Bti, as they adhere less to suspended particulates. The spores can remain viable for months in the field (Yousten et al. 1992). *Bacillus sphaericus* may undergo limited recycling (reproducing itself within the larval gut of *Culex* species), especially in rich organic environments (Abbott Laboratories 1996, Karch et al. 1990).

*Bacillus sphaericus* is ingested by other filter-feeding arthropods (*Daphnia pulex* and *Cypris* sp.) It can germinate within the gut of these animals and may be spread by them through the environment. It does not seem to adversely affect them and they may play a role in recycling *B. sphaericus*, thereby increasing the length of control achieved from a single application. *Bacillus sphaericus* does not affect populations of the predator species *Cleon*

*dipterium*, *Corixa punctat* and *Nepa cinerea*. (Karch et al. 1990). *Bacillus sphaericus* can be used in conjunction with the fungus *Lagenidium gigateum* (Orduz and Axtell 1991).

Effects on non-target organisms. *Bacillus sphaericus*, technical material, had acute oral and dermal LD<sub>50</sub> values for rats of >5g/kg and >2g/kg respectively, making it a class IV larvicide. The technical material can be moderately irritating to the skin and eyes of people.

Toxicity tests on mallards, bluegills, and rainbow trout showed the material to be extremely safe to these animals. Acute toxicity tests on freshwater invertebrates (daphnia and mayfly larvae) and salt marsh and bay species (sheephead minnow, shrimp and oysters) all indicate that the material is essentially non-toxic. Honey bees are not affected by *B. sphaericus* (Abbott Laboratories 1996).

*Bacillus sphaericus* is not phytotoxic (Abbott Laboratories 1996).

#### b. Methoprene

Mode of Action. Methoprene is an insect growth regulator (i.e., a synthetic analog of the juvenile hormone) which does not allow insects to mature from the larval stages into reproductively capable adults. It shows little or no effect on the adult or pupal stages of insect development. Unlike ordinary insecticides, this relatively non-persistent chemical exhibits morphological rather than direct toxic activities. Although its exact mode of action is not completely known, three modes have been investigated: 1) methoprene binds JH receptors resulting in extended juvenile forms which are not reproductively competent, 2) methoprene competitively inhibits catabolic metabolism of JH which extends juvenile forms etc., and 3) methoprene binds to its own receptors and extends juvenile forms (Matsumura 1985).

Fate in the Environment. Methoprene is relatively stable but nonpersistent in the environment (SCAMP 1987). It does not biologically magnify. In soil, methoprene rapidly degrades with a half-life of approximately 10 days. In plants its degradation principally involves ester hydrolysis, O-demethylation, and oxidative cleavage of the double bond of the C4-position. In lucerne and rice, the principle metabolite is 7-methoxycitronellal. Methoprene is very susceptible to photolytic decomposition under environmental conditions. It is degraded to many photoproducts which are present in relatively low yield (<10%). The rapid degradation of methoprene and multiplicity of photolytic products are indicative of extensive photodegradability in the natural environment (Quistad et al. 1975).

Technical grade methoprene is stable >4 yr. in glass in the dark at 43° (Worthing & Walker 1983). Sterile aqueous 0.5 ppm solutions at 98% pure [C<sub>5</sub>-<sup>14</sup>C] methoprene, buffered at various pH values, were found to be

extremely stable to hydrolysis over 4 weeks at 20°C in the dark. No degradation (detectable limit 1%) was seen in sterile water at pH 5.7 methoprene was rapidly photoisomerized ( $t_{1/2}$ -30 min.) to a final 2E:2% isomeric mixture of 44:56. The 2% isomer of methoprene has a much lower biological activity than 2E-methoprene itself, hence photoisomerization of methoprene in the field should quickly result in a nondegradative loss of about half the biological activity. Solutions of methoprene (0.5 ppm) were found to undergo photoinitiated decomposition to more polar products, to the extent of 15% in 2 days and 33% in 3 days at 20°C. Earlier experiments at 0.1 ppm at two temperatures (24 and 40°C) had shown no breakdown at 24°C and only 5% breakdown at 40°C, in 1 day (Schooley et al. 1975).

In pond water, the half-life of methoprene is approximately 30 hr at 0.001 ppm and 40 hr at 0.01 ppm. Incubation of (2E)-[C<sub>10</sub>-<sup>14</sup>C] methoprene for 3 days at 0.42 ppm generated three primary metabolites, the result of ester hydrolysis and/or O-demethylation. These metabolites and recovered methoprene were photoequilibrium mixtures of 2-ene double bond isomers (e.g., E and %). In another incubation experiment with (2E)-[C<sub>5</sub>-<sup>14</sup>C] methoprene at 0.66 ppm in a pond water sample of presumably different microflora, a completely different metabolite was identified as resulting from oxidative scission of the 4-ene double bond. The principle metabolite in the latter experiment was 7-methoxycitronellic acid.

Methoprene was rapidly degraded when a thin film (0.1  $\mu$ ) on glass was exposed to sunlight through glass. The half-life under these conditions was 6 hr. After exposure to sunlight for 27 hr, only 3% of the applied dose remained as methoprene and it was isomerized to a 50:50 mixture of (2E,4E) and (2%,4E)-methoprene. The recovery of only 72% of the applied radioactivity after 27 hr suggested photolysis of methoprene to volatile products which were lost by vaporization. Collection of vapors above the photolysate resulted in recovery of 13% of the applied radioactivity. The volatile constituents were resolved into methoxycitronellal (4%), methoprene (0.2%) and <sup>14</sup>CO<sub>2</sub> (6%). Since only a trace amount of methoprene (0.2%) was detected in the condensed vapors, volatility of methoprene is not considered a major route for loss of radioactivity.

Effects on Non-target Organisms. Methoprene is non-toxic to bees, and relatively non-toxic to non-target species but shrimp and crabs may be killed (Hartley & Kidd 1983, SCAMP 1987). Methoprene does have some toxicity to the saltmarsh copepod *Apocyclops spartinus*, but the concentration of methoprene in the water required to cause transient reductions in the early life stages of the copepod is above that which should occur during routine mosquito control (Bircher & Ruber 1988). Methoprene did not adversely affect the copepods *Cyclops vernalis* and *Cyclops*

*navus* in a Minnesota study but did reduce chironomids, tipulids, ceratopogonids and stratiomyids (SPRP 1996). As for Bti applications, reducing chironomid populations may have a long-term effect on nesting ducks.

The acute toxicity (96hr-LC<sub>50</sub>) of methoprene to a variety of fish ranged from 1.6 ppm for rainbow trout to greater than 100 ppm for channel catfish (Johnson & Finley 1980).

In a model ecosystem study on the uptake and degradation of methoprene by bluegills, the fish had a surprisingly large amount of radioactivity after 4 to 6 weeks of exposure. If the radioactivity was due to the presence of parent methoprene, one would have concluded that the compound was bioaccumulated several thousand times. However, less than 0.1% of the measured radioactivity was in the form of methoprene and its primary metabolites, with the rest being present in such natural products like cholesterol, proteins, free fatty acids, and glycerides. With this correction factor, methoprene was bioconcentrated by bluegills to a moderate extent. In clean water, bluegills eliminated 93 to 95% of the accumulated body burden of methoprene in less than 2 weeks.

Two formulations of methoprene were applied aerially (0.1 lb a.i./acre) to rice fields in the Sacramento Valley of California. The level of control of *Cx. tarsalis* was assessed in emergence cages established before and after spraying in these and control fields. After spraying, the treated fields had about one half the rate of *Cx. tarsalis* emergence as did control fields. None of the non-target organisms examined exhibited population fluctuations which could be statistically attributed to the methoprene applications (Case & Washino 1978).

Locomotor activities of mosquitofish (*Gambusia affinis*) and goldfish (*Carassus auratus*) were monitored for a 2-week period in the presence of methoprene at concentrations approximately 10-fold greater than those generally recommended for application. Methoprene, the active ingredient in Altosid SR-10, at 0.2 ppm did not significantly alter the locomotor activity of either mosquitofish or goldfish (Ellgaard et al. 1979).

The acute oral toxicity (LD<sub>50</sub>) of methoprene to mallard ducks was assessed as greater than 2000 ppm. However, treatment levels as low as 520 ppm produced signs of intoxication in mallards (Smith 1987). The eight-day dietary LC<sub>50</sub> for chickens was greater than 4640 ppm (Hartley & Kidd 1983). Thus, methoprene shows only slight toxicity to fish and birds and is relatively non-toxic to non-target species (SCAMP 1987).

When the metabolic fate of methoprene was studied in a guinea pig, a steer, and a cow, a rather large percentage of the radiolabel was incorporated in the tissues and respired by the animals. In the urine and feces, a small amount of radiolabel was metabolized into free primary metabolites, somewhat more was incorporated into simple glucuronides, and a considerable quantity of radiolabel was found in polar compounds, possibly complex

conjugates or polar biochemicals. No parent methoprene was found in the urine, but approximately 40% of the radiolabel in feces was contributed by unmetabolized methoprene. The formation of conjugates and the metabolism of methoprene was more extensive in the steer than in the guinea pig (Chamberlain et al. 1975). Samples of fat, muscle, liver, lung, blood, and bile from a steer which received a single dose of [C<sub>5</sub>-<sup>14</sup>C]methoprene were analyzed for radioactive residues. No primary methoprene metabolites could be characterized, but the majority (16-88%, depending on tissue) of the total tissue radioactivity was positively identified as [<sup>14</sup>C]-cholesterol. Seventy-two percent of the bile radioactivity was contributed by cholesterol, cholic acid, and deoxycholic acid. Radioactivity from catabolized methoprene was also associated with protein and cholesteryl esters of fatty acids (Quistad et al. 1975).

Acute oral toxicity (LD<sub>50</sub>) of methoprene in rats is greater than 34,000 mg/kg. That for dogs is greater than 5000-10,000 mg/kg. Dermal LD<sub>50</sub> values of methoprene are greater than 3500 mg/kg for rabbits. It is nonirritating to both the skin and eyes of rabbits (Hartley & Kidd 1983, Worthing & Walker 1983). Methoprene has an inhalation LC<sub>50</sub> value of greater than 210 mg/l in rat (Sine 1984). In 2-yr feeding trials, no methoprene-related effects were observed in rats at 5000 mg/kg diet and in mice at 250 mg/kg diet. No effects were observed at the highest rates tested: in 3-generation reproduction studies in rats (2500 mg/kg diet); teratogenicity in rabbits (500 mg/kg) or rats (1000 mg/kg); mutagenicity in rats (2000 mg/kg) (SCAMP 1987). Hollingsworth calculated the VSR for methoprene to be greater than 1,730,000. Methoprene is one of the most selective insecticides presently available (Wilkinson 1976).

Methoprene is not phytotoxic.

#### c. Oils

Petroleum oils are also known as mineral oils and refined grades have been called white oil. Petroleum oils are prepared by the distillation and refinement of crude mineral oils. Those used as pesticides generally distill >310°C to 335°C, namely; "light" (67-79%), "medium" (40-49%), and "heavy" (10-25%) (SCAMP 1987). They consist largely of aliphatic hydrocarbons, both saturated and unsaturated, the content of the latter being reduced by refinement. Recently, highly refined oils have been used as adjuvants to increase the effectiveness of some other pesticides (Hartley & Kidd 1983). Petroleum oils may be used as diluents for pesticides or by themselves

Petroleum oils are also used alone as insecticides. Inhalation of oils from the surface of water attacks mosquito larvae through their respiratory system. Death occurs due to reduced oxygen levels (hypoxia) and lack of

feeding. Petroleum oils also enhance penetration of the insect cuticle by the insecticide. In bioassays of insecticidal compounds, selective toxicity has been noted to vary for the same compound depending on the nature of the carrier solvent and site of application. The degree to which a solvent induces rapid penetration through the insect cuticle has been termed its carrier efficiency. This carrier efficiency can be directly related to and is dependent on the physical properties of the solvent-insecticide combination. Differences in carrier efficiency have been attributed to the ability of the solvent to dissolve the outer layer of the insect epicuticle (Schouest et al. 1983).

Petroleum oils are used as surface treatments to prevent mosquitoes from breathing at the surface. Petroleum oils have been shown to be phytotoxic to plants so use instructions should be followed and oil used only during the dormant period (Hartley & Kidd 1983).

Petroleum oils/Flit ML0 are mineral oils used as diluent/adjuvant oils or as a physical toxicant which kills mosquito larvae via suffocation. Both are prepared by the distillation and refinement of crude mineral oils. Those used as pesticides generally distill  $>310^{\circ}\text{C}$ . They may be classified by the proportion distilling at  $335^{\circ}\text{C}$ , namely: 'light' (67-79%), 'medium' (40-49%), and 'heavy' (10-25%) (Worthing & Walker 1983). Acute oral  $\text{LD}_{50}$  for rats and mice  $>4300$  mg 'Actipron'/kg. No toxicological problem due to petroleum oils has been reported in practice. Tests have shown there is no risk of polynuclear aromatic compounds entering the food chain using mineral oils for insect control (Hartley & Kidd 1983).

In 1968, the Medical Research Council in the United Kingdom published its report, "The Carcinogenic Action of Mineral Oils," based on work done in several British universities. The most biologically active fractions (boiling range of  $300\text{-}400^{\circ}\text{C}$ ) were distilled further. The carcinogenic activity appeared to occur in materials which boiled above  $350^{\circ}\text{C}$  (presence in lower-boiling fractions was possibly the result of azeotropism), and activity was still present in fractions boiling at  $420^{\circ}\text{C}$ . Over 40 chemical compounds were isolated from mineral-oil fractions, many for the first time, by repeated chromatography, complexing with picric acid and trinitrobenzene, and fractional crystallization. Further studies of the nature of the active compounds did not identify any single highly potent carcinogen. Several of the compounds separated were structurally similar to very potent carcinogens, and the total activity of the oil could be caused by the combined effect of several individually weak carcinogens (Kipling & Cooke 1984).



Albino rats were dosed orally by stomach tube at varying rates up to 10,000 mg FLIT MLO/kg BW and observed for mortality and signs of systemic toxicity for 14 days. The LD<sub>50</sub> was never reached and it was concluded that the LD<sub>50</sub> for FLIT MLO is above 10,000 mg/kg BW. A dose of one pint of FLIT MLO administered to a horse by stomach tube showed no gross reactions.

FLIT MLO was applied to the exposed abdominal skin of albino rabbits at dosage levels up to 3.16 g/kg BW. After 24 hr, the residue was removed, and the animals were observed for a total of 14 days after application. There were no deaths or signs of systemic toxicity at any dosage level tested. There was no dermal irritation to unabraded skin on any of the animals. A slight to moderate redness appeared on abraded skin. At the end of the 14-day period, the skin of all animals was completely clear of any signs of irritation.

Albino rats (10 male and 10 female) were exposed continually for 1 hr to an atmosphere containing an average of 200 mg FLIT MLO (aerosol) per liter (1) of air. Observations of mortality or signs of acute toxicity were made at intervals throughout the exposure, and the animals were observed for 14 days after exposure. There were no deaths during exposure or in the 14 days thereafter. During the post-exposure period the respiration rates of the animals seemed elevated initially, but returned to normal within 24 hr. A slight loss of fur occurred after a few days, but this effect had disappeared in all but four animals by the end of the study. Other superficial effects (exudate around the eyes, etc.) were observed but quickly disappeared.

FLIT MLO (0.1 ml) was placed in the left eyes (the right eyes served as a control) of nine albino rabbits. The eyes of three of the rabbits were irrigated after two seconds; those of another three after four seconds; the eyes of the remaining three were left unirrigated. Periodic observations for signs of eye irritation were made, with the last observation made 7 days after application. There was no evidence of systemic effect due to any of the applications. Eye irritation was slight and transient in all animals. All signs of the effect had cleared within four days following application. Examination on the seventh day confirmed the absence of any corneal damage. Ratings on the Draize scale, a common scoring system, were as follows: 4 at one and four hours, 2 at 24, 48, and 72 hours, and zero thereafter (Exxon 1973).

Bonide Mosquito Larvicide is a low-order, low-viscosity, highly saturated petroleum hydrocarbon mineral oil that enters mosquito larval breathing tubes and spreads over main tracheal trunks. These respiratory pathways twist and collapse reducing oxygen levels to tissues. Death is by hypoxia and lack of feeding. It is virtually identical to Flit MLO.

The residual periods of petroleum oils are relatively short. Tests have shown there is no risk of polynuclear aromatic compounds entering the food chain. No toxicological problems due to the use of petroleum oils for mosquito control have been reported in practice (Worthing & Walker 1983). It has some hazard to fish but little or no hazard to birds. Flit MLO has been evaluated to determine its effects on the aquatic environment. In a series of laboratory tests on grass shrimp, fiddler crabs, goldfish, bluegill, sunfish, fathead minnows, coho salmon, killifish, and domestic ducks, in which field applications of the larvicide were simulated and exaggerated, Flit MLO was found to have no adverse effects even at concentrations well above the recommended application rates of 1 to 5 gallons per acre (Exxon 1973).

Little or no hazard has been associated with the application of petroleum oils to beneficial and non-target invertebrates. Other Diptera that land on pools treated with oils will be pulled into the water due to the loss of surface tension and water beetles can often be observed crawling out of treated pools (Christie, personal observation). The flies clearly die, the beetles probably do not. Some hazard has been determined for honey bees.

Isooctadecanol (Arosurf MSF), is a surface-acting ethoxylated fatty alcohol (i.e., a nonionic surfactant) which forms a thin film over the surface of water creating a physical barrier due to reduced surface tension. This results in the suffocation of aquatic larvae and pupae (EPA 2/15/84). Foliar absorption, translocation in plants, and metabolism and persistence in plants is not available (EPA 2/15/84). Data on microbial breakdown shows that Arosurf MSF is degraded by unacclimated, mixed cultures of microorganisms from natural sources by shake culture methods. Although not available for Arosurf MSF, similar ethoxylates degrade under field conditions with the major route of degradation being hydrolysis at the ether linkage and subsequent oxidation of the alkyl chain to lower molecular polyethylene glycole-like materials which are ultimately mineralized to CO<sub>2</sub> and H<sub>2</sub>O. The resultant average persistence for isooctadecanol has been determined to be 2-10 days (EPA 2/15/84). Although incomplete, the 96 hr - LC<sub>50</sub> for *Daphnia* is reported as 1.9 ppm. Bioaccumulation data is also lacking but studies on closely related fatty acid and fatty alcohol ethoxylates in shellfish support the contention that the compound is rapidly cleared from aquatic invertebrates as inert metabolites.

Arosurf MSF was used in small amounts for several years in the late 80's and early 90's and is not currently being sold. It is included here both for historical purposes and in the off chance that it might become available again. As a surfactant, it forms a thin film over the surface of the water. Its mode of action is physical rather than chemical in that it reduces the water surface tension resulting in suffocation of the larvae and pupae.

These nonionic surface-active films spread into uniform, nearly monomolecular layers, and can not be seen because they are too thin to absorb light or cause iridescence due to reflective interference. Periodic observations indicated that wind velocity as low as 2-3 mph can push the film over the water surface to the downwind side or corner of a pond within minutes after treatment, thereby establishing areas of highly compressed film and areas of essentially no film (Levy et al. 1980). Average persistence for Arosurf MSF has been determined to be 2 to 10 days at recommended dosage rates (Miller 1984). Monomolecular films are biodegradable and have shown no adverse effects on mammals and several species of vertebrate and invertebrate aquatic organisms so these materials should not pose a threat to the environment or a health hazard to man.

An exemption from the requirement of a tolerance for the pesticide in or on fish, shellfish, irrigated crops, meat, milk, poultry and eggs, when used as a mosquito control agent in aquatic areas has been established under 40 CFR 18Q.1078. Studies on closely related fatty acid and fatty alcohol ethoxylates having various degrees of ethoxylation supported the clearance of the chemical as an inert ingredient and were also used in support of the exemption as an active ingredient (EPA 1984). Arosurf MSF demonstrates low mammalian toxicity (category III). Isooctadecanol has demonstrated low toxicity to fish, birds and other wildlife and non-target organisms. Acute toxicity (96hr-LC<sub>50</sub>) of isooctadecanol to fish range from 98 ppm (rainbow trout) to 290 ppm for bluegill (Miller 1984).

Qualitative data on non-target animals and plants exposed to various dosages of Arosurf MSF during field trials to control mosquitoes have indicated that this film will cause little or no adverse effects to the environment. Some mortality of pupae and/or emerging adults of certain midge species (Chironomidae) breeding in aeration and decomposition ponds at sewage treatment systems was noted. However, significant mortality of midges was also observed in some control (untreated) sewage ponds containing a similar layer of natural surface scums. Therefore, the true impact of Arosurf MSF on the reduction of the midge population is not known. Adult dragonflies were observed to oviposit in water treated with Arosurf MSF and a *Gambusia* sp. was observed eating large lenses of floating Arosurf MSF with no apparent adverse affects. Field and laboratory tests indicated that predation and asexual reproduction of the mosquito planarian *Dugesia dorotocephala* (Woodworth) and the infectivity and development of the mosquito nematode *Romanomermis culicivora* (Ross and Smith) were not adversely affected by Arosurf MSF at surface dosages of 0.4-0.5 ml/m<sup>2</sup>. Although no quantitative data were obtained concerning the

effects of Arosurf MSF on the natural populations of animals and plants, general observations indicated that there appeared to be few long-term effects (Ward 1966).

Arosurf MSF was effective in reducing 3rd and 4th instars of *Ae. nigromaculis* and *Ae. melanimon* populations at 0.5 and 1.0 ml/m<sup>2</sup> surface rates, averaging 88% and 96% mortality reduction, respectively. Non-target arthropods which showed acute lethal effects were corixids (*Corisella* spp.). Notonectids (*Notonecta unifasciata*), clam shrimp (*Eulimnadia* sp.), and *Tropisternus lateralis* beetle adults. Non-targets that did not exhibit mortality were mayfly (*Callibaetis* spp.) naiads, chironomid larvae and copepods (Takahashi et al. 1984).

Under field conditions, this film-forming substance produced a high level of control (90%+) of larvae and pupae of *Cx. tarsalis* at the rate of 0.5 to 0.75 gal/acre with no apparent effect on non-target organisms such as mayfly naiads, diving beetle adults, ostracods and copepods (Mulla et al. 1983).

#### d. Others

Temephos (Abate) is a non-systemic insecticide used as a relatively selective larvicide against mosquitoes, midges, gnats, punkies sand flies, thrips and black flies (SCAMP 1987). It has not been used in Massachusetts for several years. It is non-phytotoxic when used as recommended but is not generally used on plants (Hartley & Kidd 1983. SCAMP 1987). It has relative long residual action but short residual time in soils and water (SCAMP 1987). In plants, temephos is oxidized to the sulfoxide and, to a lesser extent, to the sulfone and mono- and di-orthophosphates. Further degradation proceeds very slowly (Hartley & Kidd 1983). In insects, the thiophosphate group and sulfur atom in the sulfide group of temephos undergoes a step wise oxidation. The oxidative compound is then hydrolyzed, and the final metabolite is 4,4'-dihydroxy diphenyl sulfone (Aizawa 1982). These reactions are highly species dependent which provides temephos's selective action.

Temephos has some hazard to non-target and beneficial insect but is highly toxic to honeybees by direct contact (topical LD<sub>50</sub> is 1.55 ug/bee). It is also hazardous to crustaceans such as shrimp and crabs (SCAMP 1987). At 100-1000 ppb, temephos causes some reduction in <sup>14</sup>C evaluation by algae (Verschueren 1983). The 96 hr - LC<sub>50</sub> for temephos to crustaceans range from 45-82 ppb and the median threshold limit (TLm) for shrimp range from 249 ppb to 2550 ppb. The LC<sub>50</sub> values of temephos to non-target aquatic insects range from 1-2 ppb (Ephemeroptera) to 500-1000 ppb (Tricoptera) (1 hr-LC<sub>90-95</sub>). The 96hr-LC<sub>50</sub> value for stoneflies is 10 ppb (Verschueren 1983). Temephos (Abate 4E) did reduce populations of copepods and cladocerans in a test in man-made ponds but the populations recovered within several weeks. Ostracods were not affected (Fortin et al 1987).

Temephos is stable when stored at room temperature, moderately stable to hydrolysis in contact with aqueous alkali, but the rate of hydrolysis increases with pH. Optimum stability is at pH 5-7 with increased hydrolysis occurring at  $\text{pH} > 9$  at rates depending on temperature. Temephos may be oxidized to the sulfoxide, the sulfone, and the mono- and dioxygen analogs. The sulfoxides and sulfones of the oxygen analogs have not been observed. Hydrolysis products include the thiophenol and the corresponding sulfoxide and sulfone (Reed 1982).

In a study using  $^3\text{H}$ -labeled temephos topically applied to bean plants, unchanged temephos comprised more than 70% of the terminal residues after 28 days. The sulfoxide comprised about 4% of the residues. The other oxidative metabolites were present in very small amounts. Conjugates of the hydrolysis products comprised the remaining residues. More than 90% of the applied activity remained after 28 days, indicating that residue decline is primarily the result of growth dilution (Reed 1982).

In laboratory studies, temephos has a half-life of 108 days in aqueous media. However, temephos degrades more rapidly in samples of reservoir, polluted brook, and puddle waters with half-lives ranging from 9-32 days. Temephos rapidly photodegrades to the sulfoxide and then to other oxidative and hydrolytic products. Sanders et al., (1981) reported concentrations ranging from 0.15  $\mu\text{g/l}$  to 10  $\mu\text{g/l}$  in small freshwater ponds 24 hr after application. Henry et al. (1971) measured temephos concentrations after simulating saltmarsh application rates (26 to 131  $\mu\text{g/l}$ ) and reported disappearance rates as a function of temperature. Temephos concentrations ranging from 16  $\mu\text{g/l}$  to 34  $\mu\text{g/l}$  were found in stagnant ponds after application by helicopter, with a half-life of approximately 5 hr (Lores et al. 1985). Peak concentration of temephos in water during early May occurred within 1 hr of treatment of a 4 EC formulation (mean approx. 20  $\mu\text{g/l}$ ) and within 8 hr of treatment with the 2G formulation (mean approx. 9  $\mu\text{g/l}$ ). The rate of degradation in water appeared to be affected by the initial solubility of temephos which is itself influenced by the application site, the water temperature and the formulation applied. These findings support a general half-life disappearance of 24 hr for the active ingredient under conditions found in local spring *Aedes* breeding habitat (Mackenzie et al. 1983).

Little data exists for soil degradation, but there are indications that certain soil bacteria are capable of causing rapid degradation of temephos to water-soluble hydrolysis products. The mean peak concentrations of temephos residues in sediments were in the 250-500  $\mu\text{g/kg}$  range following a single early spring application of 90-100 g/ha and declined to negligible levels in 10 days (Mackenzie et al. 1983).

From an animal metabolism study, data from cattle indicate that temephos and its sulfoxide are interconvertible in animals. The sulfoxide was present in the milk of cows fed 20 ppm temephos and temephos was present in the milk of cows fed 5 ppm of the sulfoxide (EPA 1986). All of the oxidative metabolite of temephos are expected ChE inhibitors, but terminal residues appear to consist primarily of temephos and its sulfoxide (EPA 1981). When <sup>3</sup>H-temephos was orally administered to rats, radioactivity reached a peak in the blood between 5 and 8 hrs and then dissipated with a half-life of about 10 hrs. Appreciable radioactivity was found only in the gastrointestinal tract and fat. Both in feces and in fat, most of the activity came from unchanged insecticide, but small amounts of the sulfoxide were present also. While traces of temephos were found in the urine, the principal urinary metabolites were sulfate ester conjugates of 4,4'-thiodiphenol, 4,4'-sulfinyl-diphenol, and 4,4'-sulfonyldiphenol. At least 10 other components could be extracted but were not identified. In the guinea pig, absorption apparently was less than in the rat, and biliary excretion of metabolites was demonstrated (Hayes 1982).

Temephos was dermally applied to cattle as a 0.1% spray and the cattle were slaughtered 7, 14, 28, 42, and 56 days after treatment. Temephos was not detected (<0.05 ppm) in any sample of muscle or liver, but was detected in the kidney at 0.08 and 0.13 ppm in 2 of the 3 animals slaughtered after 7 days. Temephos averaged 1.19 ppm in the fat of the 3 animals killed at 7 days and declined thereafter with a half-life of 6 days. Temephos sulfoxide was detected at levels of 0.39 and 0.09 ppm only in the fat of cattle killed 7 and 14 days, respectively, after treatment. In a study where <sup>14</sup>C-labeled temephos was sprayed on lactating goats, residues in the back fat were predominantly temephos. Residues in omental fat and milk were predominantly the sulfoxide with some sulfone and, in liver, residues of the sulfoxide and the sulfone were roughly equal. Other oxidative and hydrolytic metabolites were present in only small amounts. Therefore, metabolism and elimination of temephos is rapid with about 95% of a single oral dose eliminated in the urine and feces within 96 hours (EPA 1981).

The acute oral toxicity (LD<sub>50</sub>) of temephos in rats is 770-13,000 mg/kg to 8600 mg/kg, and 4000 mg/kg for mouse. Temephos has an acute dermal toxicity (LD<sub>50</sub>) for rabbit of 1300-1930 mg/kg and greater than 4000 mg/kg for rats (Hartley & Kidd 1983, Worthing & Walker 1983). Temephos and malathion showed an approximately fourfold potentiation in rats when they are given together at levels approaching their LD<sub>50</sub> values (Gaines et al. 1967). However, under other conditions no potentiation was observed between temephos and 23 other organic phosphorus compounds (Levinskas & Shaffer 1970). Temephos has a TWATLV value of 10 mg/m<sup>3</sup>. The threshold limit value of 10 mg/m<sup>3</sup> indicates that occupational intake at a rate of 1.4 mg/kg/day is considered safe.

In a neurotoxicity study, hens were fed 230, 460, and 920 ppm temephos for 30 days. Microscopic examination of nerve tissues revealed no demyelination (Reed 1982). Temephos in a dosage of 500 mg/kg produced a rapid onset of leg weakness in chickens, from which survivors recovered within 36 days or less. An intake of 125 mg/kg produced very mild leg weakness, followed by prompt recovery. This sign was produced in 30 days at a dosage of 15.3 mg/kg/day but not in 108 days at a level of 7.4 mg/kg/day (Gaines et al. 1967). Thus, the effect of temephos in chickens is similar to that of malathion, and the lack of myelin loss in hens fed the compound at a dietary level of 920 ppm (about 53 mg/kg/day) for 30 days, as reported by Levinskas & Shaffer (1970), would be expected (i.e., low OPIDN hazard).

The subacute and chronic toxicity of temephos is detailed below. Temephos has been shown to inhibit ChE activity *in vivo*. In rats, subchronic oral doses as low as 6 ppm resulted in depressed red blood cell ChE activity. In dogs, subchronic oral doses of 500-700 ppm caused depressed red blood cell, plasma, and brain ChE activities. Subchronic oral dosing with temephos caused no observed effect on survival, food consumption, or tissue and organ histopathology in rats given up to 350 ppm in the diet, and in dogs given 500 or 700 ppm in the diet for 90 days. Weight gain was reduced in rats given 350 ppm in the diet. In a 30-day study with male rats fed 0, 250, 500, and 1000 ppm technical temephos, the NOEL was 250 ppm (which is equivalent to 12.5 mg/kg B.W./day) for any effect other than ChE inhibition, which was noted at all dose levels. Weight gain depression was the effect noted at 500 ppm and above. No adverse effects were noted at feeding levels up to 300 ppm in a 2-year rat study. However, ChE activities were not measured (EPA 1981).

Over a 90-day period, rats tolerated a diet containing 9 ppm of the sulfoxide with no apparent effect except inhibition of RBC ChE activity. No inhibition occurred at 3 ppm (Levinskas and Shaffer 1970). In a study with human volunteers, increasing doses of temephos (2 to 256 mg/day) or constant doses of 64 mg/day were ingested over a four week period. No alterations in red blood cell or plasma ChE activity and no clinical symptoms were observed (EPA 1981).

The reproductive and teratogenic effects of temephos are as follows. Male and female rats were started on diets containing 500 ppm temephos at mating. Dosage was maintained throughout mating, gestation, parturition, and lactation. There were no significant differences in number of litters produced, litter size, viability of young or the incidence of congenital defects between treated and control groups even though the dosage caused signs of poisoning in some rats (Gaines et al. 1967). In a second reproduction study, no compound-related effects on

reproductive capacity were noted in rats fed 25 or 125 ppm of temephos. No effects were noted in the offspring (EPA 1981). Temephos at oral dosage levels as high as 2.5 mg/kg/day for 422 days or at 5 mg/kg/day for 186 days caused no ill effects in sheep or their lambs (McCarty et al. 1968).

No oncogenic effects were noted in a 2-year study with rats fed 0, 10, 100, and 300 ppm. There exists no mutagenicity data for temephos. The EPA Registration Standard for temephos states that toxicological data gaps related to reregistration of temephos for the food uses are a second oncogenicity study (in mouse) and teratogenicity and mutagenicity testing.

Acute toxicity (96hr-LC<sub>50</sub>) of temephos in a variety of fish species range from 0.16 ppm (rainbow trout) to 34.1 ppm (fathead minnow). Test conditions and size did not appreciably change the toxicity of temephos to fish. Variations in pH from 6.0 to 9.0, hardness from 40 to 162 ppm, or size from 1 to 20 g gave a range of less than 4 mg/l in 96hr-LC<sub>50</sub> values. Flow-through tests for up to 15 days with cut-throat trout and lake trout produced TILC<sub>50</sub> (time-independent LC<sub>50</sub>) values for temephos of 0.20 and 1.05 mg/l (ppm) and cumulative toxicity indices (96hr-LC<sub>50</sub> value divided by TILC<sub>50</sub> value) of 5.0 and 1.0, respectively. This indicates little or no cumulative action of temephos in fish (Johnson & Finley 1980).

Following the treatment of the Upper Volta River with Abate for the control of Simulium larvae, no fish died, but fish in the treated area were prone to easy capture. The catch per unit effort, and the number of species captured in the 24-hr period following treatment, were higher. Evidently, sublethal stress leading to under- or hyper-activity, make the fish an easy prey in nature and affect the stability of the population. When Abate was applied to River Oti in Ghana to control Simulium larvae, fish 300 m upstream from the point of application were normal, whereas fish at the site of application swam erratically. Fewer numbers and species were collected in the 24-hr period following application than before application, suggesting possible avoidance reaction of fish to Abate. Exposure to 96hr-LC<sub>50</sub> of fenitrothion for 24 hr completely inhibited the learning ability of salmon parr; Abate at 5 mg/l retarded learning (Murty, Vol. II, 1986).

Acute oral toxicity (LD<sub>50</sub>) of temephos for the bullfrog is greater than 2000 ppm (Smith 1987).

Acute oral toxicity (LD<sub>50</sub>) of temephos for a variety of birds ranges from 18.9 ppm (California quail) to 270 ppm (chukar). Its dietary LC<sub>50</sub> values range from 92 ppm (northern bobwhite) to 894 ppm (mallard duck). There are few data on its dermal toxicity. Mallard ducklings (12-24 hr of age) were fed diets treated with temephos



for 7 days and housed in either heated or unheated brooders. High mortality occurred in the 100 ppm group housed in unheated brooders, but diets containing up to 10 ppm did not affect duckling survival (Fleming et al. 1985).

A reproductive study conducted with game-farm mallards fed 0, 1, and 10 ppm Abate 4E (temephos) did not result in treatment effects for hatching success, clutch size, fertility, nest attentiveness of incubating hens, and duckling avoidance behavior. However, the mean interval between eggs laid was greater for the 10 ppm group, and duckling survival to 21 days was significantly lower for both treatment groups than for controls (Franson et al. 1983).

There have been no published accounts of wildlife die-offs that have been related to field applications of temephos (Smith 1987). However, the 30-day EMLD (empirical minimum lethal dose, lowest daily oral dosage that produces 1-2 deaths over a 30 day period) for mallards (n = 22) is 2.5 mg/kg per day for both sexes. The resulting cumulative toxicity index for temephos is  $79.4/2.5 = 32$ , indicating a high degree of cumulative action for an organophosphate in birds (Hudson et al. 1984).

### 3. Adulicides

#### a. Pyrethrum and Synthetic Pyrethroids

Pyrethrin I is insoluble in water, has appreciable vapor pressure but an extremely large partition coefficient (K<sub>ow</sub>) indicating that it is immobile in soil and migrates slowly, if at all. It is unstable in sunlight and rapidly hydrolyzed by alkali with loss of insecticidal properties. Photodecomposition of pyrethrin I as thin films on glass yields 11-15 products, none of which are insecticidal or of toxicological significance. Saponification (alkalhydrolysis) of the pyrethrum mixture of ester products liberates 12-16 acids, again with the subsequent loss of insecticidal activity.

Many invertebrates and microorganisms are capable of metabolically detoxifying this natural insecticide. Although the levels of Pyrethrin I in water, air, and soil are not known, little is expected to persist because of its photolabile and biodegradable nature.

Similar to pyrethrin I, resmethrin decomposes rapidly on exposure to air and sunlight and is unstable in alkaline media. However, photolysis of the ester bond is a significant reaction for trans- and cis-resmethrin but apparently not for pyrethrin I. Resmethrin and other type I pyrethroids generally yield a large number and great variety of photoproducts, most of which originate from further reactions of the primary cleavage products. For example, the alcohol moiety liberated on photolysis of resmethrin degrades further to benzyl alcohol, benzaldehyde,

benzoic acid and phenylacetic acid, the latter contributing to the unpleasant odor of photodecomposed resmethrin. The environmental and toxicological impact of these breakdown products are unknown at this time but due to the low rates and quantities used in mosquito control, they are not expected to be particularly significant.

As with pyrethrin I, resmethrin is rapidly biodegraded by invertebrates and microorganisms by established metabolic pathways. Although the levels of resmethrin in water, air, and soil are not known, little is expected to persist because of its photolabile and biodegradable nature.

Pyrethrins are potent, non-systemic contact insecticides causing rapid paralysis and knockdown with death occurring at a later stage of intoxication. Insecticidal activity is markedly increased by the addition of synergists such as PBO. Pyrethrins are not phytotoxic. Although toxic to bees, pyrethrins show less hazard than indicated by topical bioassays (approximately 1 ug/housefly) due to their repellent effect (Hartley & Kidd 1983). Pyrethrins are degraded in the environment by sunlight, chemical hydrolysis and by many invertebrate organisms via hydrolytic and oxidative metabolism. The approximate residual period for pyrethrins is 1-3 days on plants and similar short duration in soil and water (SCAMP 1987). No biological magnification has been shown. Degradative products have not been determined to be as toxic or more toxic than the parent pyrethrin compounds. Its toxicity (96hr-LC<sub>50</sub>) to crustaceans range from 11 to 42 ppb and for nontarget aquatic insects approximately 1.0 ppb (Verschuere 1983).

The synthetic pyrethroid resmethrin is also a non-systemic, contact insecticide with fast knockdown similar in action to the natural pyrethrins (Hartley & Kidd 1983). Although sometimes formulated with synergists (e.g., piperonyl butoxide), resmethrin's toxicity is not enhanced in their presence (SCAMP 1987). Resmethrin is not phytotoxic if used as recommended. Resmethrin is toxic to bees, but field data indicates that ULV sprays of resmethrin are not hazardous to honeybee colonies in the area treated for adult mosquito control (Scourge Tech. Bull. 1986). *Daphnia magna* has an LC<sub>50</sub> of 0.1 ppm (48 hr), *Penaeus* shrimp 1.25 ppb and the American oyster 1.79 ppm.

Piperonyl butoxide (3,4-methylene-dioxy-6propylbenzyl(heptyl) diethylene glycol ether) is used as a synergist in conjunction with pyrethrins and pyrethroids such as resmethrin. It is relatively stable to hydrolysis and UV irradiation. It is not toxic to bees or other beneficial insects and is degraded by invertebrates by oxidative attack on the carbon atom of the methylenedioxy group forming dihydroxyphenyl compound. Side-chain oxidations also occur. Elimination is by glucoside or amino acid conjugations (SCAMP 1987, Hayes 1982).

Pyrethrum and the synthetic pyrethroid resmethrin share a common mode of action as direct nerve toxins which interfere with nerve impulse generation via modulation of voltage-gated ion channels, particularly sodium and possibly calcium channels (Narahashi 1987, Clark & Brooks 1988).

As summarized by Hayes (1982), pyrethrum may be absorbed from the gastrointestinal tract and by the respiratory route. It is not absorbed to a significant degree through the skin. However, allergic reactions may result from this route of exposure. The esters constituting pyrethrum mixtures are rapidly detoxified by hydrolysis in the gastrointestinal tract and to some extent in the tissues of adult warm-blooded animals. The chrysanthemum monocarboxylic acid formed is excreted in the urine. Pyrethrins or their metabolites are not known to be stored in the body or to be excreted in the milk, but no study of the matter has employed modern methods. Partly because of their ready excretion, these compounds exhibit little clinical effect in animals following repeated exposure to moderate doses, but they or their metabolites do lead to liver changes in rats. Studies describe the very extensive metabolism that pyrethrins undergo, mainly in the liver. Various active ingredients undergo significantly different biotransformation. For example, within 48 hours of oral administration of  $^{14}\text{C}$ -pyrethrin II to rats, 53% of the  $^{14}\text{C}$  was recovered as exhaled  $\text{CO}_2$ , whereas only 0.3% of  $^{14}\text{C}$  pyrethrin I was recovered in that form under the same circumstances. The corresponding proportions of  $^{14}\text{C}$  recovered from the urine were 7 and 46%, for pyrethrins II and I, respectively. Some of the orally administered material is excreted in the feces, at least partially in metabolized form. Three compounds have been isolated from urine and identified by NMR and mass spectra. All three are produced by both pyrethrin I and II. All three are the result of oxidation of both the acid and alcoholic moieties leaving the main structure of the molecule intact (Elliott et al. 1972a, 1972b). So far the numerous compounds that result from hydrolysis of the esters have not been identified. The fact that the most severe cases of poisoning have been reported in infants suggests that very young children may not hydrolyze the pyrethrum esters efficiently. In any event, mammals show approximately the same susceptibility to injected pyrethrins as do cold-blooded animals, including insects (Gaudin 1937).

Acute oral  $\text{LD}_{50}$  values of pyrethrum in rats range from 584- 1500 mg/kg, 272-796 mg/kg for mice and 1500 mg/kg for rabbit (Farm Chemical Handbook 1988, Hartley & Kidd 1983, Hayes 1982). Lehman (1952) estimated that the fatal human dose might be 100 grams (1430 mg/kg) for a 70-kg man. Acute dermal  $\text{LD}_{50}$  values for rats are greater than 1500 mg/kg and for rabbits 5000 mg/kg. Dermatitis is possible in sensitive individuals from constituents of the flowers. Rats and dogs inhaled a concentration of  $16 \text{ mg/m}^3$  of pyrethrins for 30 min. periods

during 31 calendar days with only slight lung irritation (SCAMP 1987). Inhalation of pyrethrum at 6000 mg/m<sup>3</sup> for 30 min. caused only moderate lung congestion (Carpenter et al. 1980).

The subacute and chronic toxicity of pyrethrum is also low. Groups of 12 male and 12 female rats were fed pyrethrin in soybean oil at dietary levels of 0, 200, 1,000, and 5,000 ppm for 2 years. The daily dosages were, therefore, approximately 0, 10, 50, and 250 mg/kg, respectively. Even at the highest level pyrethrin had no significant effect on growth or survival. A slight though definite liver damage was observed, especially at the higher dosage levels (Lehman 1965). Dogs fed pyrethrins at a dietary level of 5,000 ppm for 90 days showed tremor, ataxia, labored respiration, and salivation during the first month of exposure (Griffin 1973). Ambrose & Robbins (1951) reported no effect in rats fed pyrethrins at a dietary level of 1000 ppm for two years, but tissue damage and gross signs of intoxication appeared at 5000 ppm. When pyrethrins were fed to rats at a dietary level of 1,000 or 5,000 ppm for 2 years, the liver lesions included bile duct proliferation and focal necrosis of the liver cells (Lehman 1965). Pyrethrins, especially synergized pyrethrins, produced enlargement, margination, and cytoplasmic inclusions in the liver cells of rats. At a dietary level of 1,000 ppm, pyrethrins and 10,000 ppm piperonyl butoxide, the changes were well developed in only 8 days, but were not maximal. The changes were proportional to dosage and similar to those produced by DDT. The effects of the two materials were additive (Kimbrough et al. 1968). No relevant pathology was detected in rats fed pyrethrins at a dietary level of 8,000 ppm for 5 weeks (Griffin 1973).

The sensitizing property of pyrethrins is apparently more evident in humans than in other animal vertebrates (Hayes 1982). When 200 people (177 women and 23 men) were patch tested with a 1% water dispersion of pyrethrins, no evidence of primary irritancy or of sensitization was found (unpublished report cited by FAO/WHO 1971). It must be noted, however, that both the formulation used and the duration of exposure were different from those that have caused the most severe dermatitis or sensitivity of any kind under practical conditions of exposure. There is little doubt that commercially available pyrethrin extracts are less sensitizing than native pyrethrum. However, apparently no study has been made to measure the difference in way that would be statistically valid. Mitchell et al., (1972) found all the active ingredients of pyrethrum, except pyrethrin II, to be inactive on patch test in a patient who had a history of allergenic dermatitis to pyrethrum and who reacted positively to pyrethrum flower heads, powder extracts of the plant, and pyrethrosin. The no-effect level corresponds to a rate of 3600 mg/man/day (SCAMP 1987).

Pyrethrum has little or no reproductive or teratogenic hazard (Hayes 1982). Rabbits that received pyrethrins orally on days 8 to 16 of gestation at dosages as high as 90 mg/kg produced litters with no statistical increase in abnormalities (unpublished report cited by FAO/WHO 1971). When rats were fed pyrethrins at a dietary level of 5,000 ppm beginning 3 weeks before first mating, reproductive performance was not reduced, but the weights of weanlings were significantly lower than those of controls (Griffin 1973).

The synthetic compounds are more photostable, sometimes providing control for many weeks following a single field application. In addition, some of the synthetic pyrethroids are metabolized more slowly by both insects and mammals and hence have increased toxicity. Enhanced environmental and biological stability of these compounds have triggered needs for residue analysis and for understanding their potential toxicity to mammals (Marquis 1986).

Resmethrin was one of the first or "early" pyrethroid synthesized which is not halogenated nor does it contain a cyanophenoxybenzyl alcohol. Thus, its metabolism and pharmacokinetics are more similar to the pyrethrins than the more stable type II pyrethroids (e.g., fenvalerate, cypermethrin, deltamethrin).

Resmethrin has an acute oral LD<sub>50</sub> of 2700 mg/kg in rats and 4250 mg/kg in rats exposed to technical resmethrin (SBP-1382). The dermal LD<sub>50</sub> value in rabbits is greater than 2000 mg/kg and greater than 3000 mg/kg in rats. Resmethrin was found to be non-irritating to skin and eyes (rabbits). SBP1382 is classified as a very mild conjunctival irritant. The inhalation (4 hr) for rats and dogs is established at greater than 9.49 g/m<sup>3</sup>. No effect level was 420 mg/m<sup>3</sup> (4 hr). The NOEL in rats for a subchronic inhalation study (90 days) is 0.1 g/m<sup>3</sup>.

In 90 day feeding trials, rats receiving 3000 mg resmethrin/kg diet showed no ill effects. The NOEL (no observable effect level) for resmethrin in a 180-day subchronic feeding study in dogs is 10 mg/kg/day.

In three generation reproduction tests for resmethrin, the NOEL for rats is 500 ppm. No teratogenic effects are observed in rats receiving resmethrin at dosages up to 80 mg/kg/day, up to 25 mg/kg/day for rabbits and up to 50 mg/kg/day for mice. The NOEL for fetotoxicity is 40 mg/kg/day.

Although limited, two studies support the contention that resmethrin has little carcinogenicity hazard. In an 85-week mouse feeding/oncogenicity study, SBP-1382 was not oncogenic at 1000 ppm. In a 2-yr rat chronic feeding/ oncogenicity study, SBP-1382 was not oncogenic at 5000 ppm, the highest dose tested. The above data have been compiled from the following sources: Hartley & Kidd 1983, Worthing & Walker 1983, SCAMP 1987,

Penick-Bio UCLAF Corp. 1986. Although overall evidence for chronic toxicity in mammals is limited, pyrethrin/pyrethroids still represents one of the safest classes of pesticides present available.

Because piperonyl butoxide (PBO) is included in various pyrethrum and pyrethroid (i.e., resmethrin) products used for mosquito control, the following mammalian toxicology is included. The acute oral toxicity ( $LD_{50}$ ) for PBO in various mammal range from 3800 mg/kg (mouse) to 11,502 mg/kg in rat. Dermal  $LD_{50}$  for PBO in rabbit is greater than 1880 mg/kg. Although dermal absorption is poor, multiple inunction at 200 mg/kg may be fatal to animals. In a subchronic feeding study, rats tolerated without harmful effects 5000 ppm in the diet for 17 weeks; 10,000 ppm were endured through 3 successive generations with moderate toxic effects and; 25,000 ppm in the diet were fatal to rats in from 4 to 46 weeks. In 2-yr chronic feeding trials, rats receiving 100 mg/kg diet suffered no ill-effect. PBO is noncarcinogenic and the safe human tolerance for chronic ingestion is estimated at 42 mg/kg diet (Negherbon 1959, Worthing & Walker 1983).

Pyrethrins (pyrethrum) are highly toxic to fish (Hartley & Kidd 1983). Acute toxicity of pyrethrum as judged by 96hr- $LC_{50}$  ranged from 13 ppb (channel catfish) to 58 ppb (bluegill). Temperature and pH of test solutions affected the biological activity of natural pyrethrins. Toxicity to channel catfish was 12-fold higher at 18°C than at 12°C. Toxicity increased in acid water (low pH); the 96hr- $LC_{50}$  for bluegills was 41 ug/l (ppb) at pH 6.5 and 87 ug/l (ppb) at pH 9.5. Water hardness (44-314 ppm) had little influence on toxicity (Johnson and Finley 1980). Excellent control of larvae of *Cx. tarsalis* in experimental ponds was obtained at the rate of 1 pyrethrin tossits/ m<sup>2</sup> (44 B ai/ha). At the same rate, satisfactory control (92%) of *Cx. peus* larvae was obtained in dairy waste-water lagoons in Riverside County, Ca. At the effective mosquito larvicidal rate (44 g/ha), mosquito fish, *G. affinis*, was affected when some tossits were placed with the fish in 0.9 m<sup>3</sup> screened cages. Five successive weekly treatments of *G. affinis* at the larvicidal rate and double that rate (44 and 88 g/ha) resulted in 37% and 67 % reduction of fish yield harvested 42 days after the first treatment compared to the yield obtained from the untreated ponds (controls), respectively. No marked effects on some of the non-target organisms such as dragonfly naiads, ostracoda, copepods, and Dixa midge larvae were noted. Mayfly naiads were eliminated at all rates applied, but recovered within 2-3 weeks after treatment.

Pyrethroids have very high insecticidal activity and have found wide use in the last few years. Though they are not persistent in the environment, their acute toxicity to fish is high. The cyano-substitution of the phenoxybenzyl alcohol moiety (as in cypermethrin and fenvalerate) enhances the toxicity of pyrethroids to fish.

Similarly, pyrethroids with (1R)-cis-isomers for the acid moiety are more lethal than the corresponding cis-,trans-racemates. Fenvalerate and permethrin are more toxic than many OC compounds, the 96hr-LC<sub>50</sub> being 0.5 to 12.0 ug/l (ppb). Cold water fish were reportedly more susceptible than warm water fish to natural permethrins as well as synthetic pyrethroids.

Pyrethroids are metabolized in fish by both hydrolytic and oxidative processes. It appears that, in general, esterases are more important in metabolizing the trans-chrysanthemates of primary alcohols whereas oxidases are more important with the cis-chrysanthemates of primary alcohols. Pyrethroids are rapidly metabolized by fish (Murty, Vol. II 1986). Resmethrin (SBP-1382) is toxic to fish (Hartley & Kidd 1983) with an acute toxicity (96hr-LC<sub>50</sub>) to freshwater fish ranging from 1.7 ppb (lake trout, bluegill) to 16.6 ppb (channel cat fish) (Johnson and Finley 1980). Resmethrin has a 96hrLC<sub>50</sub> value of greater than 150 ppb for coho salmon (Verschuere 1983). Resmethrin was the least toxic pyrethroid against frogs (*Rana pipiens pipiens*) with a subcutaneous LD<sub>50</sub> value of greater than 60 ppm (Cole and Casida 1983).

The acute oral toxicity of resmethrin to California quail has been determined to be much greater than 2000 ppm (Johnson and Finley 1980).

b. Malathion

Malathion is a non-systemic insecticide and acaricide that acts as a cholinesterase inhibitor. It is generally non-phytotoxic but may damage glasshouse cucumber, beans and certain ornamentals. Some varieties of apple, pear and grape may also be injured (Hartley & Kidd 1983). Its approximate residual period on plants is 5-10 days but has a short residual time in soils. Malathion is rapidly degraded *in vitro* by saltmarsh bacteria to malathion monocarboxylic acid (monoacid), malathion dicarboxylic acid (diacid) and various phosphothionates as a result of carboxyesterase cleavage. In addition, some expected phosphatase activity produces desmethyl malathion, phosphomono- and phospho-dithionates, 4-carbon dicarboxylic acids and the corresponding ethylesters (Verschuere 1983). In insects, malaoxon is the major metabolite formed by oxidative desulfuration at about 25% of all total. Predominant degradation is caused by hydrolysis in insects (Aizawa 1982). Biological magnification of malathion is not likely (SCAMP 1987).

Malathion presents some toxicity to beneficial insects including honey bees (Hartley & Kidd 1983). The topical LD<sub>50</sub> for honey bees is 0.71 ug/bee (SCAMP 1987). The 96 hr-LC<sub>50</sub> for non-target aquatic insects range

from 1.1 to 10 ppb, and for crustaceans 0.76 ppb to 3000 ppb. The median tolerance limit (TLM) for the American oyster is 9070 ppb (48 hr) for egg and 2600 ppb (14d) for larval (Verschueren 1983).

Degradation of malathion in soil is quite rapid. It was found that after the application of malathion at 5 lb/acre only 15% of the applied dosage was recovered 3 days later. After 1 week, 95% of the applied malathion had disappeared. In Ludhiana, Palamput, and Kamma soils, malathion was 100% degraded 4 days with higher temperatures producing more rapid degradation (Laveglia & Dahm 1977).

Walker & Stojanovic (1973) also found that malathion was quite stable under neutral or acid pH conditions and that susceptibility to hydrolysis increased with increasing alkalinity. They demonstrated that malathion disappeared more rapidly under nonsterile than sterile conditions and indicated that malathion disappearance is stimulated by various microbiological systems in soil. Under sterile conditions, the observed malathion remaining in the Trinity loam, Okolona clay, and Freestone sandy loam after 10 days of incubation was 91%, 77% and 95%, respectively. In nonsterile soil, complete disappearance of malathion had occurred. Getzin and Rosefield studied the degradation of malathion in nonsterile, heat-sterilized, and gamma-sterilized soil. After 1 day, 97% of the malathion was degraded in the nonsterile soil, 90% in the irradiated soil, and only 7% in the autoclaved soil. Because of the rapid degradation in nonsterile soil, it was suggested that microorganisms were partly responsible for the degradation of the compounds. The difference between the amount of malathion decomposed in irradiated soil and autoclaved soil was attributed to nonviable, heat-labile substances. A stable, heat-labile enzyme, which catalyzed the hydrolysis of malathion to its monoacid, was partially purified. This esterase was extracted from irradiation-sterilized soil as well as from nonirradiated soil. When the partly purified enzyme was applied to the soil, it possessed many of the characteristics necessary for prolonged activity in a cell-free state in soil. It was less heat-labile than most enzymes, lost little or no activity upon prolonged storage, and survived desiccation in the soil. There have been many reports of microorganisms metabolizing malathion to various products. Carboxyesterase activity, which degrades malathion to its monoacid and diacid, is the predominant degradative pathway. Phosphatase activity also has been reported. Oxidative desulfuration and demethylation seem to be rather minor metabolic routes (Laveglia & Dahm 1977).

Walker (1976) reported that malathion dissipated rapidly in both sterile and nonsterile water in that only 3% of the added insecticide could be detected after 18 days incubation. The similarities between malathion degradation in the presence and absence of biologically active systems dictates that malathion disappearance is by



purely chemical mechanisms. The effect of salinity on the degradation of malathion in estuarine water was determined by incubating the insecticide in sterilized seawater varying in salinity from 0 to 25 ppt. The degradation of malathion was found to be directly proportional to the length of incubation and to increasing salinity in this sterile system indicating direct chemical hydrolysis.

The organophosphate insecticides included in this classification share a common mode of action as potent indirect nerve toxins via inhibition of cholinesterases (ChE).

Acetylcholine (ACh), the neurotransmitter secreted by cholinergic neurons, allows for chemical transmission of nerve impulses across the synapse. Acetylcholinesterase (AChE) is the enzyme responsible for the breakdown of ACh, thereby terminating the electrochemical connection between two nerve cells. Organophosphorus insecticides (OP) phosphorylate AChE in an irreversible reaction that inhibits the activity of AChE to hydrolyze the neurotransmitter at the nerve synapse. The accumulation of ACh results in continuous nerve firing and eventual failure of nerve impulse propagation. Respiratory paralysis is generally the immediate cause of death. Brain ChE activity is used in the diagnosis of OP poisoning with a reduction of 20% of normal activity indicating exposure. A ChE inhibition of 50% or more is considered the diagnostic threshold for determining the cause of death. Because of the irreversible nature of the pesticide-enzyme binding reaction characteristic of OP poisoning, recovery from a sublethal OP exposure depends on synthesis of more ChE. There is evidence that recovery of ChE activity in plasma is as fast or faster than in the brain (Hayes 1982).

In addition to anticholinesterase activity, some OP pesticides have other adverse biochemical effects. A number of have been determined to inhibit neurotoxic esterase, an enzyme that has not been isolated and whose biochemical and physiological functions in nerve tissue are unknown (Metcalf 1982). Organophosphate-Induced Delayed Neuropathy (OPIDN) has been recently reviewed (Marquis 1986). Little information is available on delayed neurotoxicity in wildlife species. However, species differences can be great and the capacity to produce delayed neurotoxicity is widespread among OP (Metcalf 1982).

Other sublethal biochemical effects have been described for OP, such as impaired reproduction in birds through possible hormonal effects and reduced tolerance to cold stress (Rattner et al. 1982a, 1982b). Embryotoxicity and teratogenic effects have been demonstrated for some OP (Hoffman & Albers 1983), and some have been shown to cause changes in brooding behavior or nest attentiveness of birds which could result in death of

the young (Grue et al. 1983, Smith 1987). Central nervous system toxicity and noncholinergic effects of OP in mammals have been recently reviewed by Marquis (1986).

As summarized by Hayes (1982), the most striking difference between the metabolism of malathion and that of the majority of organic phosphorus compounds used as insecticides depends on the presence of two carboxy groups in malathion. Malathion is subject to the various kinds of metabolism that other organic phosphorus insecticides undergo, but the splitting of either carboxyester linkage renders the compound non-toxic. Details of the metabolism have been reported by O'Brien (1960) and by Heath (1961).

The excretion of absorbed malathion is rapid. When 25 mg of  $^{14}\text{C}$ -labeled malathion was administered to each of six male rats, activity appeared in the urine within 2 hr, and 91.72% was eliminated within 24 hr while an additional 7.75% remained in the gastrointestinal contents. Excretion was mainly via the urine (83.44%) and partly by the feces (5.51%), but 2.77% was exhaled as carbon dioxide. No unmetabolized malathion could be detected 8 hrs after dosing (Bourke et al. 1968). In *in vitro* studies of human and rat liver, no important difference was found in ability to degrade malathion (Matsumura & Ward 1966) concentrations of 1 ug/l (ppb) of methoxychlor or MDE, *Daphnia* exposed for three generations grew and reproduced normally, but the emergence of mayfly nymphs was drastically reduced. In ponds treated with 10 to 40 ug/l (ppb) of methoxychlor, the total number of benthic organisms increased and species composition changed with chironomids becoming the dominant species. Neither survival nor growth of bluegills in the treated ponds was affected. However, most of the fish examined showed nonspecific liver degeneration and an accumulation of a muco- or glyco-proteinaceous material inside the major blood vessels. Regression of these effects had occurred by 56 days after treatment (Johnson & Finley 1980).

Greater sensitivity of young striped mullet than older juveniles to methoxychlor has been reported. Adult striped mullets had larger amounts of the residues, owing to the presence of a relatively higher percentage of body fat in the adult when compared with the juveniles. That it is the lipid content that confers a greater degree of tolerance of the larger fish to the DDT-type compounds is confirmed by the toxic concentrations of DDT, endrin, and malathion, to brook trout, cut-throat trout, and coho salmon of 1-g size and 1- to 2-g size. With DDT and endrin, there was reduction in the toxicity with increase in size, whereas no such difference in the toxicity of malathion to smaller and larger fish was found. Exposure to a 96hr- $\text{LC}_{50}$  value of fenitrothion for 24 hr completely inhibited the learning ability of salmon parr and Abate at 5 mg/l retarded learning. DDT (OC) at 0.07 mg/l mildly enhanced learning whereas methoxychlor had no effect (Murty, Vol II, 1986).

Organophosphate insecticides have negligible chronic toxicity, but some have moderate to high acute toxicity in fish. The 96hr-LC<sub>50</sub> of several organophosphate compounds to various species of North American freshwater fish are listed below:

The acute toxicity (96hr-LC<sub>50</sub>) of malathion for a variety of fish species ranged from 62 ppb (red ear sunfish) to 12,900 ppb (black bullhead). In lake trout, fry (0.3 g) were twice as sensitive as fingerlings (4.5 g) to malathion. An increase in temperature from 10° to 21°C caused an 11-fold decrease in toxicity to the daphnid *Simocephalus*. However, an increase from 7° to 29°C caused a fourfold increase in toxicity to bluegills. Variations in water hardness did not appreciably alter the toxicity to fish or invertebrates. Mixtures of malathion with Baytex (fenthion), parathion, EPN, Perthane (ethylan), or carbaryl were synergistic in their toxicity to rainbow trout and bluegills. Combinations of malathion with DDT or toxaphene were only additive. Malathion has more-than-additive toxicity with more than half of the pesticides with which it was combined (Murty 1986) and is no longer used as a larvicide in Massachusetts.

Organophosphate compounds, because of their relatively higher water solubility, are in general taken up to lesser extent than organochlorines and eliminated faster. The time for achieving the highest levels of uptake and the extent of retention of OP residues by fish was directly related to the extent of persistence of a compound in water. Mutsugo fish exposed to 0.6 to 1.2 ppm malathion, attained the highest body concentrations 2.4 mg/kg of malathion after 1 day. No malathion residues were found 24 hr after the exposure of pinfish to 75 ppb concentration. Only malathion monoacid was recorded in the gut (Murty 1986). With DDT and endrin, there was reduction in the toxicity with increase in size, whereas no such difference in the toxicity of malathion to smaller and larger fish was found. The natural degradation products of pesticides, in general, are less toxic than the parent compounds. But occasionally, the primary degradation product may be more toxic. The hydrolysis product of malathion, diethyl fumarate, was more toxic than the parent compound to the fathead minnows (Murty 1986).

Acute oral toxicity (LD<sub>50</sub>) of malathion is 4100 mg/kg for rabbits and greater than 4000 mg/kg for rat, (Hartley & Kidd 1983, Worthing & Walker 1983, Smith 1987).

Based upon the low toxicity of this compound, it would appear that a time-weighted average TLV of 10 mg/m<sup>3</sup> is sufficiently low to prevent systemic effects (Hartley & Kidd 1983). Workers exposed to 84.8 mg/m<sup>3</sup> experienced moderate eye irritation (SCAMP 1987). Hollingsworth has established a VSR for malathion of 38 (Wilkinson 1976).

Under subacute and chronic experimental conditions, malathion has been fed to rats for 104 weeks at levels as high as 5000 ppm in the diet with no gross effects. In 1.75 yr trials rats receiving 100 mg tech./kg diet showed normal weight gain. There was no effect found on blood ChE when malathion was fed to human volunteers for 47 days at the rate of 16 mg/man/day. Volunteers dosed dermally showed no change of blood ChE or other injury while exceeding an average of 47 mg/man/day and a maximum of 220 mg/man/day.

Whereas there is no evidence that malathion is teratogenic in mammals, it does cause a characteristic syndrome of deformity in chickens that apparently depends on tryptophan metabolism and may be peculiar to the egg, which is closed system (Hayes 1982). When 1.17 to 29.20 mg of malathion was injected directly into the yolk of each egg, it often killed the embryo and deformed many of those that survived. When injections were made on days 8 to 12, half of the survivors lacked feathers or had only a few feathers on the abdominal region. When injections were made on days 6 or 7, 95% of the survivors were smaller than normal. When injections were made on days 4 or 5, the legs were half the proper size and the phalanges were permanently flexed in 95% of the survivors, and the maxilla was curved downward over the shortened mandible in 50% of cases. All of the hatched chicks had abnormal feathers, and some lacked feathers completely or in some areas. Most of the hatched chicks were about two-thirds normal size, and 6% were only one-fourth normal size (Greenberg & LaHam 1969). Tryptophan prevented the hind limb, beak, and feather defects and overall growth retardation in malathion-dosed embryos (Greenberg & LaHam, 1970). Application of 0.1 ml of 0.2 M nicotinamide or 0.1 ml of 0.1 M tryptophan simultaneously with malathion prevented development of the syndrome in all survivors, although the nicotinamide increased mortality. Tryptophan was also effective when injected into the yolk, and it was not toxic. It was speculated that malathion interferes with mobilization of tryptophan from yolk proteins, consequently decreasing synthesis of nicotinamide, which is essential for pyridine nucleotide-dependent reactions involved in differentiation of legs, beak, feathers, and other organs. Deformities of chicks can also be produced by feeding malathion to laying hens for 3 weeks at dietary concentrations up to 600 ppm (Ghadiri et al. 1967). However, the most likely problem for poultry is not teratogenicity but simple toxicity.

The hatchability of eggs was reduced when the hens were fed dietary levels of 1.0 ppm or higher but not 0.1 ppm (about 0.0058 mg/kg/day) (Sauter & Steele 1972).

Although malathion has been reported to produce delayed neuropathological syndrome in a human suicide attempt (Hierons & Johnson 1978), these findings are not substantiated in more sensitive animal models (Hayes 1982).

None of the 8 mutagenicity studies reported in NCI were positive for malathion. In general carcinogenicity/oncogenicity studies carried out by NCI, malathion was not implicated as a carcinogen (personal communication, Pesticide Bureau, DFA, Boston, MA). IARC concluded that malathion and its metabolite malaoxon were tested for carcinogenicity in mice and rats by administration in the diet. No evidence of carcinogenicity was found. Maddy (1984) ranks malathion in category 3 (negligible oncogenic potential, controversy as to any such potential, or not oncogenic).

Salmonids exposed to 120 to 300 ug/l (ppb) malathion showed AChE inhibition of 70 to 80%, and activity indices were reduced by 50 to 70% of that of unexposed fish. Goldfish exposed to sublethal levels of malathion showed a significantly reduced frequency of avoidance response at levels below that causing a reduced AChE activity. One-hour bath exposures of rainbow trout to sublethal levels of malathion caused severe tissue damage to the gills and minor nonspecific liver lesions. Ponds given four semi-monthly treatments during May-July at levels up to 0.02 mg/l (20 ppb) produced no discernible effects on resident bluegill and channel catfish populations. Aquatic insect populations were significantly depressed at the high treatment level but not at the lower levels (Johnson & Finley 1980).

Irrespective of the exposure time and exposure concentration, the AChE activity of dying pinfish was reduced by 72 to 79%. Enzyme inhibition was higher in lethal exposures than in sublethal exposures. The observed correlation between brain AChE activity and deaths was considered to be of diagnostic value. After 3.5, 24, 48, or 72-hr exposure to 575, 142, 92, and 58 ppb of malathion, 40 to 60% of the pinfish died and the extent of inhibition of AChE was in the range of 72 to 79%. Following the exposure of brook trout and rainbow trout to malathion, the AChE levels were approximately 24 and 28% of those of the controls, and the fish had less than one third the ability of the controls to do work. Thus malathion, though not directly and immediately toxic, had a deleterious effect, owing to the impairment of the ability of the fish to maintain equilibrium, to search for food, and to avoid predators (Murty 1986). In their examination of population and ChE responses in Walleye exposed to malathion aerial sprays, Lockhart et al. (1985) found that cholinesterase activity fell to about 25% of prespray values within 12 hr after the first spray, and then gradually recovered to about 80% of prespray values over 2 weeks. The same pattern

was evident after the second spray, but the inhibition of ChE activity was not quite as large. Fish captured for analysis showed no indication of the symptoms of OP poisoning. The population statistics indicated small temporary decreases in both catch per unit effort and weight gains after the first spray. Evidently a reduction in ChE activity to about 25% of preexposure levels was the approximate threshold for population effects caused by malathion.

The acute oral toxicity ( $LD_{50}$ ) of malathion to various species of birds range from not toxic at 100 ppm (European starling, red-winged black bird) to 1485 ppm for mallard duck. Dietary  $LC_{50}$  values range from 2639 ppm (ringnecked pheasant) to greater than 5,000 ppm for mallard duck. Acute dermal  $LD_{50}$  values are not available (Smith 1987).

Malathion applied at 0.6 to 1.1 kg/ha (0.5 to 1.0 lb/acre) resulted in no observed mortality of wild birds in one study conducted, and bird counts after spraying were either higher or nearly the same as before spraying (Black & Zorb 1967). At applied rates of 852-1140g malathion/ha (12-16 oz/acre) to fields containing caged quail, no evidence of mortality or population changes of wild birds were observed (Smith 1987). After malathion was aerially applied to a forested watershed at the rate of 0.81 kg/ha, birds reacted to the spraying for 2 days without lasting effects and no effects on reptiles and amphibians were observed. However, populations of mice and chipmunks were reportedly reduced by at least 30% (Giles 1970). Tadpoles exposed to 5 mg/l (5 ppm) malathion through a continuous-flow apparatus did not bioaccumulate levels that were toxic when fed to 2-week-old mallard ducklings in a single meal (Hall & Kolbe 1980). The predicted bioconcentration factor (BCF) for malathion is low.

Thus, malathion has moderate to slight acute oral toxicity to birds and there are no published reports of wildlife die-offs that can be attributed to the use of malathion. Its persistence and toxicity to birds is relatively low. However, malathion's toxicity has been reported to be potentiated by EPN treatment (Murphy 1969), and the interaction of these and other pesticides is not fully understood (Smith 1987).

## B. Biological Control.

### 1. Introduction

Biological control includes attacks on the pest species by other species and manipulation of the pest species itself. The former includes the traditional biological control agents, predators, pathogens and parasites, whereas the later includes such techniques as sterile-male release and genetic manipulations.

Biological control agents are grouped into three categories: predators, parasites and pathogens. Predators include both vertebrates and invertebrates and may attack both adult and immature stages of mosquitoes. Helminth, protozoan and fungal parasites and microbial pathogens generally only invade immature stages, though mortality may not occur until the early adult stage. Parasitic water mites are an exception in that they attach to certain adults as they emerge from the pupal stage and apparently reduce adult survivorship if they are abundant (Lanciani & Boyett 1980) In general, biological control is much more feasible in managing permanent water mosquitoes than temporary water forms.

There are three basic strategies for utilizing all biological control agents: (1) increasing existing natural enemy populations by habitat alteration, (2) one-time introduction of sustainable exotic agents from other regions or habitats, and (3) augmentation of natural or exotic enemy populations by repeatedly releasing non-sustainable, lab-reared (or field collected) organisms. To date only the first, increasing fish habitat through OMWM, has been used in Massachusetts. Bti is sometimes classified as a biological control agent but its application technique and mode of action as a stomach poison more closely resemble a pesticide than a biological control agent *per se*. *Bacillus sphaericus* may more closely fit the model of repeatedly releasing non-sustainable lab-reared organisms as there is evidence to suggest that it recycles within the environment.

No other biological control agent is currently available for general field use, though experiments continue with many different organisms (see below). There are important reasons why biocontrol is not more widely used against mosquitoes. First, the differences in biology of the various species of mosquitoes make it unlikely that any one control agent will operate across a wide range of species. Second, mosquito breeding is wide spread, making it difficult for a biological control agent to find, or be placed in, all breeding areas. Third, predators such as bats and purple martins, may eat mosquitoes but prefer to eat other, larger insects. Further, even when abundant, they do not drive mosquito populations below levels that people generally find intolerable. Finally, there is a high cost associated with sustained releases of a biological control agent and there are not, at this time, control agents available that require a single, or a few, releases to become established.

Because of the limited application of biological control to mosquitoes in Massachusetts, the following discussion will focus primarily on the feasibility of control agents currently being studied for mosquito control. An important point to make regarding biological control is that the mosquito control have limited research capabilities. While conducting field evaluations of new control techniques is a desirable practice for any mosquito control

program, the projects should not be expected to find and bring forward biological control agents without substantial help from research institutions such as the state university.

## 2. Predators

### a. Introduction

In order for any predator to independently be an effective control agent, it must meet two important criteria: (1) its size and abundance in relation to the target species must be sufficient for it to kill or consume a high percentage of the prey population within a relatively short time period, and (2) its feeding behavior should be selective toward the prey species when it is present but allow it to utilize other food items when the target species is absent. These criteria are rarely met in full. Predators that are sufficiently large and/or abundant to have a major impact, usually lack feeding specificity. Conversely, those with feeding specificity are usually less abundant because their populations are regulated by a more restricted food supply. Vertebrate predators of insects have a clear size advantage but invertebrate predators tend to exist in much greater numbers.

Because of the limitations of predators as biocontrol agents, it is normally essential to continuously raise and release the predator to achieve field densities high enough to cause real reductions in the prey species. However, in some cases, manipulation of the environment to the advantage of natural predator populations can provide an adequate augmentation effect.

### b. Vertebrate predators

#### Fish

Larvivorous fish are the oldest and perhaps most effective traditional biological control agent used against mosquitoes. In certain habitats they may, by themselves, provide adequate larval control throughout the breeding season. If not, pesticides such as BTI or methoprene which are non-toxic to vertebrates can be used in an integrated fashion with fish.

As already mentioned, the main reason open saltmarsh management strategies effectively control many saltmarsh *Aedes* in the Northeast is because this method provides access for the abundant estuarine populations of larvivorous killifish (*Fundulus* spp.) into the mosquito breeding pools in the high saltmarsh (Hruby & Montgomery 1986).

The mosquitofish *Gambusia affinis* is distributed widely throughout the warmer parts of the world and is the species most often reared and introduced into fresh water habitats for mosquito control. The biology and use of



this fish in mosquito control was reviewed by Meisch (1985). It is an opportunistic feeder and avidly eats pupae and late-instar larvae of culicines and chironomids. It is most effective against *Culex* in unvegetated, permanent ponds but has been widely used in California and the Gulf States against ricefield *Aedes* and *Psorophora*.

Because *Gambusia* is so aggressive and fecund, it may replace important commercial or rare native fish species. This has raised environmental concern over the introduction of this fish into natural waters where it does not already occur. A recent article by Rupp (1996) has renewed this debate, both emphasizing real successes and real concerns over *Gambusia* use (see “Comments on ‘adverse Assessments of *Gambusia affinis*’” (JAMCA 1996) and Boklund 1997, Eliason 1997, and Rupp 1997). Because it is not a native species of Massachusetts it may not be imported and released in state waters.

Outside of the mosquitofish, the common guppy (*Poecilia reticulata*) has received the most attention for mosquito control (Bay 1985). Comparative studies indicate that it is a less effective predator than *Gambusia* but it is more tolerant of polluted waters. Many other native fish have been explored for their mosquito control potential (Bay 1985). Studies in North Africa (Allo et al. 1985) suggest that malaria may be controlled through the annual introduction of native fish from streams into the manmade water storage tanks which produce the vector *Anopheles* in this region.

### Birds

Many birds depend on insects as food and those which capture insects on the wing (e.g., the swallows), have been credited with consumption of significant numbers of mosquitoes. Purple martins specifically have been promoted on the basis of the claim that they often consume 10-12 thousand mosquitoes per day but Kale (1968) concluded on the basis of existing evidence that all claims of martins significantly reducing mosquito populations were unsubstantiated and, because of several biological facts, were unlikely to ever be demonstrated. The facts on which these conclusions were drawn are as follows:

- (1) Mosquitoes were a negligible item in the diet of martins in the only two published studies in which the contents of martin gizzards were examined.
- (2) None of the published statements appearing in the popular ornithological literature which attributes a mosquito-eating habit to martins is based on factual study or scientific reference. In fact, there is evidence that martins feed more on larger insects including species of dragonflies which may be predators of adult mosquitoes.

- (3) Behavior patterns of mosquitoes and martins are such that they tend to not fly at the same height or at the same time. Thus, contact between the two is minimal.
- (4) There is no evidence that any avian species can effectively control a pest insect upon which it feeds when the insect is at or near peak abundance.

### Other Vertebrates

Mosquito control by insectivorous bats was tried in the early part of this century but without success (Kale 1968, Storer 1926). Bats continue to appear in the popular press as legitimate mosquito-control agents (Wright 1996) but are not considered worthwhile agents in Common-sense Pest Control (Olkowski et al. 1991), which is very thorough in its coverage of non-chemical control options, or in mainstream mosquito control (Mitchell 1992).

#### c. Invertebrate predators

### Predators of mosquito eggs.

Evidence exists of predation on diapausing flood water mosquito eggs by mites and beetles, and on *Culex* egg rafts by fish (Collins & Washino 1985). Nonetheless, egg predation appears to be a relatively minor component of natural mosquito mortality and is not being studied for biological control.

### Terrestrial insect and spider predators of mosquito adults

Predation on emerging and indoor resting adult mosquitoes has been readily observed but the impact of this mortality on populations is extremely difficult to assess. Certain spiders (especially Tetragnatha) and predatory flies (mainly Empididae, Anthomyiidae and Dolichopodidae) have been shown by precipitation tests to have consumed emerging mosquitoes (Collins & Washino 1985). In one British study, up to 28% of the spiders tested had eaten mosquitoes (Service 1973). Certain adult dragonflies reportedly capture mosquitoes on the wing but these observations have not been backed up by any controlled field studies. Synanthropic emesine bugs (Reduviidae) appears to be potentially important predators of indoor resting mosquitoes in the tropics. In sum, the prospect for enhancing or managing invertebrate predators for more effective adult control is not encouraging, especially in temperate regions.

### Aquatic insect predators of mosquito larvae & pupae

Aquatic insect predators seldom occur in significant numbers in the temporary floodwaters that produce most pest mosquitoes. Studies of predation have therefore largely taken place in permanent ponds or semi-permanent habitats such as rice fields, rock pools or vernal woodland pools. The adult stage of most predaceous aquatic beetles and true bugs can fly (usually at night) so natural colonization of newly flooded habitats can occur in a matter of days. Development time for these insects is usually from several weeks to several months.

Among the beetles, the dytiscids (predaceous diving beetle), which are predaceous both as larvae and adults, are the most effective predators of mosquitoes. There is evidence from studies in rice fields that adult

dytiscids selectively locate and colonize sites with locally high concentrations of mosquito larvae (Collins & Washino 1985). Mass production methods for dytiscids have never been developed. Whirligig beetles (Gyrinidae) only feed at the surface where they may prey on concentrations of emerging adult mosquitoes. Hydrophilids are only predaceous as larvae and seem to feed mainly on chironomid midges.

Only two aquatic families of true bugs, the back swimmer (Notonectidae) and pigmy back swimmer (Pleidae) have received serious consideration as mosquito control agents. Pleids are generally not abundant enough to have significant impact but notonectids can become quite dense in certain habitats. Mass rearing of the latter appears to be possible. Water boatmen (Corixidae) are also common and similar in appearance to backswimmers but they are mostly detritus feeders. Other predaceous aquatic Hemiptera that have been suggested as mosquito predators but which normally occur in insufficient densities to have much value as natural control agents include the giant water bugs (Belostomatidae), water scorpions (Nepidae), water measuring bugs (Hydrometridae) and the two family of surface-feeding, water striders (Gerridae and Veliidae).

Dragonfly naiads have been marketed commercially for mosquito control and at least one town in Massachusetts, and others in New Hampshire and Maine, have purchased dragonflies for mosquito control. In northern climates these insects require 1-5 years to mature, so they normally occur in permanent waters only. Furthermore, many are bottom feeders that seldom if ever come in contact with mosquito species that feed at the surface or in the water column. Most bottom-feeding mosquitoes (i.e. *Aedes*) occur in temporary water containing few if any dragonfly naiads. Another factor weighing against the mosquito control efficiency of these aquatic predators is the fact that they normally occur at low densities. Adults of many species are territorial and this seems to spatially limit population densities of naiads as well as adults. In certain habitats such as rice fields, naiads may become quite abundant but populations fluctuate greatly and their role in limiting populations of rice field mosquitoes is limited at best. No controlled, field studies have been done in which naiads have performed well as biological control agents.

The trichopteran *Limnephilus indivisus* may be an important predator of early spring *Aedes* in woodland swamps in Ontario but most caddisflies are omnivorous shredders rather than predators (Collins & Washino 1985, Merritt & Cummins 1985). Prospects for mass rearing and manipulating caddisfly larvae are not very bright. Many of the aquatic nematoceran relatives of mosquitoes contain groups with predaceous larvae. These include the families Chaoboridae, Chiromomidae, Ceratopogonidae, Tipulidae, Anthomyiidae, and others. Most are too small

to consume many mosquito larvae. Predation on small, early instars occurs but it is far less efficient in reducing the numbers of adults than is predation on late instars and pupae. *Mochlonyx* (Chaoborid) predation on spring *Ae. communis* populations has been observed in woodland pools in Massachusetts (Edmans, personal communication) and in Europe (Chodorowski 1968). The impact of this small but voracious predator is unknown.

The insect predator with the most promise in mosquito control is another mosquito. Larvae of the non-biting genus *Toxorhynchites* are large and effective predators of mosquitoes that develop in natural and man-made containers such as tires, tree holes, metal cans, and leaf axils (Steffan & Evenhuis 1981). Unfortunately, none of the 70 some species in this mainly tropical genus can survive the winters as far north as Massachusetts. Their use here would therefore require repeated, annual releases of lab-reared females. This is not warranted at the present time since container-breeding species generally do not represent the major nuisance or health threat in Massachusetts. This situation could change if *Ae. albopictus* becomes well established in used tires in the Northeast. Focks (1985) states that although it is possible to control or reduce certain species of container-breeding mosquitoes with inoculative or inundative releases of *Toxorhynchites*, the usefulness of this genus in practical, operational control programs has yet to be demonstrated.

#### Other invertebrate predators of larvae and pupae.

Among the non-insect predators of the immature stages of mosquitoes, only hydra (Cnidaria: Hydrozoa), flatworms (Platyhelminthes: Turbellaria) and copepods (Cyclopoida) have been studied in any detail.

Both hydra and flatworms can be easily mass produced and, unlike most predaceous insects, they can be maintained at high densities without cannibalism. In the laboratory, they kill far more larvae than they consume. Both groups produce semi-dormant eggs so they occur naturally in shallow temporary pools as well as permanent swamps and ponds. Detrimental effects on young fish have been reported when these predators are at high densities (Mulla & Tsai 1978).

Both of these predators have good potential as control agents in the Northeast but additional long-term field evaluations are needed. As with mosquito fish, the laboratory production, storage, and field translocation of these organisms requires a certain degree of sophistication, which is usually lacking at the local level. Currently, there are no commercial sources for the quantities that would be required for mosquito control applications.

Natural populations of predaceous cyclopoid copepods appear to limit the distribution of container breeding mosquitoes in certain tropical settings (Marten 1984). However, they have never been shown to be

important predators in temperate regions or in other types of aquatic habitats. Therefore their potential for the biological control of pest and vector mosquitoes in Massachusetts appears to be nil.

### 3. Parasites and Pathogens

#### a. mermithid nematode parasites

Outside of bacteria, parasitic nematodes are the only natural parasites and pathogens that have ever achieved operational status in mosquito control. Known parasitic roundworms of mosquitoes now number over 20 species. The free-living, aquatic, preparasitic stage which hatches from the nematode egg, seeks out and penetrates the cuticle of host mosquito larvae. Larvae are usually killed in the last instar. In a few species, the mature worms are carried over in the adult mosquito and cause mortality when they exit during attempted pseudo-oviposition.

*Romanomermis culicivorax* (including most references to *Reesimermis nfelsenii*) is the species that has been most extensively studied. Methodologies for the mass production and commercial preparation of this species have been developed and it was briefly marketed as Skeeter Doom in the late 1970's. Low sales and problems with the shipping and shelf-life of viable eggs appear to have been the major factors which led to the marketing failure of this agent (Service 1983). Other economic drawbacks include host specificity which limits its effectiveness to only certain species (e.g., it is ineffective in cold, polluted or brackish water), and the lack of patent protection for companies investing in the production and marketing of this product. In addition, applicators need some special skill and training to effectively use this biocontrol agent. The tendency of this agent to naturally recycle once it is introduced into favorable aquatic habitats is beneficial from a control viewpoint, but it further reduces the long-term marketability and profit potential for private producers. It remains under study, however, as a recent article (Mijares et al. 1997) discussed the establishment of *R. culicivorax* in sewage settling ponds and natural ponds in Cuba.

On the biological plus side, mermithids appear to be highly compatible with a wide range of chemical pesticides and growth regulators. Moreover, they: 1) are non-specific and well suited to the life cycle of their mosquito host, 2) produce high levels of infection and mortality, 3) can be easily mass-produced and applied with standard spray equipment, and 4) offer no threat to non-target organisms or the environment.

There are species of mermithids which appear to be highly specific to spring snow-pool *Aedes* and to saltmarsh *Ae. sollicitans* (Petersen 1985). However, these worms have not been established and studied in the

laboratory. Such species may have greater control potential in northern coastal states like Massachusetts than the more tropically adapted *R. culicivora*.

Since the technology for using mermithids in mosquito control already exists, and there are numerous field trials documenting their control potential, the future availability and use of these biocontrol agents in operational programs seems to depend on changing economic and market forces (Petersen 1985).

b. Microsporidia

Virtually all mosquito species carefully examined have been found to harbor these parasites. Larvae are infected by ingesting the spore stage. Spores, which are produced at the end of the life cycle, have often proven difficult to induce and to reinfect larvae in lab cultures. Few microsporidian life cycles are well enough known to assess biocontrol potential. Life cycles vary from simple to complex and often form the basis for the non-taxonomic grouping of these protozoan parasites. The simplest forms (Type I) occur mainly in terrestrial insects and even the one aquatic species known from mosquitoes (*Nosema algerae*), does not cause mortality until the adult stage. For this reason *N. algerae* has limited potential for reducing pest problems but may impact on disease transmission by reducing survival and fecundity. Wild strains only persist in larval populations for short periods and cause little direct mortality. Type II microsporidia have simple, asexual life cycles similar to Type I forms and they also show little promise in mosquito control (Hazard 1985). Type III forms are dimorphic, have binucleate spores, and kill mosquitoes in the larval stage. Only one species (*Hazardia milleeri*) is known from mosquitoes and it seems to have low infectivity (Hazard 1985). Type IV microsporidia include many species from mosquitoes and have the most control potential. Infected larvae are usually killed but a few females survive and carry the infection via the ovary to the next generation. However, transovarial infection ceases after a single generation. Non-ovarian modes of transmission must exist but this issue along with the possibility of sexual reproduction and alternate hosts are as yet unresolved. Additional basic research is required before any microsporidian can be considered in the biocontrol of mosquitoes (Hazard 1985).

*Tetrahymena* and *Lambornella* (tetrahymenid ciliates)

The lack of a resistant resting stage and difficulty in culturing these ciliated protozoa has prevented the thorough study and evaluation of this group of potential biocontrol agents. Ongoing studies of *Lambornella clarki* in California treehole *Ae. sierrensis* should help to better assess the control value of these parasites in the near future (Anderson et al. 1986).

### Helicosporidium

There is still debate concerning the proper classification of this spore-forming group of parasites; some believe they are primitive Ascomycetes fungi. There are only 3 reports of natural infections in mosquitoes. They appear to infect a wide range of species but also may infect non-target insects as well. High dosages of spores are required to infect older instars in the lab. Continuous cultures of these parasites do not exist so the basic research needed to evaluate their biocontrol potential is not forthcoming.

#### c. Fungal Pathogens

### Coelomomyces

Many forms of pathogenic fungi have been known from both larval and adult stages of mosquitoes since the 1930's. However, it was not until the discovery of the obligate alternate host (i.e., microcrustacea) in the mid-70's that cultures and full-scale laboratory investigations were possible with *Coelomomyces* (Whisler et al. 1974, 1975). The rather complex life cycles of some species have recently been described and methods for in vivo culturing established. Before wide-scale field application can occur, mass in vitro cultivation of the infective biflagellate zygote stage needs to be developed. If *Coelomomyces*, and perhaps their copepod and ostracod intermediate hosts, are introduced into favorable habitats, the potential for natural recycling exists. However, too little is known about the host ranges and habitat requirement of most *Coelomomyces* to recommend any such introduction at the present time. Moreover, species of *Coelomomyces* do not routinely provide high and predictable levels of mosquito control. It is, premature to critically assess their potential as control agents to be used independently or as part of an integrated control program (Federici et al. 1985). Studies related to their evaluation as operational control agents have only recently been initiated and it is not likely that any *Coelomomyces* will be operational within the near future.



### *Lagenidium giganteum.*

This mosquito-specific water mold is a very promising biological control agent, especially in fresh water and in warmer climates. This fungal pathogen can be mass produced on artificial media and can recycle in as little as 3 hours (McCray 1985). It has a resistant, dormant stage and infects a wide range of mosquito species. Unfortunately, it is not effective in polluted, brackish or cold waters. This limits its commercial value and its potential usefulness in northern and coastal states like Massachusetts (Service 1983).

### Other Fungi

Fungi are among the commonest pathogens of insects and many other genera besides *Coelomomyces* and *Lagenidium* have been reported from mosquitoes. Of these, *Culicinomyces* and *Metarhizium* have received the most attention. Both groups infect a wide range of mosquito species but relatively high concentrations are required to cause infection. They can tolerate organic pollution and salinity but not high temperatures (i.e. above 30°C). These fungi can be grown on inexpensive artificial media but no resistant resting stage has been found. Difficulty in achieving long term storage of infective stages and formulation problems remain as barriers to commercial production. Nonetheless, these fungi are a promising group of biocontrol agents which may provide new mosquito control tools in the future.

### *Erynia aquatica*

*Erynia aquatica* is an Entomophthorales fungus, and is the only species of the genus known to infect the immature aquatic stages of mosquitoes. It was first discovered infecting *Ae. canadensis* and *Cs. moristans* larvae in woodland pools in Hartford County, Connecticut from late May to early June (Anderson & Ringo 1969). It has since been recovered in early May from *Ae. stimulans* in woodland pools near the village of Cambridge, New York (Molloy & Wraight 1982) and from *Ae. cantator* on May 21, 1981 in a shallow salt meadow pool in Milford, Connecticut (Andreadis & Magnarelli 1983). Most recently, it was discovered in 1995 and 1996 in a woodland pool in Bristol, Rhode Island (Christie 1997).

Steinkraus and Kramer (1989) collected *Ae. fitchii* larvae infected with *E. aquatica* from a semi-permanent woodland pool in Tompkins County, New York. They used infected pupae to successfully transmit the disease to emerging adult *Ae. aegypti*, on which resting spores of the fungus developed.

*Erynia aquatica* has characteristics which make it attractive as a microbial agent: it is capable of causing epizootics, has been found in both freshwater and brackish water mosquitoes and has a resting spore stage that may

survive well in storage. Operating against it is the fact that it has only been found in cooler, springtime waters. One thought is that infected pupae may be removed from the original infestation site and placed in other, nearby pools. A fungus that kills in the pupal stage works against its own spread.

d. Bacterial pathogens

*Bacillus thuringiensis* var. *israelensis*

Bti Serotype H-14 has become an important biological larvicide following its discovery in the Negev desert in 1976. Within the last 5 years it has become widely used by the mosquito control projects in Massachusetts. It is now the larvicide of choice in many situations because of its host specificity, high and rapid mortality to many mosquitoes species, and its environmental safety. It is quite distinct from the Bt strains which infect lepidopterous insects. Its track record in controlling polluted-water *Culex* is mixed, apparently because it sinks to the bottom and the active moiety rapidly binds to organic particulates. Consequently, higher dosages are required to achieve good control in highly organic and deep-water situation. Saltmarsh mosquitoes generally require rates at the high end of the labeled application rates for effect control. Liquid, powder, granular and slow-release briquet formulations are commercially available.

Bti must be ingested to cause toxicity to filter-feeding mosquito larvae; pathology occurs in cells of the midgut wall. It is least toxic to 4th instars since they cease feeding at least 12 hours prior to pupation. The mosquito toxic ingredient of Bti preparations is the heat labile deltaentotoxin located in the proteinaceous parasporal crystalline inclusions synthesized concomitantly with the spore during sporogenesis. Once released in the environment, it biodegrades rapidly (it is usually only active 1-3 days) and this bacterium (gram negative Bacillaceae) does not recycle. This is considered the only major drawback of this highly effective mosquito pathogen but it has enhanced its commercialization (Lacey & Undeen 1986).

The biocidal activity of Bti toxin appears to be limited to larvae of certain families of nematoceros Diptera. A large number of laboratory and field tests have confirmed that all non-nematoceros, non-target organisms are virtually unaffected (Lacy 1985). All existing data indicate that the unaltered protoxin of Bti is also safe to vertebrates including humans (Lacey 1985). Further improvements in the efficacy and price competitiveness of this control agent, brought about by formulation changes and genetic engineering, are likely to occur in the near future. In addition, formulation of this agent with other compatible and perhaps synergistic agents such as juvenile hormone analogs (e.g. Altosid) is currently underway. Such mixture have two important advantages. They reduce

the likelihood of mosquitoes developing resistance to either agent and, secondly, they widen the window for control since Bti is most effective against early instars and growth regulators against later instars.

*Bacillus sphaericus*.

Although only recently available, *B. sphaericus* may have greater control potential than Bti because of its ability to continue to recycle once it is introduced. It has many of the same beneficial attributes of Bti but all strains isolated to date are less effective against a wide range of species than is Bti. At the moment it is primarily marketed against *Culex* in high-organic waters but research is on-going in expanding its control potential.

e. Viral Pathogens

The biological control potential of both non-accluted (iridoviruses and densoviruses) and occluded (baculoviruses, cytoplasmic polyhedrosis viruses and entomopox viruses) viruses pathogenic to mosquitoes has recently been reviewed by Federicci (1985). It is sufficient here to simply paraphrase from the summary of this thorough review as follows:

Analysis of research conducted to date makes it clear that none of the viral pathogens of mosquitoes can currently be considered good candidates for mosquito management programs. The two main reasons for this assessment are that viruses discovered so far possess low infectivity for original or alternate hosts and there is no readily available method for mass production of virions. This apparent lack of control potential is most obvious when one considers that few field trials have been conducted with these agents even though the first one was discovered over 15 years ago. While it appears unlikely that viruses will be developed for mosquito control in the near future, they may prove to be extremely useful microbial agents in the long term, perhaps in 2 or 3 decades, once we learn how to manipulate them effectively. Biotechnology has the greatest potential for engineering new more useful biocontrol organisms among the viruses because of their simple molecular structure.

4. Pest Species Manipulation

A somewhat different approach to biological control is manipulation of the mosquito genome to either 1) induce reproductive failure (leading to population elimination) or 2) bring about permanent changes in the behavior or physiology of the target population so it no longer vectors disease or bites people. There are numerous theoretical mechanisms by which this could be accomplished. Following is a brief discussion of the three approaches that have been most commonly discussed and researched.

a. Sterile Insect Technique

The early and continuing success of the sterile male release program in eliminating the screwworm fly from the U.S. and Mexico has given rise to many investigations and new ideas for controlling other insects (inoculating mosquitoes) through the use of this or some other genetic technique. Except for fruit flies, this method has not yet been successfully applied to other insects in operational programs and the technique is not currently being pursued for mosquito control.

b. Incompatibility

Incompatibility resulting from a lack of fertility in sexual unions may occur due to a variety of genomic failures or due to the effort of bacteria-like symbionts to control the reproduction of host (Barr 1985). The feasibility of suppressing *Culex pipiens* through cytoplasmic incompatibility was demonstrated in a Burmese Village over 20 years ago (Laven 1967) but the practicality of this method has not been confirmed by any other field tests. Moreover, incompatibility factors have been isolated in only a few mosquito species to date.

c. Chromosomal Aberrations

There are several heritable chromosome rearrangements that can, in theory, be used to inject genetic load into a mosquito population and/or to effect a permanent change in the genetic makeup of the population. These aberrations can be used to 1) increase genetic lethal load (serility is limited to 50-80% because of the low chromosome number (three, in most mosquitoes) or 2) replace noxious species with harmless strains if appropriate viable homozygous rearrangements are available. The latter is perhaps only applicable in the case of important disease vectors. Naturally occurring aberrations can be screened for in wild populations but their frequency is greatly increased through exposure to mutagens.

Controlling mosquitoes through the use of chromosomal aberrations requires a major basic research effort and a target species that is easily colonized. This later requirement automatically eliminates many important species from consideration.

#### d. Competitive Displacement

The final, and somewhat abstract, strategy for controlling mosquitoes biologically is the ecologically based notion of displacing a noxious species by introducing a benign but more competitive (i.e. better adapted) exotic one. This idea has been suggested primarily to control container-breeding species like *Ae. aegypti*. However, benign mosquito species are difficult to find, as most mosquitoes that have become established (*Ae. aegypti*, *Ae. albopictus*) are as bad or worse than the species with which they compete (container-breeding *Culex* and *Ae. triseriatus*).

#### 5. Other Control Approaches

a. Trap out techniques. All experience to date indicates that while various traps may be good sampling devices for adult mosquitoes, they are too inefficient and limited in their range to provide any benefit in reducing biting annoyances (Nasci et al. 1983). Work is ongoing on attractants (octanol) that would both greatly increase trap collections of mosquitoes and reduce non-targets trapped, but no products are marketed for this purpose to date.

Electrocutor traps ("Bug Zappers") continue to be a popular item, with an estimated 1.75 million sold in the United States each year (Mitchell 1992) but they are extremely non-specific (mosquitoes generally make up less than 5% of the catch, and may be harmful to other insect species. They cannot be considered a part of any mosquito-control program.

b. Repellents. Personal protection through the use of mosquito repellents is an appropriate alternative to controlling the mosquito populations before they bite. This is especially true if the periods of annoyance are infrequent and brief and where the land areas are too vast and unpopulated to economically consider control programs.

The most commonly used mosquito repellent is DEET (N,N)(diethyl-metatoluamide) which has been formulated and sold under a variety of trade names (e.g., Off, Muskol, Cutters), in a variety of concentrations and as both aerosol sprays (usually ca 15%) and lotions (up to 100%). Laboratory tests have shown that maximum repellent duration (ca. 1-2 hours) is obtained with a concentration of ca. 50% so that higher concentrations do not provide appreciable advantages. The major disadvantage of DEET are:

1) relatively short protection time

2) somewhat offensive odor

- 3) damage to some fabrics and surfaces at higher concentrations
- 4) high cost
- 5) possible hazards from heavy use

Small children frequently have skin reactions to DEET. Small children are also most likely to be the individuals that develop neurotoxicological symptoms from DEET. Thirteen of 14 cases of encephalopathy (toxicity of the central nervous system), found in publicly available reports by a recent review (Osimitz and Grothaus 1995), were in children 8 years old or younger. Three of these children died, all having used “heavy” amounts of repellent, even though the repellent in each case had DEET concentrations of 20% or less. Oral ingestion may have played a role in some of the cases. Osimitz and Grothaus (1995) concluded that there is no evidence that increased DEET concentration has an effect on the severity of symptoms. They also compared reports that the Poison Control Center received for DEET (6,724 in children under 6 years old during a five-year period) to laundry detergent reports (10,789 in 1989) and household bleach (16,169 in 1989), concluding that accidental exposure, while undesirably high, is in line with, or lower than, exposure to other household chemicals.

There is one unsubstantiated report in the Russian literature of carcinogenic effects in rats at high doses.

Three other repellent materials are in common use. Dimethyl phthalate (generally sold as 6-12) is not as effective as DEET against mosquitoes but still has a share of the market. Citronella-based repellents have long been marketed as candles or in oil-burning lamps. Citronella is also available as a repellent to be sprayed on skin and clothing (Natrappel). The third material (Avon skin-so-soft) is a popular bath oil that is not marketed as a mosquito repellent but has, through word of mouth, been recognized for its as yet uncharacterized mosquito repellent effect. It is as effective as DEET but it does not persist as long. On the other hand, it is much cheaper, smells better, and apparently does not damage any fabrics or surfaces. The active ingredient(s) of skin-so-soft has not been isolated or identified to date. It also has not undergone the EPA safety testing that other repellents have because it is marketed as a beauty aid rather than a pesticide (Note: repellents are classified as pesticides by EPA).

Electronic mosquito repellent devices which are periodically marketed in the U.S. (usually by mail order houses) are completely ineffective and are not based on any biological rationale (Foster & Lutes 1985, Mitchell 1992, Curtis 1994).

## C. Physical Control

### 1. Types of Habitat Modification

#### a. Open marsh water management (OMWM)

Originally developed for New Jersey salt marshes (Ferrigno 1970, Ferrigno & Jobbins 1968, Ferrigno et al. 1969), this strategy basically attempts to overcome the limitations of ditching by the incorporation of other water management strategies. In particular, champaign pools or reservoirs (which permanently hold water and sustain larvivorous fish) are created (by backhoe, dragline or rotary ditcher) in selected tidal pools or large shallow pans and are connected via small shallow ditches to surrounding mosquito breeding depressions. If old ditches are re-dug and used as reservoirs, then plugs must be inserted at the tidal end to prevent drainage. This customized approach to marsh management represents the least deleterious and most efficient nonpesticidal method for controlling saltmarsh mosquitoes and has been adapted to New England conditions (Boyes and Capotosto 1980, Hruby et al. 1985, Christie 1990). A manual outlining this method was developed by the Massachusetts Audubon Society (Hruby and Montgomery 1986) and OMWM is currently being practiced by coastal projects in Essex and Plymouth Counties in Massachusetts. As of 1996, OMWM had become the accepted technique for new salt-marsh water management work, though maintenance work remains dominated by cleaning existing ditching, as opposed to conversion to OMWM.

#### b. Other Modification Strategies

Mosquito control efforts in Massachusetts predate modern chemical insecticides. Early control efforts consisted of source reduction work, mostly in salt marshes (see “History of Cape Cod Mosquito Control Project, 1928-1971”). This emphasis was largely abandoned when cheap and seemingly more effective organo-chlorine insecticides became available in the early 1950's. Early control programs capitalized on cheap WPA labor but they failed to achieve the level of control that the public has come to expect of modern control programs. Nonetheless, they serve as a reminder that mosquito control, from its earliest inception, considered and practiced control alternatives to synthetic chemical pesticides.

Except for new OMWM projects in salt marshes, mosquito control source reduction work now consists primarily of maintenance work on existing culverts, storm sewers and ditches. Very little new ditch construction has taken place in recent years. Ditch cleaning, which often involves excavating spoil with a backhoe or plow, is an activity which has drawn great concern when it takes place in estuarine environments. This is because many of the

old grid ditches in the saltmarsh served no real purpose for mosquito control but they must be re-cleaned periodically or they themselves become shallow breeding areas for saltmarsh mosquitoes.

c.        Origination of Requests for Physical Control.

The exemption from certain regulations enjoyed by mosquito control is a two-edged sword. On the one hand, it enables mosquito control projects to more quickly respond to drainage problems. On the other hand, it makes the heading “Mosquito Control” particularly desirable for drainage projects in which mosquito control is, at best, a marginal goal. This pressure can come from property owners, public officials, or from within control projects themselves. The pressure to conduct drainage work that does not have a mosquito-control component must be resisted.

The best interests of mosquito control programs are served by conservative application of the definition of mosquito control, as over-use of the wetlands exemptions may result in the loss of those exemptions. To this end, mosquito control programs, in conjunction with state and federal wetlands protection agencies, must develop a strong set of guidelines for alterations exempt from Wetlands Protection Act.

2.        Ecosystem changes of non-target biota as a result of physical controls.

a.        Salt Marsh .

New England coastal wetlands have been heavily impacted by man (Shisler 1990). However, evidence concerning the negative impact of saltmarsh ditch maintenance activity is mixed. The principal concern is with disposal of the spoil on the marsh and the invasion of upland plants that can occur with even slight elevation increases (i.e. 1-2 inches) (Miller and Egler 1950, Buchsbaum 1994). Ditching also permits *Spartina alterniflora* to invade the upper marsh (dominated by *Spartina patens* and *Distichlis spicata*) along the edges of the newly created ditches. There is evidence to support the claim that this increases marsh productivity (Buchsbaum 1994).

In 1979, staff biologists from the New England Division of the U.S. Army Corps of Engineers (DeSista & Newling 1979) carried out preliminary investigation in several Massachusetts salt marshes to explore the issue of spoil deposition and upland plant invasion. In many instances they found little plant invasion despite evidence of previous spoil deposition of 2 inches or more in depth on the marsh. In a few locations with minimal tidal influence, some invasion by species such as common ragweed (*Ambrosia artemisiifolia*), march elder (*Iva frutescens*), and seaside goldenrod (*Solidago sempervirens*) had occurred over time. However, they concluded that it was not obviously correlated with the spoil itself but was perhaps caused by some other factor associated with the



disturbance to the marsh. They recommended the sort of long-term monitoring studies which have as yet not been carried out in Massachusetts salt marshes.

Clarke et al. (1984) studied the effect of ditching and vegetation changes on the use of the saltmarsh by birds in Rowley, Massachusetts. They concluded that bird use of the marsh was negatively impacted by mosquito control ditching. This is in contrast to the studies of Shisler & Jobbins (1977) in New Jersey marshes where increase productivity was observed in ditched marshes. Daiber (1986) noted that, where ditches drain pannes, birds that need a constant water supply (American bittern, pied-billed grebe and American coot) may decline. Also noted was a case where spoil ridges invade by *Baccharis* and *Iva* caused gulls to seek less brushy areas for nesting. Scheirer (1994) encouraged mosquito control programs to develop water management partnerships with the U. S. Fish and Wildlife Service, especially for OMWM-type marsh restorations designed to improve migrant waterfowl habitat.

In a series of investigative reports by staff biologists from the National Park Service, the environmental impact of ditching and diking of salt marshes in the Herring River basin of Cape Cod National Seashore were investigated (Soukup & Portnoy 1983, Portnoy 1984a, 1984b). These reports held that Cape Cod marsh management practices were responsible for the freshening, stagnation, acidification and high sulfate and aluminum concentration in diked marshes. These authors suggest that the destruction of a thriving eel and herring fishery in Wellfleet was the direct result of these marsh disruptions. However, the main dike across the mouth of the Herring River (from Griffin Island to the Mainland) was constructed in 1909 with the main objective of providing for agricultural use of the basin. These structures are not typical of early mosquito marsh management practices in other coastal regions in Massachusetts. The impact may be largely reversible if dikes are removed and normal tidal flow is allowed to return to these areas. OMWM tailored to the unique characteristics of these small estuaries should be explored as a way to manage the *Ae. sollicitans* populations which are likely to replace the present *Ae. cantator* populations if tidal flow and normal vegetation and salinity are restored in these marshes.

Perhaps the most damaging assessment of ditching in salt marshes was the report of Bourn and Cottam (1950) in which they blamed open ditching for converting up to 90% of the *Spartina alterniflora* marsh along the Mispillion River in Delaware to *Baccharis*. However, Provost (1977) reported that the area had returned to *Sp. alterniflora* after navigational dredging of the river had ceased and concluded that it was the dredging of the main channel, not the marsh ditching, which had permitted *Baccharis* to invade the marsh (see Daiber 1986 and

Buchsbaum 1994 for reviews of this debate). Arguing against significant water-table lowering in salt marshes is the strong affinity for water exhibited by salt marsh soils. Because permeability is so low, the water table may be lowered only within a meter or so of the ditch itself (Balling and Resh 1982).

Grid ditching, even if not the marsh destroyer some claim it to be, still reduces standing water on the marsh and creates an unnatural and aesthetically unpleasing view. Open marsh water management was designed to more closely approximate the diversity of the natural marsh while eliminating the shallow pannes in which mosquitoes breed.

Wolfe (1996) reviewed the effects of OMWM on numerous tidal marsh resources. OMWM systems tended to enhance tidal exchange and salinity in marshes that were converted from grid ditching. Except where spoil piles were left (improperly) on the marsh, vegetation change was slight and favored salt-marsh species such as *Spartina patens* and *Distichlis spicata*. Small changes in elevation due to spoil deposition sometimes result in invasion by *Iva*, *Baccharis*, and *Phragmites*. Salt-marsh snails (*Melampus bidentatus*) have declined in some OMWM sites but not in others. Similar mixed observations have been made for marsh periwinkle (*Littorina irrorata*) and fiddler crabs (*Uca* species). Marsh fish populations are, by design, enhanced by OMWM. However, changes in species composition may occur where existing pools are deepened. Mummichogs (*Fundulus heteroclitus*) and spotfin killifish (*Fundulus luciae*) should decrease while sheepshead minnows (*Cyprinodon variegatus*), inland silversides (*Menidia menidia*) and rainwater killifish (*Lucania parva*) should increase (Talbot et al. 1986). The small reservoirs are not particularly attractive to birds and the minor changes in hydrology, flora and invertebrate fauna caused by OMWM do not cause significant changes in bird use on OMWM sites. Effects on mammals are not well documented. OMWM has had no long-term detrimental effects on water quality. As a result, Wolf concludes that, "Open Marsh Water Management is an environmentally focused management tool that is designed to be compatible with nature rather than compete with it." Of course, the technique is new enough that long-term monitoring is required to ensure that altered sites remain functional salt marshes.

b. Freshwater Wetlands (exclusive of Vernal Pools). Palustrine wetlands, including emergent, scrub-shrub and forested wetlands, are the dominant system in which Massachusetts freshwater physical control take place. In the vast majority of cases, this work consists of maintaining existing ditching designed to remove standing water from the wetland, thereby reducing mosquito-breeding habitat. For most MCPs, this type of work (source reduction) makes up a large percentage of their control effort. Though reducing standing water

certainly reduces mosquito breeding, there has been little research concerning the overall effects of these alterations on the modified wetland. Ditch systems can become problems in their own right, producing mosquitoes if left unmaintained. Most of these systems were never designed specifically for mosquito control and their other, primary function, such as removing runoff from large parking lots, may cause considerable damage to the ecosystem, leaving the MCP to clean up, or at least deal with, someone else's mess.

The majority of drainage systems currently maintained by MCPs were not initially constructed by MCPs and the effort of MCPs today is almost entirely restricted to removing blockages to existing flows, rather than enlarging or straightening channels to increase flow. Most freshwater drainage is an inherited problem which requires intervention not because of mosquito-control activities but because of the activities of others. Road sand and yard waste represent two of the most common obstructions MCPs are called upon to remove from existing streams and drainage networks. New developments also can cause dramatic changes in the sediment load in streams, despite regulations designed to prevent such problems. Road sand, yard waste and increased sediment load from development can all have impacts on a stream that are as greater or greater than regular ditch maintenance.

Because MCPs are often involved in removing manmade sediments from streams, a system under appropriate ditch maintenance may function more closely to a natural system than one in which manmade wastes are allowed to accumulate unabated. The appropriate response by the MCP in such cases is not obvious because, although the problem, and its cause, may not be mosquito related, the mosquito control program may be the organization best equipped, both from and equipment and a training perspective, to rectify the situation.

Since mosquito control projects came into being in Massachusetts, the perceived values of wetlands have changed. Once shunned as disease-bearing waste lands, best suited for dumping, draining or filling, wetlands are now viewed in a much more positive way. They are important wildlife habitats, perform a myriad of water quality maintenance functions, and serve as flood control, erosion, ground-water recharge and water supply regulators (Tiner 1989). Mosquito control programs have been slow in adapting to the increased value accorded wetlands. On the one hand, long-residual and/or broad-spectrum pesticides are no longer used in wetlands in Massachusetts. On the other hand, there is real resistance to halting maintenance work in drainage networks that may be seriously damaging wetland habitat.

There has not been a great deal of work done specifically on the effects of physical control for mosquitoes on non-target organisms. Therefore the following discussion is based on the general effects that can be expected

when wetland alterations are made. Care should be taken in extending these general concerns to mosquito control in Massachusetts. For example, channelization (straightening) of natural streams is not permitted in Massachusetts, where programs are required to follow the natural meanders of the stream. The three broad categories of wetlands alteration are outright loss, changes in the abiotic system and changes in the biotic community. Filling and/or draining wetlands to convert them to upland is a mosquito-control practice that has been all but eliminated in Massachusetts. There is no indication that MCPs are intentionally reducing wetland acreage in order to control mosquitoes. However, the fact that the wetlands boundary remains essentially unmoved by a mosquito-control alteration does not mean that profound alterations have not occurred.

Changes in the abiotic system and biotic community are deeply intertwined, though physical control most often causes abiotic changes which then cause biotic changes. For channels changes in flow rates, microhabitats, sediment load, sedimentation, and groundwater interactions can all occur. For wetlands (outside of channels) changes can include lost water-storage capacity, increased sedimentation and pollutant load, changes in water depth, and changes in groundwater hydrology.

When a stream is altered to improve water flow for the purpose of removing standing water, either within the stream or from adjacent wetlands, a number of changes may take place. By definition, improving water flow increases runoff. This, in turn, may decrease the surface-water storage capacity of the wetland system and decrease the capability of the wetland to retain load (suspended solids). This may increase the load of the water moving through the stream (Brown 1988). Increasing runoff into a given stream tends also to increase erosion, which further increases load (Williams & Feltmantle 1992). Not only is total flow increased, but alteration tends to increase peak flow, which is associated with reductions in faunal diversity (Hynes 1972).

The effects of increased flow and loading are many. At its most obvious, higher peak flows increase scouring of the stream bed by gravel and sand being transported by the water. Bottom dwelling animals are either affected on-site or swept downstream, leaving an impoverished community as flows return to normal (Hynes 1972). Increased flows can also remove organic matter, leaving a sandy bottom on which macrofauna is reduced (Ward 1992). As stated earlier, higher peak flows also encourage erosion, a process that can increase stream load long after sediment controls put in place during the actual drainage work are removed.

High levels of suspended solids alter the stream habitat by:

1. reducing light penetration

2. reducing primary production
3. altering the trophic structure
4. altering nutrient dynamics
5. changing thermal conditions
6. reducing oxygen levels (Ward 1992).

These effects can, in turn can have the following impacts on the stream fauna:

1. abrading respiratory epithelia
2. clogging respiratory structures
3. reducing feeding rates
4. reducing feeding efficiency
5. increasing exposure to toxins
6. reducing vision
7. inducing organisms to drift.

All of these effects can alter behavior patterns, change predator/prey interactions and change the outcome of inter and intra-specific competition (Ward 1992).

Maintenance for the purpose of reducing mosquito breeding also includes removing obstructions within streams. Tree branches and fallen trees are a particularly important part of the stream environment, providing food, living space, concealment from predators, protection from abiotic conditions and emergence sites (Ward 1992). They also provide varied microhabitats by deepening and slowing flow on the upstream side and often creating deeper pools on the downstream side. Removing these obstructions diminishes the variability of the stream ecosystem.

At the other end of the flow-rate spectrum, increasing peak flow may lead to faster drying in intermittent streams. The insect fauna (and biotic community in general) of intermittent streams does not overlap to a great extent with that of permanent streams (Ward 1992). As a general rule, this is because intermittent stream dwellers have evolved to deal with the drying down stream. Survival mechanisms include leaving the stream (swimming down stream or emerging as land-living forms), surviving in crayfish burrows or remaining pools of water, burying either at shallow depths or quite deeply into the substrate or hiding under rocks or leaf litter within or along the stream course (Ward 1992). Streams that dry more quickly, and stay drier longer, may disrupt all of these techniques

as insects that emerge as adults may not have enough time to complete development (the primary example being mosquitoes themselves), remaining pools may decrease in size and number and shallow burrows and hiding sites under rocks and leaf litter may become too dry to support the fauna hiding there.

The effect of rapid drying brings up the aspect of the hyporheic zone. This is the interstitial space between the substrate particles in a stream bed. Within the hyporheic zone the macrofauna can find shelter from floods, drought and extreme temperatures, can find suitable and predictable conditions for immotile stages such as eggs, pupae and diapausing nymphs and larvae and, particularly for early instars, protection from predation. Gravel beds provide the best hyporheic zones and animals can often be found many feet down (Ward 1992). This hyporheic zone fauna can be an important source of recolonizing organisms after a stream bed is denuded (Williams & Feltmantle 1992). Excessive drying can reduce the viability of the hyporheic zone.

Sedimentation, both within stream beds and in wetlands into which streams flow, is a problem because it can alter the stream bed composition, thereby altering the fauna, and can clog interstitial spaces, thereby reducing the hyporheic zone and/or reducing groundwater recharge. Sediments can also increase exposure to pollutants as they provide additional sites for pollutant binding while suspended, and then carry the pollutants to the benthic fauna.

MCPs routinely conduct maintenance to remove sediments. Therefore, there is good reason to expect the overall effect of maintenance may be to reduce the negative impacts of sedimentation within the stream. In such cases, it would be preferential to develop systems designed to prevent the deposition of road sand into drainage systems, rather than to prevent the removal of that sand, once it has entered the system. Removing the dense, rotting masses of grass clippings that are dumped into streams by property owners should also improve overall stream quality.

Up to this point the discussion has focused on the stream itself, but freshwater wetland alterations are typically designed to change standing-water wetlands to soil-saturated wetlands (New Jersey DEP 1997). The obvious problem is that any organism that requires standing water for periods other than peak flooding, the wetland may become unusable. Mosquitoes fit this definition perfectly, but so do many other organisms. Many species of amphibians use temporary standing water for breeding and are becoming scarce as these habitats are eliminated.

Increased drainage also may have an effect on groundwater. Precipitation and inflow determine the amount of water initially available to a wetland for ground water recharge (Todd 1972). Increasing the amount of

water removed from the wetland prior to percolation downwards can decrease the capacity of the wetland to recharge groundwater supplies. Not only can groundwater recharge within the wetland lowered, it can be lowered with the outflowing stream as well. During peak flows, water moves from the stream into the substrate and raises the water table (increases groundwater). As the flow declines, groundwater percolates back into the stream (if it does not, the stream dries out). By eliminating upstream reservoirs (wetlands), more water flows out of the system initially, leaving less water within the system. As water levels fall, groundwater discharge occurs sooner than might otherwise be the case. If nothing else, stream depth is liable to vary more widely after adjacent wetlands are reduced to soil saturation.

What is most needed is a comprehensive understanding of the true ecological costs of physical alterations for mosquito control. This is particularly important because, although the environmental effects of pesticides receive the lion's share of concern, it is possible that the long-term effects of physical controls may have a greater effect on the environment than does pesticide use (Buchsbaum 1994). This may be especially true today with the switch from broad-spectrum, more-persistent pesticides to methoprene and Bti.

New Jersey has recently updated its Best Management Practices for Mosquito Control and Freshwater Wetlands Management and Massachusetts should look to such guidelines to establish a protocol for physical control in freshwater wetlands. At a minimum the common-sense requirements that all alterations be planned (not random), necessary, and desirable should be rigorously applied to all MCP water management projects regardless of whether they are defined as new or maintenance work. The North East Massachusetts MCP Standards for Ditch Maintenance (Appendix F) can be viewed as a starting point for a statewide protocol, though it fails to mention the need for sediment controls during maintenance work and leaves the MCP superintendent with wide latitude for determining the necessity of a given project. A response from the Massachusetts Audubon Society (also Appendix F) to these Standards provides additional comments which deserve consideration when a protocol is established.

c. Vernal Pools. Vernal pools form in contained depressions in which water stands for a period of several months, generally from mid- to late winter through the spring. Water either comes in the form of snow melt or spring precipitation or can be a result of a rising water table. Some pools dry down within two or three months, others may only dry when the water table is lower than normal, resulting in a pool that is semi-permanent. Regardless, a key feature of vernal pools is that they undergo periods of dry down. Vernal pools may have permanent inlets but do not have permanent outlets (Kenney 1995).

There are numerous obligate species for vernal pools, the most visible of which include fairy shrimp, the wood frog (*Rana sylvatica*) and several species of salamander (*Ambystoma* spp.).

The Massachusetts Division of Fisheries and Wildlife (Publication #15498-10-600-6-1-88C.R) has created guidelines for certifying vernal pool habitat on the grounds that many vernal pool species cannot successfully survive without vernal pools and that vernal pools are under pressure from continued development within the state. The certification program is coordinated by the Natural Heritage and Endangered Species Program (NHESP - see next section). “Automatic” protection is given to vernal pools only if they

1. occur either (a) within the 100 year inland flood plain or (b) on “isolated land subject to flooding” (as defined in the regulations at 310 CMR 10.57 (2)(b)); and
2. its existence and location has been certified by the Massachusetts Division of Fisheries and Wildlife.

Curiously, upland vernal pools are not granted the same protection but may be certified as vernal pools. The NHESP does not seek out pools to certify; it certifies submissions from the public. A guidebook for vernal pool certification (Wicked Big Puddles) is available to help those who wish to submit a vernal pool site for certification (Kenney 1995).

Mosquitoes, particularly *Ae. canadensis*, also use vernal pools for breeding. From a control perspective, vernal pools are important because, due to increasing protection, vernal pool habitat is often left undeveloped while the land adjacent to the pool is built up. As a result, many new developments surround known breeding sites. Regardless of the wisdom of developing so close to vernal pools, mosquito-control personnel are charged with controlling the mosquitoes coming from the pool.

d. Rare and Endangered Species. Hynes (1972) states three axioms of running water biotic communities:

1. The greater the diversity of the abiotic system, the greater the number of species within the system.
2. The more conditions deviate from the normal, the fewer species will be present, but those remaining will be present in greater numbers.
3. The longer a system is left undisturbed, the richer and more stable is the biotic community.

Operating under the assumption that it is rare and endangered species which are most likely to be lost from the system first, the above statements would suggest that reductions in habitat diversity, alterations from the natural state, and frequent disturbances will all work against these species. Channelization of streams reduces diversity by



removing obstructions, straightening the channel and increasing flood levels. Wetlands changed from standing-water to saturated-soil regimes have been drastically changed from their natural state. Maintenance is ongoing, as is the disturbance it causes.

The key question is, however, to what extent does mosquito control contribute to the above problems. First, Massachusetts MCPs do not channelize streams, as their certification manual calls for following the existing meanders. Second, MCPs work neither in historically undisturbed, nor currently undisturbed streams. There is every reason to argue that there is no specific “natural” state that can be assigned a ditch dug by man and intermittently filled with road sand and grass clippings. Even with natural streams, the “natural” habitat in which they flow has long been altered and continues to be altered.

The Natural Heritage and Endangered Species Program (NHESP) was created in order to conserve and protect those plants and animals not hunted, fished, trapped or commercially harvested in the state. The program’s highest priority is to inventory rare and endangered species and to develop conservation plans through research, management and habitat protection for those species. One such program that directly impacts mosquito control is the vernal pool certification program mentioned above.

The NHESP also reviews proposed alterations to wetlands habitats under the Wetlands Protection Act (M.G.L. c. 131, s. 40 and regulations 310 CMR 10.00). NHESP has produced a series of estimated habitat maps for rare and endangered species (Massachusetts Natural Heritage Atlas) which proponents of a given alteration are required to check. Should a project fall within an estimated habitat, NHESP will then determine if the area to be altered is actual wetland habitat for a state-listed species. The results of the NHESP determination are given to the inquiring MCP.

The Massachusetts Endangered Species Act (M.G.L. c. 131A and regulations 321 CMR 10.00) prohibits the “taking” of rare plants or animals. From a mosquito-control perspective, the most important definition of “taking” is disrupting nesting, breeding or feeding sites of animals or killing or cutting a plant. Aside from directly protecting rare or endangered species, this Act also allows areas to be designated “significant habitats.” Alterations in “significant habitats” require a permit from the Division of Fisheries & Wildlife.

In Massachusetts, the species that have caused modifications in mosquito control practices are the Blue-spotted salamander (*Ambystoma laterale*), Mystic Valley amphipod (*Crangonyx aberrans*), and banded bog

skimmer (*Williamsonia lintneri*). In addition, ditch maintenance in vernal pond areas has been curtailed to protect this type of habitat. Other animals for which concerns have been raised are the yellow-spotted turtle and osprey.

The presence of a threatened species need not prevent water management, however. In the East Volusia Mosquito Control District in Daytona Beach, Florida, OMWM was carried out in a salt marsh that contained the Atlantic salt marsh snake (*Nerodia clarkii taeniata*). An observer walked in front of the ditching machinery and work was halted until observed snakes left the area. In practice the snakes were difficult to spot and several were seen in the freshly cut ditches behind the ditcher. Two dead snakes were found, and assumed killed by the work. The dead snakes were handled as “incidental take” and placed on ice for delivery to the Florida Game and Fresh Water Fish Commission (Goode 1996). With an increased understanding of the ecosystems in which mosquito control takes place, mosquito control projects should improve their ability to work in areas containing endangered species with minimal impact to those species.

Under the current system mosquito-control maintenance activities are exempt from the Massachusetts Wetland Protection Act, leaving only the federal 401 Water Quality Certification Act and both the Massachusetts and federal Endangered Species Act as methods for regulating maintenance. Unfortunately, water quality, while important, does not address the issue of changing habitat and the presence or absence of a rare and endangered species has little to do with the merits of a given project.

Rare and endangered species will probably increase in their impact for several reasons. First, as data is collected on these species, additional species and additional habitats will most likely come under protection. Second, residential areas are creeping closer to wetlands areas. The net effect is that the clash between economic development and environmental protection will likely increase, with mosquito control being one component of an intense debate. Again, a more comprehensive understanding of the true ecological effects of mosquito control is required to better determine the cost/benefit ratio for different types of mosquito control.

#### D. Food Web Effects of Mosquito Control.

Throughout their life cycle mosquitoes are a part of the food web, both as consumers and prey. As larvae most species feed on algae, protozoa, and organic debris (Pennak 1953). As adults they drink nectar and the females of most species take blood meals from a wide variety of animals. Larval mosquitoes are eaten by an impressive array of animals (Bates 1949) while adult mosquitoes are taken by spiders, predatory flies, odonates,

bats and birds (Collins and Washino 1985). Given that mosquitoes are so thoroughly embedded in the food web, the question arises as to the effect of removing a large percentage of the mosquitoes from that food web.

At first glance, the effect would appear to be large, particularly in habitats with dense larval populations. However, mosquitoes are r-strategists, in that they produce large numbers of eggs that develop quickly (when immersed in water) to adulthood. Most pest species seek out temporary and spatially disconnected habitats for breeding and can complete a breeding cycle long before an adequate predator complex can develop. Mosquitoes are, therefore, a highly unpredictable food source for predators, and predation rates must be equally unpredictable (this section will focus on predation, as parasites and pathogens are not widespread control agents in Massachusetts). This is borne out by the fact that mosquito predators are generalists which can readily switch to other prey when mosquitoes become scarce (Collins and Washino 1985). In a study in Florida, immature mosquitoes made up over 50% of the diet of several species of saltmarsh fish (*Fundulus confluentus*, *Lucania parva*, *Gambusia a. holbrooki*). But each of these species fed on other items as well, including copepods, shrimp, other fish and even some plant material (Harrington and Harrington 1961).

One important point regarding predation and larval broods is that the concepts of handling time and satiation come in to play (Varley et al. 1973). A predator must spend a certain amount of time with each prey. Hydrophilid larvae took several minutes to feed on larvae and pupae taken in a panne in Tiverton, Rhode Island (Christie, pers. observation). Not only does it take time to catch and eat the prey, but invertebrate predators are typically not much larger than the mosquito larvae they are attacking. Satiation must play a role in limiting predator take, particularly when mosquito numbers are high.

Predators can play an important role in regulating mosquito numbers in some situations. That mosquitoes are absent from waters with fish populations is well known. Less well known is the influence of chaoborid larvae, which can severely reduce mosquito populations in vernal pools (Morrison and Andreadis 1990). In this case the larvae are present in snow-melt pools for approximately two months, so predation has time to operate as a regulating mechanism.

Although adult mosquitoes are eaten by numerous predators, it is rare that they make up an appreciable proportion of the diet of any one predator. One exception is the spider *Tetragnatha montana* for which, in Poland, mosquitoes made up 74% in June and 62% in July of all prey captured (Collins and Washino 1985). Bats and purple martins are not effective mosquito predators nor do mosquitoes form a significant part of their diet.

From a mosquito-control perspective, the mosquitoes to be controlled are, for the most part, those which have escaped predation to become predators in their own right. In the absence of scientific data supporting the necessity of mosquitoes in the diets of specific animals, removing mosquitoes from the food web by chemical, biological or physical control remains an easily justifiable activity. Even so, control personnel should take care to avoid chemical applications where mosquito larvae are not present or are present in very small numbers, should use control measures that do not harm existing predator complexes, and should limit control to areas where control is necessary, allowing natural cycles to continue in areas where human activity and the risk of disease transmission is slight. One argument made in favor of Altosid is that it does not kill the young larvae, leaving them available as food for the existing predator complex.

Up to this point, the discussion has focused on the effect of chemical control (including *Bacillus* products and IGRs) of mosquito populations on other species. Within the context of biological control, one of the primary reasons *Gambusia* are not being used in Massachusetts is the fear that they might displace native species of fish, thus altering the natural biota, not by predation but by competition for the same resource.

Physical control by water management may increase predation, as in OMWM, or may eliminate predator and prey as when wetlands are drained to soil saturation. Mosquito breeding must be thoroughly documented before new work is done. Because disturbances may displace some species, and because predator species tend to rebound more slowly than their prey, maintenance work should be conducted only when necessary.

#### E. No Program

Another alternative strategy to current mosquito control practice is no control. Many communities in Massachusetts have chosen this option. These towns are usually outside of the enzootic EEE zone so the risk of human diseases transmitted by mosquitoes is viewed as practically nil by these communities. In addition, they are not located near salt marshes and their attendant pest mosquito problems. The mid-section of Massachusetts, where most no-control communities occur, also has a more rural character, less wetland, lower human populations, and a lower mean family income than most eastern areas with organized mosquito control programs. In general, the view of these communities is that the anticipated benefits from mosquito control do not outweigh the anticipated costs and perceived risks.

A more precise way of polling the public and confirming this opinion would be through establishing the Human Annoyance Threshold (HAT) for the town. Communities in which the HAT is below the actual annoyance

level should be persuaded to choose the no control option since justification for control is lacking unless a documented disease threat exists.

In some communities, biting annoyance is created by a combination of biting insects (i.e. mosquitoes, black flies, biting midges and tabanids) which require completely different control approaches. Many people do not recognize the difference between these insects, especially in dim evening light. It is critical to accurately identify the biting insects actually responsible for the human annoyance. In general, black flies and tabanids only cause annoyance during the daytime while mosquitoes and biting midges are most annoying from 6-10 PM. The HAT level for tabanids and biting midges is likely to be less than for mosquitoes because of their more painful bites.

Perhaps part of the reason why Berkshire County communities have supported a MC program while other Western Massachusetts communities have not, lies in the fact that this region supports many summertime outdoor activities (e.g. camps, resorts, golf courses, Tanglewood, etc.). In addition, this more mountainous region has a significant black fly annoyance problem superimposed on top of the mosquito problem. Vacationers are likely to have a lower HAT than permanent residents since they are spending more time and money on outdoor recreational activities.

In addition to risk-benefit considerations, other criteria for weighing the control/no control option are 1) the feasibility of successfully reducing annoyance below the HAT level, and 2) the adequacy of community resources for reducing annoyance to acceptable levels.

Towns with large areas of mosquito-producing freshwater wetland should recognize that effective mosquito control in these habitats is difficult at best and often impossible. As indicated earlier in this report, permanent wetlands do not usually produce large numbers of pest mosquitoes but in situations where they do, the public needs to be aware that these wetlands are a valuable resource that must be protected from significant perturbation and that options for mosquito control are therefore few. Community planning boards and zoning laws can and should be used to restrict residential development and other human activities from such wetland areas.

Towns with annoyance problems but with large land areas and thinly, scattered populations must understand that the same level of mosquito control achieved in more populated communities will cost them significantly more per capita. In many such cases, the economic status of the population means that insufficient tax dollars can be generated to adequately deal with the problem.

Nantucket provides an interesting case study. Mosquito control had lapsed in the early eighties. However, by 1989 saltmarsh mosquitoes were becoming a significant problem in the west and north areas of the island. The town began applying Altosid (including aerial applications of pellets in 1992) to control the mosquitoes while getting the requisite permits for OMWM in several west-end marshes (Madaket, Warren's Landing Eel Point). The OMWM systems were dug in January 1993 (Christie 1993). It was hoped that the OMWM alone would be sufficient to control mosquitoes but large-scale breeding continued in the north end (Pocomo) and the low tidal range has hindered the effectiveness of the OMWM as dug. As a result, some larviciding continues. The conclusion on Nantucket is that mosquito control against saltmarsh mosquitoes must be continued at some level in order to provide residents with the summer environment they want. However, Nantucket does not target freshwater mosquitoes at all and does no adulticiding. Nantucket and Cape Cod together indicate that adulticiding is not a requirement even in high-tourist areas.

It is difficult to measure the impact of choosing the no control option. The example of towns that have left, and left and later rejoined MC projects is perhaps the only available basis for estimating public opinion concerning such impact. No documentation of annoyance levels, cases of disease, recreational dollars spent, etc., was ever attempted in these towns when they had mosquito control versus when they did not. Thus, public complaints were apparently the main indication of the impact of the no control option that was used in guiding town decision making.

In the 1986 questionnaire, only about 10% of the towns in organized projects indicated that there had been controversy relative to leaving, joining, or rejoining a project. Those that had experienced controversy indicated that 4 factors were about equally involved. These were: 1) monetary constraints, 2) concern over the effectiveness of project control programs, 3) concern over the safety of the methods used by the project, and 4) concern over the environmental impact of the control procedures. Paradoxically, 86% of these towns indicated they were unwilling to spend more money (if legally possible) to obtain more effective control and 67% indicated unwillingness to spend more money to support less hazardous control methods. However, the bias of the people filling out the questionnaire sent to each town may have influenced these responses; no town in Massachusetts has actually polled their citizens on these questions. In fact, some towns within projects have taken advantage of new provisions which allow towns to collect additional money for MC activities which projects can then only spend in these towns.

Among towns that actually withdrew from MC programs, monetary constraints played a role in 73% of these decisions, followed by environmental concerns in 32% and concern over effectiveness in 27% of these cases. Towns that contemplated withdrawing but did not do so, indicated that monetary and effectiveness considerations dominated their concern.

The number of towns in MCPs declined in the late eighties. Economic factors, not environmental concerns, were the dominant reason given for withdrawal. This trend has reversed itself significantly in the last several years. The 1990 EEE problem is probably one reason, coupled with the fact that several coastal programs tried the no-control option and found mosquito numbers rose quickly.

Many towns in the Berkshire County and South Shore projects withdrew in 1981 mainly in response to Proposition 2-1/2 monetary constraints. The remaining 8 towns in Berkshire County subsequently chose to continue as a multi-town Project (Note: the town of Lanesborough rejoined in 1986, the city of Pittsfield rejoined in 1988, and other towns are contemplating reentry). South Shore actually disbanded as a project but by 1988 most towns had joined neighboring MC projects in Norfolk and Plymouth Counties. These actions suggest that these communities were not content with the no control option that resulted from the disbanding of their former project. Towns that voluntarily withdrew from MC projects but then later rejoined a project, did so for a variety of reasons. These were in order of their importance: increased public support for MC, increased mosquito annoyance, alleviation of monetary constraints, threat of EEE, improved methods and effectiveness of the project.

The Environmental Impact Statement (Sjogren 1977) prepared for the Metropolitan Mosquito Control District in Minnesota attempted to quantify the no control option for their community. Any attempt to develop similar estimates for Massachusetts would be meaningless given the lack of appropriate baseline data.

## B. Biological Control.

### 1. Introduction

Biological control includes attacks on the pest species by other species and manipulation of the pest species itself. The former includes the traditional biological control agents, predators, pathogens and parasites, whereas the latter includes such techniques as sterile-male release and genetic manipulations.

Biological control agents are grouped into three categories: predators, parasites and pathogens. Predators include both vertebrates and invertebrates and may attack both adult and immature stages of mosquitoes. Helminth, protozoan and fungal parasites and microbial pathogens generally only invade immature stages, though mortality may not occur until the early adult stage. Parasitic water mites are an exception in that they attach to certain adults as they emerge from the pupal stage and apparently reduce adult survivorship if they are abundant (Lanciani & Boyett 1980) In general, biological control is much more feasible in managing permanent water mosquitoes than temporary water forms.

There are three basic strategies for utilizing all biological control agents: (1) increasing existing natural enemy populations by habitat alteration, (2) one-time introduction of sustainable exotic agents from other regions or habitats, and (3) augmentation of natural or exotic enemy populations by repeatedly releasing non-sustainable, lab-reared (or field collected) organisms. To date only the first, increasing fish habitat through OMWM, has been used in Massachusetts. Bti is sometimes classified as a biological control agent but its application technique and mode of action as a stomach poison more closely resemble a pesticide than a biological control agent *per se*. *Bacillus sphaericus* may more closely fit the model of repeatedly releasing non-sustainable lab-reared organisms as there is evidence to suggest that it recycles within the environment.

No other biological control agent is currently available for general field use, though experiments continue with many different organisms (see below). There are important reasons why biocontrol is not more widely used against mosquitoes. First, the differences in biology of the various species of mosquitoes make it unlikely that any one control agent will operate across a wide range of species. Second, mosquito breeding is wide spread, making it difficult for a biological control agent to find, or be placed in, all breeding areas. Third, predators such as bats and purple martins, may eat mosquitoes but prefer to eat other, larger insects. Further, even when abundant, they do not drive mosquito populations below levels that people generally find intolerable. Finally, there is a high cost



associated with sustained releases of a biological control agent and there are not, at this time, control agents available that require a single, or a few, releases to become established.

Because of the limited application of biological control to mosquitoes in Massachusetts, the following discussion will focus primarily on the feasibility of control agents currently being studied for mosquito control. An important point to make regarding biological control is that the mosquito control have limited research capabilities. While conducting field evaluations of new control techniques is a desirable practice for any mosquito control program, the projects should not be expected to find and bring forward biological control agents without substantial help from research institutions such as the state university.

## 2. Predators

### a. Introduction

In order for any predator to independently be an effective control agent, it must meet two important criteria: (1) its size and abundance in relation to the target species must be sufficient for it to kill or consume a high percentage of the prey population within a relatively short time period, and (2) its feeding behavior should be selective toward the prey species when it is present but allow it to utilize other food items when the target species is absent. These criteria are rarely met in full. Predators that are sufficiently large and/or abundant to have a major impact, usually lack feeding specificity. Conversely, those with feeding specificity are usually less abundant because their populations are regulated by a more restricted food supply. Vertebrate predators of insects have a clear size advantage but invertebrate predators tend to exist in much greater numbers.

Because of the limitations of predators as biocontrol agents, it is normally essential to continuously raise and release the predator to achieve field densities high enough to cause real reductions in the prey species. However, in some cases, manipulation of the environment to the advantage of natural predator populations can provide an adequate augmentation effect.

### b. Vertebrate predators

#### Fish

Larvivorous fish are the oldest and perhaps most effective traditional biological control agent used against mosquitoes. In certain habitats they may, by themselves, provide adequate larval control throughout the breeding season. If not, pesticides such as BTI or methoprene which are non-toxic to vertebrates can be used in an integrated fashion with fish.

As already mentioned, the main reason open saltmarsh management strategies effectively control many saltmarsh *Aedes* in the Northeast is because this method provides access for the abundant estuarine populations of larvivorous killifish (*Fundulus* spp.) into the mosquito breeding pools in the high saltmarsh (Hruby & Montgomery 1986).

The mosquitofish *Gambusia affinis* is distributed widely throughout the warmer parts of the world and is the species most often reared and introduced into fresh water habitats for mosquito control. The biology and use of this fish in mosquito control was reviewed by Meisch (1985). It is an opportunistic feeder and avidly eats pupae and late-instar larvae of culicines and chironomids. It is most effective against *Culex* in unvegetated, permanent ponds but has been widely used in California and the Gulf States against ricefield *Aedes* and *Psorophora*.

Because *Gambusia* is so aggressive and fecund, it may replace important commercial or rare native fish species. This has raised environmental concern over the introduction of this fish into natural waters where it does not already occur. A recent article by Rupp (1996) has renewed this debate, both emphasizing real successes and real concerns over *Gambusia* use (see "Comments on 'adverse Assessments of *Gambusia affinis*'" (JAMCA 1996) and Boklund 1997, Eliason 1997, and Rupp 1997). Because it is not a native species of Massachusetts it may not be imported and released in state waters.

Outside of the mosquitofish, the common guppy (*Poecilia reticulata*) has received the most attention for mosquito control (Bay 1985). Comparative studies indicate that it is a less effective predator than *Gambusia* but it is more tolerant of polluted waters. Many other native fish have been explored for their mosquito control potential (Bay 1985). Studies in North Africa (Allo et al. 1985) suggest that malaria may be controlled through the annual introduction of native fish from streams into the manmade water storage tanks which produce the vector *Anopheles* in this region.

### Birds

Many birds depend on insects as food and those which capture insects on the wing (e.g., the swallows), have been credited with consumption of significant numbers of mosquitoes. Purple martins specifically have been promoted on the basis of the claim that they often consume 10-12 thousand mosquitoes per day but Kale (1968) concluded on the basis of existing evidence that all claims of martins significantly reducing mosquito populations were unsubstantiated and, because of several biological facts, were unlikely to ever be demonstrated. The facts on which these conclusions were drawn are as follows:

- (1) Mosquitoes were a negligible item in the diet of martins in the only two published studies in which the contents of martin gizzards were examined.
- (2) None of the published statements appearing in the popular ornithological literature which attributes a mosquito-eating habit to martins is based on factual study or scientific reference. In fact, there is evidence that martins feed more on larger insects including species of dragonflies which may be predators of adult mosquitoes.
- (3) Behavior patterns of mosquitoes and martins are such that they tend to not fly at the same height or at the same time. Thus, contact between the two is minimal.
- (4) There is no evidence that any avian species can effectively control a pest insect upon which it feeds when the insect is at or near peak abundance.

#### Other Vertebrates

Mosquito control by insectivorous bats was tried in the early part of this century but without success (Kale 1968, Storer 1926). Bats continue to appear in the popular press as legitimate mosquito-control agents (Wright 1996) but are not considered worthwhile agents in Common-sense Pest Control (Olkowski et al. 1991), which is very thorough in its coverage of non-chemical control options, or in mainstream mosquito control (Mitchell 1992).

#### c. Invertebrate predators

##### Predators of mosquito eggs.

Evidence exists of predation on diapausing flood water mosquito eggs by mites and beetles, and on *Culex* egg rafts by fish (Collins & Washino 1985). Nonetheless, egg predation appears to be a relatively minor component of natural mosquito mortality and is not being studied for biological control.

##### Terrestrial insect and spider predators of mosquito adults

Predation on emerging and indoor resting adult mosquitoes has been readily observed but the impact of this mortality on populations is extremely difficult to assess. Certain spiders (especially Tetragnatha) and predatory flies (mainly Empididae, Anthomyiidae and Dolichopodidae) have been shown by precipitation tests to have consumed emerging mosquitoes (Collins & Washino 1985). In one British study, up to 28% of the spiders tested had eaten mosquitoes (Service 1973). Certain adult dragonflies reportedly capture mosquitoes on the wing but these observations have not been backed up by any controlled field studies. Synanthropic emesine bugs (Reduviidae) appears to be potentially important predators of indoor resting mosquitoes in the tropics. In sum, the prospect for

enhancing or managing invertebrate predators for more effective adult control is not encouraging, especially in temperate regions.

#### Aquatic insect predators of mosquito larvae & pupae

Aquatic insect predators seldom occur in significant numbers in the temporary floodwaters that produce most pest mosquitoes. Studies of predation have therefore largely taken place in permanent ponds or semi-permanent habitats such as rice fields, rock pools or vernal woodland pools. The adult stage of most predaceous aquatic beetles and true bugs can fly (usually at night) so natural colonization of newly flooded habitats can occur in a matter of days. Development time for these insects is usually from several weeks to several months.

Among the beetles, the dytiscids (predaceous diving beetle), which are predaceous both as larvae and adults, are the most effective predators of mosquitoes. There is evidence from studies in rice fields that adult dytiscids selectively locate and colonize sites with locally high concentrations of mosquito larvae (Collins & Washino 1985). Mass production methods for dytiscids have never been developed. Whirligig beetles (Gyrinidae) only feed at the surface where they may prey on concentrations of emerging adult mosquitoes. Hydrophilids are only predaceous as larvae and seem to feed mainly on chironomid midges.

Only two aquatic families of true bugs, the back swimmer (Notonectidae) and pigmy back swimmer (Pleidae) have received serious consideration as mosquito control agents. Pleids are generally not abundant enough to have significant impact but notonectids can become quite dense in certain habitats. Mass rearing of the latter appears to be possible. Water boatmen (Corixidae) are also common and similar in appearance to backswimmers but they are mostly detritus feeders. Other predaceous aquatic Hemiptera that have been suggested as mosquito predators but which normally occur in insufficient densities to have much value as natural control agents include the giant water bugs (Belostomatidae), water scorpions (Nepidae), water measuring bugs (Hydrometridae) and the two family of surface-feeding, water striders (Gerridae and Veliidae).

Dragonfly naiads have been marketed commercially for mosquito control and at least one town in Massachusetts, and others in New Hampshire and Maine, have purchased dragonflies for mosquito control. In northern climates these insects require 1-5 years to mature, so they normally occur in permanent waters only. Furthermore, many are bottom feeders that seldom if ever come in contact with mosquito species that feed at the surface or in the water column. Most bottom-feeding mosquitoes (i.e. *Aedes*) occur in temporary water containing few if any dragonfly naiads. Another factor weighing against the mosquito control efficiency of these aquatic

predators is the fact that they normally occur at low densities. Adults of many species are territorial and this seems to spatially limit population densities of naiads as well as adults. In certain habitats such as rice fields, naiads may become quite abundant but populations fluctuate greatly and their role in limiting populations of rice field mosquitoes is limited at best. No controlled, field studies have been done in which naiads have performed well as biological control agents.

The trichopteran *Limnephilus indivisus* may be an important predator of early spring *Aedes* in woodland swamps in Ontario but most caddisflies are omnivorous shredders rather than predators (Collins & Washino 1985, Merritt & Cummins 1985). Prospects for mass rearing and manipulating caddisfly larvae are not very bright. Many of the aquatic nematoceran relatives of mosquitoes contain groups with predaceous larvae. These include the families Chaoboridae, Chiromomidae, Ceratopogonidae, Tipulidae, Anthomyiidae, and others. Most are too small to consume many mosquito larvae. Predation on small, early instars occurs but it is far less efficient in reducing the numbers of adults than is predation on late instars and pupae. *Mochlonyx* (Chaoborid) predation on spring *Ae. communis* populations has been observed in woodland pools in Massachusetts (Edmans, personal communication) and in Europe (Chodorowski 1968). The impact of this small but voracious predator is unknown.

The insect predator with the most promise in mosquito control is another mosquito. Larvae of the non-biting genus *Toxorhynchites* are large and effective predators of mosquitoes that develop in natural and man-made containers such as tires, tree holes, metal cans, and leaf axils (Steffan & Evenhuis 1981). Unfortunately, none of the 70 some species in this mainly tropical genus can survive the winters as far north as Massachusetts. Their use here would therefore require repeated, annual releases of lab-reared females. This is not warranted at the present time since container-breeding species generally do not represent the major nuisance or health threat in Massachusetts. This situation could change if *Ae. albopictus* becomes well established in used tires in the Northeast. Focks (1985) states that although it is possible to control or reduce certain species of container-breeding mosquitoes with inoculative or inundative releases of *Toxorhynchites*, the usefulness of this genus in practical, operational control programs has yet to be demonstrated.

#### Other invertebrate predators of larvae and pupae.

Among the non-insect predators of the immature stages of mosquitoes, only hydra (Cnidaria: Hydrozoa), flatworms (Platyhelminthes: Turbellaria) and copopods (Cyclopoida) have been studied in any detail.

Both hydra and flatworms can be easily mass produced and, unlike most predaceous insects, they can be maintained at high densities without cannibalism. In the laboratory, they kill far more larvae than they consume. Both groups produce semi-dormant eggs so they occur naturally in shallow temporary pools as well as permanent swamps and ponds. Detrimental effects on young fish have been reported when these predators are at high densities (Mulla & Tsai 1978).

Both of these predators have good potential as control agents in the Northeast but additional long-term field evaluations are needed. As with mosquito fish, the laboratory production, storage, and field translocation of these organisms requires a certain degree of sophistication, which is usually lacking at the local level. Currently, there are no commercial sources for the quantities that would be required for mosquito control applications.

Natural populations of predaceous cyclopoid copepods appear to limit the distribution of container breeding mosquitoes in certain tropical settings (Marten 1984). However, they have never been shown to be important predators in temperate regions or in other types of aquatic habitats. Therefore their potential for the biological control of pest and vector mosquitoes in Massachusetts appears to be nil.

### 3. Parasites and Pathogens

#### a. mermithid nematode parasites

Outside of bacteria, parasitic nematodes are the only natural parasites and pathogens that have ever achieved operational status in mosquito control. Known parasitic roundworms of mosquitoes now number over 20 species. The free-living, aquatic, preparasitic stage which hatches from the nematode egg, seeks out and penetrates the cuticle of host mosquito larvae. Larvae are usually killed in the last instar. In a few species, the mature worms are carried over in the adult mosquito and cause mortality when they exit during attempted pseudo-oviposition.

*Romanomermis culicivorax* (including most references to *Reesimermis nfelsenii*) is the species that has been most extensively studied. Methodologies for the mass production and commercial preparation of this species have been developed and it was briefly marketed as Skeeter Doom in the late 1970's. Low sales and problems with the shipping and shelf-life of viable eggs appear to have been the major factors which led to the marketing failure of this agent (Service 1983). Other economic drawbacks include host specificity which limits its effectiveness to only certain species (e.g., it is ineffective in cold, polluted or brackish water), and the lack of patent protection for companies investing in the production and marketing of this product. In addition, applicators need some special skill and training to effectively use this biocontrol agent. The tendency of this agent to naturally recycle once it is

introduced into favorable aquatic habitats is beneficial from a control viewpoint, but it further reduces the long-term marketability and profit potential for private producers. It remains under study, however, as a recent article (Mijares et al. 1997) discussed the establishment of *R. culicivorax* in sewage settling ponds and natural ponds in Cuba.

On the biological plus side, mermithids appear to be highly compatible with a wide range of chemical pesticides and growth regulators. Moreover, they: 1) are non-specific and well suited to the life cycle of their mosquito host, 2) produce high levels of infection and mortality, 3) can be easily mass-produced and applied with standard spray equipment, and 4) offer no threat to non-target organisms or the environment.

There are species of mermithids which appear to be highly specific to spring snow-pool *Aedes* and to saltmarsh *Ae. sollicitans* (Petersen 1985). However, these worms have not been established and studied in the laboratory. Such species may have greater control potential in northern coastal states like Massachusetts than the more tropically adapted *R. culicivorax*.

Since the technology for using mermithids in mosquito control already exists, and there are numerous field trials documenting their control potential, the future availability and use of these biocontrol agents in operational programs seems to depend on changing economic and market forces (Petersen 1985).

protozoan parasites

#### b. Microsporidia

Virtually all mosquito species carefully examined have been found to harbor these parasites. Larvae are infected by ingesting the spore stage. Spores, which are produced at the end of the life cycle, have often proven difficult to induce and to reinfect larvae in lab cultures. Few microsporidian life cycles are well enough known to assess biocontrol potential. Life cycles vary from simple to complex and often form the basis for the non-taxonomic grouping of these protozoan parasites. The simplest forms (Type I) occur mainly in terrestrial insects and even the one aquatic species known from mosquitoes (*Nosema algerae*), does not cause mortality until the adult stage. For this reason *N. algerae* has limited potential for reducing pest problems but may impact on disease transmission by reducing survival and fecundity. Wild strains only persist in larval populations for short periods and cause little direct mortality. Type II microsporidia have simple, asexual life cycles similar to Type I forms and they also show little promise in mosquito control (Hazard 1985). Type III forms are dimorphic, have binucleate spores, and kill mosquitoes in the larval stage. Only one species (*Hazardia milleeri*) is known from mosquitoes and it seems to

have low infectivity (Hazard 1985). Type IV microsporidia include many species from mosquitoes and have the most control potential. Infected larvae are usually killed but a few females survive and carry the infection via the ovary to the next generation. However, transovarial infection ceases after a single generation. Non-ovarian modes of transmission must exist but this issue along with the possibility of sexual reproduction and alternate hosts are as yet unresolved. Additional basic research is required before any microsporidian can be considered in the biocontrol of mosquitoes (Hazard 1985).

#### *Tetrahymena* and *Lambornella* (tetrahymenid ciliates)

The lack of a resistant resting stage and difficulty in culturing these ciliated protozoa has prevented the thorough study and evaluation of this group of potential biocontrol agents. Ongoing studies of *Lambornella clarki* in California treehole *Ae. sierrensis* should help to better assess the control value of these parasites in the near future (Anderson et al. 1986).

#### *Helicosporidium*

There is still debate concerning the proper classification of this spore-forming group of parasites; some believe they are primitive Ascomycetes fungi. There are only 3 reports of natural infections in mosquitoes. They appear to infect a wide range of species but also may infect non-target insects as well. High dosages of spores are required to infect older instars in the lab. Continuous cultures of these parasites do not exist so the basic research needed to evaluate their biocontrol potential is not forthcoming.

### c. Fungal Pathogens

#### *Coelomomyces*

Many forms of pathogenic fungi have been known from both larval and adult stages of mosquitoes since the 1930's. However, it was not until the discovery of the obligate alternate host (i.e., microcrustacea) in the mid-70's that cultures and full-scale laboratory investigations were possible with *Coelomomyces* (Whisler et al. 1974, 1975). The rather complex life cycles of some species have recently been described and methods for in vivo culturing established. Before wide-scale field application can occur, mass in vitro cultivation of the infective biflagellate zygote stage needs to be developed. If *Coelomomyces*, and perhaps their copepod and ostracod intermediate hosts, are introduced into favorable habitats, the potential for natural recycling exists. However, too little is known about the host ranges and habitat requirement of most *Coelomomyces* to recommend any such introduction at the present time. Moreover, species of *Coelomomyces* do not routinely provide high and predictable



levels of mosquito control. It is, premature to critically assess their potential as control agents to be used independently or as part of an integrated control program (Federici et al. 1985). Studies related to their evaluation as operational control agents have only recently been initiated and it is not likely that any *Coelomomyces* will be operational within the near future.

#### *Lagenidium giganteum.*

This mosquito-specific water mold is a very promising biological control agent, especially in fresh water and in warmer climates. This fungal pathogen can be mass produced on artificial media and can recycle in as little as 3 hours (McCray 1985). It has a resistant, dormant stage and infects a wide range of mosquito species. Unfortunately, it is not effective in polluted, brackish or cold waters. This limits its commercial value and its potential usefulness in northern and coastal states like Massachusetts (Service 1983).

#### Other Fungi

Fungi are among the commonest pathogens of insects and many other genera besides *Coelomomyces* and *Lagenidium* have been reported from mosquitoes. Of these, *Culicinomyces* and *Metarhizium* have received the most attention. Both groups infect a wide range of mosquito species but relatively high concentrations are required to cause infection. They can tolerate organic pollution and salinity but not high temperatures (i.e. above 30°C). These fungi can be grown on inexpensive artificial media but no resistant resting stage has been found. Difficulty in achieving long term storage of infective stages and formulation problems remain as barriers to commercial production. Nonetheless, these fungi are a promising group of biocontrol agents which may provide new mosquito control tools in the future.

#### *Erynia aquatica*

*Erynia aquatica* is an Entomophthorales fungus, and is the only species of the genus known to infect the immature aquatic stages of mosquitoes. It was first discovered infecting *Ae. canadensis* and *Cs. moristans* larvae in woodland pools in Hartford County, Connecticut from late May to early June (Anderson & Ringo 1969). It has since been recovered in early May from *Ae. stimulans* in woodland pools near the village of Cambridge, New York (Molloy & Wraight 1982) and from *Ae. cantator* on May 21, 1981 in a shallow salt meadow pool in Milford, Connecticut (Andreadis & Magnarelli 1983). Most recently, it was discovered in 1995 and 1996 in a woodland pool in Bristol, Rhode Island (Christie 1997).

Steinkraus and Kramer (1989) collected *Ae. fitchii* larvae infected with *E. aquatica* from a semi-permanent woodland pool in Tompkins County, New York. They used infected pupae to successfully transmit the disease to emerging adult *Ae. aegypti*, on which resting spores of the fungus developed.

*Erynia aquatica* has characteristics which make it attractive as a microbial agent: it is capable of causing epizootics, has been found in both freshwater and brackish water mosquitoes and has a resting spore stage that may survive well in storage. Operating against it is the fact that it has only been found in cooler, springtime waters. One thought is that infected pupae may be removed from the original infestation site and placed in other, nearby pools. A fungus that kills in the pupal stage works against its own spread.

d. Bacterial pathogens

*Bacillus thuringiensis* var. *israelensis*

Bti Serotype H-14 has become an important biological larvicide following its discovery in the Negev desert in 1976. Within the last 5 years it has become widely used by the mosquito control projects in Massachusetts. It is now the larvicide of choice in many situations because of its host specificity, high and rapid mortality to many mosquitoes species, and its environmental safety. It is quite distinct from the Bt strains which infect lepidopterous insects. Its track record in controlling polluted-water *Culex* is mixed, apparently because it sinks to the bottom and the active moiety rapidly binds to organic particulates. Consequently, higher dosages are required to achieve good control in highly organic and deep-water situation. Saltmarsh mosquitoes generally require rates at the high end of the labeled application rates for effect control. Liquid, powder, granular and slow-release briquet formulations are commercially available.

Bti must be ingested to cause toxicity to filter-feeding mosquito larvae; pathology occurs in cells of the midgut wall. It is least toxic to 4th instars since they cease feeding at least 12 hours prior to pupation. The mosquito toxic ingredient of Bti preparations is the heat labile deltaentotoxin located in the proteinaceous parasporal crystalline inclusions synthesized concomitantly with the spore during sporogenesis. Once released in the environment, it biodegrades rapidly (it is usually only active 1-3 days) and this bacterium (gram negative Bacillaceae) does not recycle. This is considered the only major drawback of this highly effective mosquito pathogen but it has enhanced its commercialization (Lacey & Undeen 1986).

The biocidal activity of Bti toxin appears to be limited to larvae of certain families of nematoceros Diptera. A large number of laboratory and field tests have confirmed that all non-nematoceros, non-target

organisms are virtually unaffected (Lacy 1985). All existing data indicate that the unaltered protoxin of Bti is also safe to vertebrates including humans (Lacey 1985). Further improvements in the efficacy and price competitiveness of this control agent, brought about by formulation changes and genetic engineering, are likely to occur in the near future. In addition, formulation of this agent with other compatible and perhaps synergistic agents such as juvenile hormone analogs (e.g. Altosid) is currently underway. Such mixture have two important advantages. They reduce the likelihood of mosquitoes developing resistance to either agent and, secondly, they widen the window for control since Bti is most effective against early instars and growth regulators against later instars.

*Bacillus sphaericus.*

Although only recently available, *B. sphaericus* may have greater control potential than Bti because of its ability to continue to recycle once it is introduced. It has many of the same beneficial attributes of Bti but all strains isolated to date are less effective against a wide range of species than is Bti. At the moment it is primarily marketed against *Culex* in high-organic waters but research is on-going in expanding its control potential.

e. Viral Pathogens

The biological control potential of both non-accluted (iridoviruses and densovirus) and occluded (baculoviruses, cytoplasmic polyhedrosis viruses and entomopox viruses) viruses pathogenic to mosquitoes has recently been reviewed by Federicci (1985). It is sufficient here to simply paraphrase from the summary of this thorough review as follows:

Analysis of research conducted to date makes it clear that none of the viral pathogens of mosquitoes can currently be considered good candidates for mosquito management programs. The two main reasons for this assessment are that viruses discovered so far possess low infectivity for original or alternate hosts and there is no readily available method for mass production of virions. This apparent lack of control potential is most obvious when one considers that few field trials have been conducted with these agents even though the first one was discovered over 15 years ago. While it appears unlikely that viruses will be developed for mosquito control in the near future, they may prove to be extremely useful microbial agents in the long term, perhaps in 2 or 3 decades, once we learn how to manipulate them effectively. Biotechnology has the greatest potential for engineering new more useful biocontrol organisms among the viruses because of their simple molecular structure.

4. Pest Species Manipulation

A somewhat different approach to biological control is manipulation of the mosquito genome to either 1) induce reproductive failure (leading to population elimination) or 2) bring about permanent changes in the behavior or physiology of the target population so it no longer vectors disease or bites people. There are numerous theoretical mechanisms by which this could be accomplished. Following is a brief discussion of the three approaches that have been most commonly discussed and researched.

a. Sterile Insect Technique

The early and continuing success of the sterile male release program in eliminating the screwworm fly from the U.S. and Mexico has given rise to many investigations and new ideas for controlling other insects (inoculating mosquitoes) through the use of this or some other genetic technique. Except for fruit flies, this method has not yet been successfully applied to other insects in operational programs and the technique is not currently being pursued for mosquito control.

b. Incompatibility

Incompatibility resulting from a lack of fertility in sexual unions may occur due to a variety of genomic failures or due to the effort of bacteria-like symbionts to control the reproduction of host (Barr 1985). The feasibility of suppressing *Culex pipiens* through cytoplasmic incompatibility was demonstrated in a Burmese Village over 20 years ago (Laven 1967) but the practicality of this method has not been confirmed by any other field tests. Moreover, incompatibility factors have been isolated in only a few mosquito species to date.

c. Chromosomal Aberrations

There are several heritable chromosome rearrangements that can, in theory, be used to inject genetic load into a mosquito population and/or to effect a permanent change in the genetic makeup of the population. These aberrations can be used to 1) increase genetic lethal load (serility is limited to 50-80% because of the low chromosome number (three, in most mosquitoes) or 2) replace noxious species with harmless strains if appropriate viable homozygous rearrangements are available. The latter is perhaps only applicable in the case of important disease vectors. Naturally occurring aberrations can be screened for in wild populations but their frequency is greatly increased through exposure to mutagens.

Controlling mosquitoes through the use of chromosomal aberrations requires a major basic research effort and a target species that is easily colonized. This latter requirement automatically eliminates many important species from consideration.

d. Competitive Displacement

The final, and somewhat abstract, strategy for controlling mosquitoes biologically is the ecologically based notion of displacing a noxious species by introducing a benign but more competitive (i.e. better adapted) exotic one. This idea has been suggested primarily to control container-breeding species like *Ae. aegypti*. However, benign mosquito species are difficult to find, as most mosquitoes that have become established (*Ae. aegypti*, *Ae. albopictus*) are as bad or worse than the species with which they compete (container-breeding *Culex* and *Ae. triseriatus*).

5. Other Control Approaches

a. Trap out techniques. All experience to date indicates that while various traps may be good sampling devices for adult mosquitoes, they are too inefficient and limited in their range to provide any benefit in reducing biting annoyances (Nasci et al. 1983). Work is ongoing on attractants (octanol) that would both greatly increase trap collections of mosquitoes and reduce non-targets trapped, but no products are marketed for this purpose to date.

Electrocutor traps ("Bug Zappers") continue to be a popular item, with an estimated 1.75 million sold in the United States each year (Mitchell 1992) but they are extremely non-specific (mosquitoes generally make up less than 5% of the catch, and may be harmful to other insect species. They cannot be considered a part of any mosquito-control program.

b. Repellents. Personal protection through the use of mosquito repellents is an appropriate alternative to controlling the mosquito populations before they bite. This is especially true if the periods of annoyance are infrequent and brief and where the land areas are too vast and unpopulated to economically consider control programs.

The most commonly used mosquito repellent is DEET (N,N)(diethyl-metatoluamide) which has been formulated and sold under a variety of trade names (e.g., Off, Muskol, Cutters), in a variety of concentrations and as both aerosol sprays (usually ca 15%) and lotions (up to 100%). Laboratory tests have shown that maximum repellent duration (ca. 1-2 hours) is obtained with a concentration of ca. 50% so that higher concentrations do not provide appreciable advantages. The major disadvantage of DEET are:

1) relatively short protection time

2) somewhat offensive odor

- 3) damage to some fabrics and surfaces at higher concentrations
- 4) high cost
- 5) possible hazards from heavy use

Small children frequently have skin reactions to DEET. Small children are also most likely to be the individuals that develop neurotoxicological symptoms from DEET. Thirteen of 14 cases of encephalopathy (toxicity of the central nervous system), found in publicly available reports by a recent review (Osimitz and Grothaus 1995), were in children 8 years old or younger. Three of these children died, all having used “heavy” amounts of repellent, even though the repellent in each case had DEET concentrations of 20% or less. Oral ingestion may have played a role in some of the cases. Osimitz and Grothaus (1995) concluded that there is no evidence that increased DEET concentration has an effect on the severity of symptoms. They also compared reports that the Poison Control Center received for DEET (6,724 in children under 6 years old during a five-year period) to laundry detergent reports (10,789 in 1989) and household bleach (16,169 in 1989), concluding that accidental exposure, while undesirably high, is in line with, or lower than, exposure to other household chemicals.

There is one unsubstantiated report in the Russian literature of carcinogenic effects in rats at high doses.

Three other repellent materials are in common use. Dimethyl phthalate (generally sold as 6-12) is not as effective as DEET against mosquitoes but still has a share of the market. Citronella-based repellents have long been marketed as candles or in oil-burning lamps. Citronella is also available as a repellent to be sprayed on skin and clothing (Natrappel). The third material (Avon skin-so-soft) is a popular bath oil that is not marketed as a mosquito repellent but has, through word of mouth, been recognized for its as yet uncharacterized mosquito repellent effect. It is as effective as DEET but it does not persist as long. On the other hand, it is much cheaper, smells better, and apparently does not damage any fabrics or surfaces. The active ingredient(s) of skin-so-soft has not been isolated or identified to date. It also has not undergone the EPA safety testing that other repellents have because it is marketed as a beauty aid rather than a pesticide (Note: repellents are classified as pesticides by EPA).

Electronic mosquito repellent devices which are periodically marketed in the U.S. (usually by mail order houses) are completely ineffective and are not based on any biological rationale (Foster & Lutes 1985, Mitchell 1992, Curtis 1994).

## C. Physical Control

### 1. Types of Habitat Modification

a. Open marsh water management (OMWM)

Originally developed for New Jersey salt marshes (Ferrigno 1970, Ferrigno & Jobbins 1968, Ferrigno et al. 1969), this strategy basically attempts to overcome the limitations of ditching by the incorporation of other water management strategies. In particular, champaign pools or reservoirs (which permanently hold water and sustain larvivorous fish) are created (by backhoe, dragline or rotary ditcher) in selected tidal pools or large shallow pans and are connected via small shallow ditches to surrounding mosquito breeding depressions. If old ditches are re-dug and used as reservoirs, then plugs must be inserted at the tidal end to prevent drainage. This customized approach to marsh management represents the least deleterious and most efficient nonpesticidal method for controlling saltmarsh mosquitoes and has been adapted to New England conditions (Boyes and Capotosto 1980, Hruby et al. 1985, Christie 1990). A manual outlining this method was developed by the Massachusetts Audubon Society (Hruby and Montgomery 1986) and OMWM is currently being practiced by coastal projects in Essex and Plymouth Counties in Massachusetts. As of 1996, OMWM had become the accepted technique for new salt-marsh water management work, though maintenance work remains dominated by cleaning existing ditching, as opposed to conversion to OMWM.

b. Other Modification Strategies

Mosquito control efforts in Massachusetts predate modern chemical insecticides. Early control efforts consisted of source reduction work, mostly in salt marshes (see "History of Cape Cod Mosquito Control Project, 1928-1971"). This emphasis was largely abandoned when cheap and seemingly more effective organo-chlorine insecticides became available in the early 1950's. Early control programs capitalized on cheap WPA labor but they failed to achieve the level of control that the public has come to expect of modern control programs. Nonetheless, they serve as a reminder that mosquito control, from its earliest inception, considered and practiced control alternatives to synthetic chemical pesticides.

Except for new OMWM projects in salt marshes, mosquito control source reduction work now consists primarily of maintenance work on existing culverts, storm sewers and ditches. Very little new ditch construction has taken place in recent years. Ditch cleaning, which often involves excavating spoil with a backhoe or plow, is an activity which has drawn great concern when it takes place in estuarine environments. This is because many of the old grid ditches in the saltmarsh served no real purpose for mosquito control but they must be re-cleaned periodically or they themselves become shallow breeding areas for saltmarsh mosquitoes.

c.        Origination of Requests for Physical Control.

The exemption from certain regulations enjoyed by mosquito control is a two-edged sword. On the one hand, it enables mosquito control projects to more quickly respond to drainage problems. On the other hand, it makes the heading “Mosquito Control” particularly desirable for drainage projects in which mosquito control is, at best, a marginal goal. This pressure can come from property owners, public officials, or from within control projects themselves. The pressure to conduct drainage work that does not have a mosquito-control component must be resisted.

The best interests of mosquito control programs are served by conservative application of the definition of mosquito control, as over-use of the wetlands exemptions may result in the loss of those exemptions. To this end, mosquito control programs, in conjunction with state and federal wetlands protection agencies, must develop a strong set of guidelines for alterations exempt from Wetlands Protection Act.

2.        Ecosystem changes of non-target biota as a result of physical controls.

a.        Salt Marsh .

New England coastal wetlands have been heavily impacted by man (Shisler 1990). However, evidence concerning the negative impact of saltmarsh ditch maintenance activity is mixed. The principal concern is with disposal of the spoil on the marsh and the invasion of upland plants that can occur with even slight elevation increases (i.e. 1-2 inches) (Miller and Egler 1950, Buchsbaum 1994). Ditching also permits *Spartina alterniflora* to invade the upper marsh (dominated by *Spartina patens* and *Distichlis spicata*) along the edges of the newly created ditches. There is evidence to support the claim that this increases marsh productivity (Buchsbaum 1994).

In 1979, staff biologists from the New England Division of the U.S. Army Corps of Engineers (DeSista & Newling 1979) carried out preliminary investigation in several Massachusetts salt marshes to explore the issue of spoil deposition and upland plant invasion. In many instances they found little plant invasion despite evidence of previous spoil deposition of 2 inches or more in depth on the marsh. In a few locations with minimal tidal influence, some invasion by species such as common ragweed (*Ambrosia artemisiifolia*), march elder (*Iva frutescens*), and seaside goldenrod (*Solidago semprevirens*) had occurred over time. However, they concluded that it was not obviously correlated with the spoil itself but was perhaps caused by some other factor associated with the disturbance to the marsh. They recommended the sort of long-term monitoring studies which have as yet not been carried out in Massachusetts salt marshes.



Clarke et al. (1984) studied the effect of ditching and vegetation changes on the use of the saltmarsh by birds in Rowley, Massachusetts. They concluded that bird use of the marsh was negatively impacted by mosquito control ditching. This is in contrast to the studies of Shisler & Jobbins (1977) in New Jersey marshes where increase productivity was observed in ditched marshes. Daiber (1986) noted that, where ditches drain pannes, birds that need a constant water supply (American bittern, pied-billed grebe and American coot) may decline. Also noted was a case where spoil ridges invade by *Baccharis* and *Iva* caused gulls to seek less brushy areas for nesting. Scheirer (1994) encouraged mosquito control programs to develop water management partnerships with the U. S. Fish and Wildlife Service, especially for OMWM-type marsh restorations designed to improve migrant waterfowl habitat.

In a series of investigative reports by staff biologists from the National Park Service, the environmental impact of ditching and diking of salt marshes in the Herring River basin of Cape Cod National Seashore were investigated (Soukup & Portnoy 1983, Portnoy 1984a, 1984b). These reports held that Cape Cod marsh management practices were responsible for the freshening, stagnation, acidification and high sulfate and aluminum concentration in diked marshes. These authors suggest that the destruction of a thriving eel and herring fishery in Wellfleet was the direct result of these marsh disruptions. However, the main dike across the mouth of the Herring River (from Griffin Island to the Mainland) was constructed in 1909 with the main objective of providing for agricultural use of the basin. These structures are not typical of early mosquito marsh management practices in other coastal regions in Massachusetts. The impact may be largely reversible if dikes are removed and normal tidal flow is allowed to return to these areas. OMWM tailored to the unique characteristics of these small estuaries should be explored as a way to manage the *Ae. sollicitans* populations which are likely to replace the present *Ae. cantator* populations if tidal flow and normal vegetation and salinity are restored in these marshes.

Perhaps the most damaging assessment of ditching in salt marshes was the report of Bourn and Cottam (1950) in which they blamed open ditching for converting up to 90% of the *Spartina alterniflora* marsh along the Mispillion River in Delaware to *Baccharis*. However, Provost (1977) reported that the area had returned to *Sp. alterniflora* after navigational dredging of the river had ceased and concluded that it was the dredging of the main channel, not the marsh ditching, which had permitted *Baccharis* to invade the marsh (see Daiber 1986 and Buchsbaum 1994 for reviews of this debate). Arguing against significant water-table lowering in salt marshes is the

strong affinity for water exhibited by salt marsh soils. Because permeability is so low, the water table may be lowered only within a meter or so of the ditch itself (Balling and Resh 1982).

Grid ditching, even if not the marsh destroyer some claim it to be, still reduces standing water on the marsh and creates an unnatural and aesthetically unpleasing view. Open marsh water management was designed to more closely approximate the diversity of the natural marsh while eliminating the shallow pannes in which mosquitoes breed.

Wolfe (1996) reviewed the effects of OMWM on numerous tidal marsh resources. OMWM systems tended to enhance tidal exchange and salinity in marshes that were converted from grid ditching. Except where spoil piles were left (improperly) on the marsh, vegetation change was slight and favored salt-marsh species such as *Spartina patens* and *Distichlis spicata*. Small changes in elevation due to spoil deposition sometimes result in invasion by *Iva*, *Baccharis*, and *Phragmites*. Salt-marsh snails (*Melampus bidentatus*) have declined in some OMWM sites but not in others. Similar mixed observations have been made for marsh periwinkle (*Littorina irrorata*) and fiddler crabs (*Uca* species). Marsh fish populations are, by design, enhanced by OMWM. However, changes in species composition may occur where existing pools are deepened. Mummichogs (*Fundulus heteroclitus*) and spotfin killifish (*Fundulus luciae*) should decrease while sheepshead minnows (*Cyprinodon variegatus*), inland silversides (*Menidia menidia*) and rainwater killifish (*Lucania parva*) should increase (Talbot et al. 1986). The small reservoirs are not particularly attractive to birds and the minor changes in hydrology, flora and invertebrate fauna caused by OMWM do not cause significant changes in bird use on OMWM sites. Effects on mammals are not well documented. OMWM has had no long-term detrimental effects on water quality. As a result, Wolf concludes that, "Open Marsh Water Management is an environmentally focused management tool that is designed to be compatible with nature rather than compete with it." Of course, the technique is new enough that long-term monitoring is required to ensure that altered sites remain functional salt marshes.

b. Freshwater Wetlands (exclusive of Vernal Pools). Palustrine wetlands, including emergent, scrub-shrub and forested wetlands, are the dominant system in which Massachusetts freshwater physical control take place. In the vast majority of cases, this work consists of maintaining existing ditching designed to remove standing water from the wetland, thereby reducing mosquito-breeding habitat. For most MCPs, this type of work (source reduction) makes up a large percentage of their control effort. Though reducing standing water certainly reduces mosquito breeding, there has been little research concerning the overall effects of these alterations

on the modified wetland. Ditch systems can become problems in their own right, producing mosquitoes if left unmaintained. Most of these systems were never designed specifically for mosquito control and their other, primary function, such as removing runoff from large parking lots, may cause considerable damage to the ecosystem, leaving the MCP to clean up, or at least deal with, someone else's mess.

The majority of drainage systems currently maintained by MCPs were not initially constructed by MCPs and the effort of MCPs today is almost entirely restricted to removing blockages to existing flows, rather than enlarging or straightening channels to increase flow. Most freshwater drainage is an inherited problem which requires intervention not because of mosquito-control activities but because of the activities of others. Road sand and yard waste represent two of the most common obstructions MCPs are called upon to remove from existing streams and drainage networks. New developments also can cause dramatic changes in the sediment load in streams, despite regulations designed to prevent such problems. Road sand, yard waste and increased sediment load from development can all have impacts on a stream that are as greater or greater than regular ditch maintenance.

Because MCPs are often involved in removing manmade sediments from streams, a system under appropriate ditch maintenance may function more closely to a natural system than one in which manmade wastes are allowed to accumulate unabated. The appropriate response by the MCP in such cases is not obvious because, although the problem, and its cause, may not be mosquito related, the mosquito control program may be the organization best equipped, both from an equipment and a training perspective, to rectify the situation.

Since mosquito control projects came into being in Massachusetts, the perceived values of wetlands have changed. Once shunned as disease-bearing waste lands, best suited for dumping, draining or filling, wetlands are now viewed in a much more positive way. They are important wildlife habitats, perform a myriad of water quality maintenance functions, and serve as flood control, erosion, ground-water recharge and water supply regulators (Tiner 1989). Mosquito control programs have been slow in adapting to the increased value accorded wetlands. On the one hand, long-residual and/or broad-spectrum pesticides are no longer used in wetlands in Massachusetts. On the other hand, there is real resistance to halting maintenance work in drainage networks that may be seriously damaging wetland habitat.

There has not been a great deal of work done specifically on the effects of physical control for mosquitoes on non-target organisms. Therefore the following discussion is based on the general effects that can be expected when wetland alterations are made. Care should be taken in extending these general concerns to mosquito control

in Massachusetts. For example, channelization (straightening) of natural streams is not permitted in Massachusetts, where programs are required to follow the natural meanders of the stream. The three broad categories of wetlands alteration are outright loss, changes in the abiotic system and changes in the biotic community. Filling and/or draining wetlands to convert them to upland is a mosquito-control practice that has been all but eliminated in Massachusetts. There is no indication that MCPs are intentionally reducing wetland acreage in order to control mosquitoes. However, the fact that the wetlands boundary remains essentially unmoved by a mosquito-control alteration does not mean that profound alterations have not occurred.

Changes in the abiotic system and biotic community are deeply intertwined, though physical control most often causes abiotic changes which then cause biotic changes. For channels changes in flow rates, microhabitats, sediment load, sedimentation, and groundwater interactions can all occur. For wetlands (outside of channels) changes can include lost water-storage capacity, increased sedimentation and pollutant load, changes in water depth, and changes in groundwater hydrology.

When a stream is altered to improve water flow for the purpose of removing standing water, either within the stream or from adjacent wetlands, a number of changes may take place. By definition, improving water flow increases runoff. This, in turn, may decrease the surface-water storage capacity of the wetland system and decrease the capability of the wetland to retain load (suspended solids). This may increase the load of the water moving through the stream (Brown 1988). Increasing runoff into a given stream tends also to increase erosion, which further increases load (Williams & Feltmantle 1992). Not only is total flow increased, but alteration tends to increase peak flow, which is associated with reductions in faunal diversity (Hynes 1972).

The effects of increased flow and loading are many. At its most obvious, higher peak flows increase scouring of the stream bed by gravel and sand being transported by the water. Bottom dwelling animals are either affected on-site or swept downstream, leaving an impoverished community as flows return to normal (Hynes 1972). Increased flows can also remove organic matter, leaving a sandy bottom on which macrofauna is reduced (Ward 1992). As stated earlier, higher peak flows also encourage erosion, a process that can increase stream load long after sediment controls put in place during the actual drainage work are removed.

High levels of suspended solids alter the stream habitat by:

1. reducing light penetration
2. reducing primary production

3. altering the trophic structure
4. altering nutrient dynamics
5. changing thermal conditions
6. reducing oxygen levels (Ward 1992).

These effects can, in turn can have the following impacts on the stream fauna:

1. abrading respiratory epithelia
2. clogging respiratory structures
3. reducing feeding rates
4. reducing feeding efficiency
5. increasing exposure to toxins
6. reducing vision
7. inducing organisms to drift.

All of these effects can alter behavior patterns, change predator/prey interactions and change the outcome of inter and intra-specific competition (Ward 1992).

Maintenance for the purpose of reducing mosquito breeding also includes removing obstructions within streams. Tree branches and fallen trees are a particularly important part of the stream environment, providing food, living space, concealment from predators, protection from abiotic conditions and emergence sites (Ward 1992). They also provide varied microhabitats by deepening and slowing flow on the upstream side and often creating deeper pools on the downstream side. Removing these obstructions diminishes the variability of the stream ecosystem.

At the other end of the flow-rate spectrum, increasing peak flow may lead to faster drying in intermittent streams. The insect fauna (and biotic community in general) of intermittent streams does not overlap to a great extent with that of permanent streams (Ward 1992). As a general rule, this is because intermittent stream dwellers have evolved to deal with the drying down stream. Survival mechanisms include leaving the stream (swimming down stream or emerging as land-living forms), surviving in crayfish burrows or remaining pools of water, burying either at shallow depths or quite deeply into the substrate or hiding under rocks or leaf litter within or along the stream course (Ward 1992). Streams that dry more quickly, and stay drier longer, may disrupt all of these techniques as insects that emerge as adults may not have enough time to complete development (the primary example being

mosquitoes themselves), remaining pools may decrease in size and number and shallow burrows and hiding sites under rocks and leaf litter may become too dry to support the fauna hiding there.

The effect of rapid drying brings up the aspect of the hyporheic zone. This is the interstitial space between the substrate particles in a stream bed. Within the hyporheic zone the macrofauna can find shelter from floods, drought and extreme temperatures, can find suitable and predictable conditions for immotile stages such as eggs, pupae and diapausing nymphs and larvae and, particularly for early instars, protection from predation. Gravel beds provide the best hyporheic zones and animals can often be found many feet down (Ward 1992). This hyporheic zone fauna can be an important source of recolonizing organisms after a stream bed is denuded (Williams & Feltmantle 1992). Excessive drying can reduce the viability of the hyporheic zone.

Sedimentation, both within stream beds and in wetlands into which streams flow, is a problem because it can alter the stream bed composition, thereby altering the fauna, and can clog interstitial spaces, thereby reducing the hyporheic zone and/or reducing groundwater recharge. Sediments can also increase exposure to pollutants as they provide additional sites for pollutant binding while suspended, and then carry the pollutants to the benthic fauna.

MCPs routinely conduct maintenance to remove sediments. Therefore, there is good reason to expect the overall effect of maintenance may be to reduce the negative impacts of sedimentation within the stream. In such cases, it would be preferential to develop systems designed to prevent the deposition of road sand into drainage systems, rather than to prevent the removal of that sand, once it has entered the system. Removing the dense, rotting masses of grass clippings that are dumped into streams by property owners should also improve overall stream quality.

Up to this point the discussion has focused on the stream itself, but freshwater wetland alterations are typically designed to change standing-water wetlands to soil-saturated wetlands (New Jersey DEP 1997). The obvious problem is that any organism that requires standing water for periods other than peak flooding, the wetland may become unusable. Mosquitoes fit this definition perfectly, but so do many other organisms. Many species of amphibians use temporary standing water for breeding and are becoming scarce as these habitats are eliminated.

Increased drainage also may have an effect on groundwater. Precipitation and inflow determine the amount of water initially available to a wetland for ground water recharge (Todd 1972). Increasing the amount of water removed from the wetland prior to percolation downwards can decrease the capacity of the wetland to

recharge groundwater supplies. Not only can groundwater recharge within the wetland lowered, it can be lowered with the outflowing stream as well. During peak flows, water moves from the stream into the substrate and raises the water table (increases groundwater). As the flow declines, groundwater percolates back into the stream (if it does not, the stream dries out). By eliminating upstream reservoirs (wetlands), more water flows out of the system initially, leaving less water within the system. As water levels fall, groundwater discharge occurs sooner than might otherwise be the case. If nothing else, stream depth is liable to vary more widely after adjacent wetlands are reduced to soil saturation.

What is most needed is a comprehensive understanding of the true ecological costs of physical alterations for mosquito control. This is particularly important because, although the environmental effects of pesticides receive the lion's share of concern, it is possible that the long-term effects of physical controls may have a greater effect on the environment than does pesticide use (Buchsbaum 1994). This may be especially true today with the switch from broad-spectrum, more-persistent pesticides to methoprene and Bti.

New Jersey has recently updated its Best Management Practices for Mosquito Control and Freshwater Wetlands Management and Massachusetts should look to such guidelines to establish a protocol for physical control in freshwater wetlands. At a minimum the common-sense requirements that all alterations be planned (not random), necessary, and desirable should be rigorously applied to all MCP water management projects regardless of whether they are defined as new or maintenance work. The North East Massachusetts MCP Standards for Ditch Maintenance (Appendix F) can be viewed as a starting point for a statewide protocol, though it fails to mention the need for sediment controls during maintenance work and leaves the MCP superintendent with wide latitude for determining the necessity of a given project. A response from the Massachusetts Audubon Society (also Appendix F) to these Standards provides additional comments which deserve consideration when a protocol is established.

c. Vernal Pools. Vernal pools form in contained depressions in which water stands for a period of several months, generally from mid- to late winter through the spring. Water either comes in the form of snow melt or spring precipitation or can be a result of a rising water table. Some pools dry down within two or three months, others may only dry when the water table is lower than normal, resulting in a pool that is semi-permanent. Regardless, a key feature of vernal pools is that they undergo periods of dry down. Vernal pools may have permanent inlets but do not have permanent outlets (Kenney 1995).

There are numerous obligate species for vernal pools, the most visible of which include fairy shrimp, the wood frog (*Rana sylvatica*) and several species of salamander (*Ambystoma* spp.).

The Massachusetts Division of Fisheries and Wildlife (Publication #15498-10-600-6-1-88C.R) has created guidelines for certifying vernal pool habitat on the grounds that many vernal pool species cannot successfully survive without vernal pools and that vernal pools are under pressure from continued development within the state. The certification program is coordinated by the Natural Heritage and Endangered Species Program (NHESP - see next section). “Automatic” protection is given to vernal pools only if they

1. occur either (a) within the 100 year inland flood plain or (b) on “isolated land subject to flooding” (as defined in the regulations at 310 CMR 10.57 (2)(b)); and
2. its existence and location has been certified by the Massachusetts Division of Fisheries and Wildlife.

Curiously, upland vernal pools are not granted the same protection but may be certified as vernal pools. The NHESP does not seek out pools to certify; it certifies submissions from the public. A guidebook for vernal pool certification (Wicked Big Puddles) is available to help those who wish to submit a vernal pool site for certification (Kenney 1995).

Mosquitoes, particularly *Ae. canadensis*, also use vernal pools for breeding. From a control perspective, vernal pools are important because, due to increasing protection, vernal pool habitat is often left undeveloped while the land adjacent to the pool is built up. As a result, many new developments surround known breeding sites. Regardless of the wisdom of developing so close to vernal pools, mosquito-control personnel are charged with controlling the mosquitoes coming from the pool.

d. Rare and Endangered Species. Hynes (1972) states three axioms of running water biotic communities:

1. The greater the diversity of the abiotic system, the greater the number of species within the system.
2. The more conditions deviate from the normal, the fewer species will be present, but those remaining will be present in greater numbers.
3. The longer a system is left undisturbed, the richer and more stable is the biotic community.

Operating under the assumption that it is rare and endangered species which are most likely to be lost from the system first, the above statements would suggest that reductions in habitat diversity, alterations from the natural state, and frequent disturbances will all work against these species. Channelization of streams reduces diversity by



removing obstructions, straightening the channel and increasing flood levels. Wetlands changed from standing-water to saturated-soil regimes have been drastically changed from their natural state. Maintenance is ongoing, as is the disturbance it causes.

The key question is, however, to what extent does mosquito control contribute to the above problems. First, Massachusetts MCPs do not channelize streams, as their certification manual calls for following the existing meanders. Second, MCPs work neither in historically undisturbed, nor currently undisturbed streams. There is every reason to argue that there is no specific “natural” state that can be assigned a ditch dug by man and intermittently filled with road sand and grass clippings. Even with natural streams, the “natural” habitat in which they flow has long been altered and continues to be altered.

The Natural Heritage and Endangered Species Program (NHESP) was created in order to conserve and protect those plants and animals not hunted, fished, trapped or commercially harvested in the state. The program’s highest priority is to inventory rare and endangered species and to develop conservation plans through research, management and habitat protection for those species. One such program that directly impacts mosquito control is the vernal pool certification program mentioned above.

The NHESP also reviews proposed alterations to wetlands habitats under the Wetlands Protection Act (M.G.L. c. 131, s. 40 and regulations 310 CMR 10.00). NHESP has produced a series of estimated habitat maps for rare and endangered species (Massachusetts Natural Heritage Atlas) which proponents of a given alteration are required to check. Should a project fall within an estimated habitat, NHESP will then determine if the area to be altered is actual wetland habitat for a state-listed species. The results of the NHESP determination are given to the inquiring MCP.

The Massachusetts Endangered Species Act (M.G.L. c. 131A and regulations 321 CMR 10.00) prohibits the “taking” of rare plants or animals. From a mosquito-control perspective, the most important definition of “taking” is disrupting nesting, breeding or feeding sites of animals or killing or cutting a plant. Aside from directly protecting rare or endangered species, this Act also allows areas to be designated “significant habitats.” Alterations in “significant habitats” require a permit from the Division of Fisheries & Wildlife.

In Massachusetts, the species that have caused modifications in mosquito control practices are the Blue-spotted salamander (*Ambystoma laterale*), Mystic Valley amphipod (*Crangonyx aberrans*), and banded bog

skimmer (*Williamsonia lintneri*). In addition, ditch maintenance in vernal pond areas has been curtailed to protect this type of habitat. Other animals for which concerns have been raised are the yellow-spotted turtle and osprey.

The presence of a threatened species need not prevent water management, however. In the East Volusia Mosquito Control District in Daytona Beach, Florida, OMWM was carried out in a salt marsh that contained the Atlantic salt marsh snake (*Nerodia clarkii taeniata*). An observer walked in front of the ditching machinery and work was halted until observed snakes left the area. In practice the snakes were difficult to spot and several were seen in the freshly cut ditches behind the ditcher. Two dead snakes were found, and assumed killed by the work. The dead snakes were handled as “incidental take” and placed on ice for delivery to the Florida Game and Fresh Water Fish Commission (Goode 1996). With an increased understanding of the ecosystems in which mosquito control takes place, mosquito control projects should improve their ability to work in areas containing endangered species with minimal impact to those species.

Under the current system mosquito-control maintenance activities are exempt from the Massachusetts Wetland Protection Act, leaving only the federal 401 Water Quality Certification Act and both the Massachusetts and federal Endangered Species Act as methods for regulating maintenance. Unfortunately, water quality, while important, does not address the issue of changing habitat and the presence or absence of a rare and endangered species has little to do with the merits of a given project.

Rare and endangered species will probably increase in their impact for several reasons. First, as data is collected on these species, additional species and additional habitats will most likely come under protection. Second, residential areas are creeping closer to wetlands areas. The net effect is that the clash between economic development and environmental protection will likely increase, with mosquito control being one component of an intense debate. Again, a more comprehensive understanding of the true ecological effects of mosquito control is required to better determine the cost/benefit ratio for different types of mosquito control.

#### D. Food Web Effects of Mosquito Control.

Throughout their life cycle mosquitoes are a part of the food web, both as consumers and prey. As larvae most species feed on algae, protozoa, and organic debris (Pennak 1953). As adults they drink nectar and the females of most species take blood meals from a wide variety of animals. Larval mosquitoes are eaten by an impressive array of animals (Bates 1949) while adult mosquitoes are taken by spiders, predatory flies, odonates,

bats and birds (Collins and Washino 1985). Given that mosquitoes are so thoroughly embedded in the food web, the question arises as to the effect of removing a large percentage of the mosquitoes from that food web.

At first glance, the effect would appear to be large, particularly in habitats with dense larval populations. However, mosquitoes are r-strategists, in that they produce large numbers of eggs that develop quickly (when immersed in water) to adulthood. Most pest species seek out temporary and spatially disconnected habitats for breeding and can complete a breeding cycle long before an adequate predator complex can develop. Mosquitoes are, therefore, a highly unpredictable food source for predators, and predation rates must be equally unpredictable (this section will focus on predation, as parasites and pathogens are not widespread control agents in Massachusetts). This is borne out by the fact that mosquito predators are generalists which can readily switch to other prey when mosquitoes become scarce (Collins and Washino 1985). In a study in Florida, immature mosquitoes made up over 50% of the diet of several species of saltmarsh fish (*Fundulus confluentus*, *Lucania parva*, *Gambusia a. holbrooki*). But each of these species fed on other items as well, including copepods, shrimp, other fish and even some plant material (Harrington and Harrington 1961).

One important point regarding predation and larval broods is that the concepts of handling time and satiation come in to play (Varley et al. 1973). A predator must spend a certain amount of time with each prey. Hydrophilid larvae took several minutes to feed on larvae and pupae taken in a panne in Tiverton, Rhode Island (Christie, pers. observation). Not only does it take time to catch and eat the prey, but invertebrate predators are typically not much larger than the mosquito larvae they are attacking. Satiation must play a role in limiting predator take, particularly when mosquito numbers are high.

Predators can play an important role in regulating mosquito numbers in some situations. That mosquitoes are absent from waters with fish populations is well known. Less well known is the influence of chaoborid larvae, which can severely reduce mosquito populations in vernal pools (Morrison and Andreadis 1990). In this case the larvae are present in snow-melt pools for approximately two months, so predation has time to operate as a regulating mechanism.

Although adult mosquitoes are eaten by numerous predators, it is rare that they make up an appreciable proportion of the diet of any one predator. One exception is the spider *Tetragnatha montana* for which, in Poland, mosquitoes made up 74% in June and 62% in July of all prey captured (Collins and Washino 1985). Bats and purple martins are not effective mosquito predators nor do mosquitoes form a significant part of their diet.

From a mosquito-control perspective, the mosquitoes to be controlled are, for the most part, those which have escaped predation to become predators in their own right. In the absence of scientific data supporting the necessity of mosquitoes in the diets of specific animals, removing mosquitoes from the food web by chemical, biological or physical control remains an easily justifiable activity. Even so, control personnel should take care to avoid chemical applications where mosquito larvae are not present or are present in very small numbers, should use control measures that do not harm existing predator complexes, and should limit control to areas where control is necessary, allowing natural cycles to continue in areas where human activity and the risk of disease transmission is slight. One argument made in favor of Altosid is that it does not kill the young larvae, leaving them available as food for the existing predator complex.

Up to this point, the discussion has focused on the effect of chemical control (including *Bacillus* products and IGRs) of mosquito populations on other species. Within the context of biological control, one of the primary reasons *Gambusia* are not being used in Massachusetts is the fear that they might displace native species of fish, thus altering the natural biota, not by predation but by competition for the same resource.

Physical control by water management may increase predation, as in OMWM, or may eliminate predator and prey as when wetlands are drained to soil saturation. Mosquito breeding must be thoroughly documented before new work is done. Because disturbances may displace some species, and because predator species tend to rebound more slowly than their prey, maintenance work should be conducted only when necessary.

#### E. No Program

Another alternative strategy to current mosquito control practice is no control. Many communities in Massachusetts have chosen this option. These towns are usually outside of the enzootic EEE zone so the risk of human diseases transmitted by mosquitoes is viewed as practically nil by these communities. In addition, they are not located near salt marshes and their attendant pest mosquito problems. The mid-section of Massachusetts, where most no-control communities occur, also has a more rural character, less wetland, lower human populations, and a lower mean family income than most eastern areas with organized mosquito control programs. In general, the view of these communities is that the anticipated benefits from mosquito control do not outweigh the anticipated costs and perceived risks.

A more precise way of polling the public and confirming this opinion would be through establishing the Human Annoyance Threshold (HAT) for the town. Communities in which the HAT is below the actual annoyance

level should be persuaded to choose the no control option since justification for control is lacking unless a documented disease threat exists.

In some communities, biting annoyance is created by a combination of biting insects (i.e. mosquitoes, black flies, biting midges and tabanids) which require completely different control approaches. Many people do not recognize the difference between these insects, especially in dim evening light. It is critical to accurately identify the biting insects actually responsible for the human annoyance. In general, black flies and tabanids only cause annoyance during the daytime while mosquitoes and biting midges are most annoying from 6-10 PM. The HAT level for tabanids and biting midges is likely to be less than for mosquitoes because of their more painful bites.

Perhaps part of the reason why Berkshire County communities have supported a MC program while other Western Massachusetts communities have not, lies in the fact that this region supports many summertime outdoor activities (e.g. camps, resorts, golf courses, Tanglewood, etc.). In addition, this more mountainous region has a significant black fly annoyance problem superimposed on top of the mosquito problem. Vacationers are likely to have a lower HAT than permanent residents since they are spending more time and money on outdoor recreational activities.

In addition to risk-benefit considerations, other criteria for weighing the control/no control option are 1) the feasibility of successfully reducing annoyance below the HAT level, and 2) the adequacy of community resources for reducing annoyance to acceptable levels.

Towns with large areas of mosquito-producing freshwater wetland should recognize that effective mosquito control in these habitats is difficult at best and often impossible. As indicated earlier in this report, permanent wetlands do not usually produce large numbers of pest mosquitoes but in situations where they do, the public needs to be aware that these wetlands are a valuable resource that must be protected from significant perturbation and that options for mosquito control are therefore few. Community planning boards and zoning laws can and should be used to restrict residential development and other human activities from such wetland areas.

Towns with annoyance problems but with large land areas and thinly, scattered populations must understand that the same level of mosquito control achieved in more populated communities will cost them significantly more per capita. In many such cases, the economic status of the population means that insufficient tax dollars can be generated to adequately deal with the problem.

Nantucket provides an interesting case study. Mosquito control had lapsed in the early eighties. However, by 1989 saltmarsh mosquitoes were becoming a significant problem in the west and north areas of the island. The town began applying Altosid (including aerial applications of pellets in 1992) to control the mosquitoes while getting the requisite permits for OMWM in several west-end marshes (Madaket, Warren's Landing Eel Point). The OMWM systems were dug in January 1993 (Christie 1993). It was hoped that the OMWM alone would be sufficient to control mosquitoes but large-scale breeding continued in the north end (Pocomo) and the low tidal range has hindered the effectiveness of the OMWM as dug. As a result, some larviciding continues. The conclusion on Nantucket is that mosquito control against saltmarsh mosquitoes must be continued at some level in order to provide residents with the summer environment they want. However, Nantucket does not target freshwater mosquitoes at all and does no adulticiding. Nantucket and Cape Cod together indicate that adulticiding is not a requirement even in high-tourist areas.

It is difficult to measure the impact of choosing the no control option. The example of towns that have left, and left and later rejoined MC projects is perhaps the only available basis for estimating public opinion concerning such impact. No documentation of annoyance levels, cases of disease, recreational dollars spent, etc., was ever attempted in these towns when they had mosquito control versus when they did not. Thus, public complaints were apparently the main indication of the impact of the no control option that was used in guiding town decision making.

In the 1986 questionnaire, only about 10% of the towns in organized projects indicated that there had been controversy relative to leaving, joining, or rejoining a project. Those that had experienced controversy indicated that 4 factors were about equally involved. These were: 1) monetary constraints, 2) concern over the effectiveness of project control programs, 3) concern over the safety of the methods used by the project, and 4) concern over the environmental impact of the control procedures. Paradoxically, 86% of these towns indicated they were unwilling to spend more money (if legally possible) to obtain more effective control and 67% indicated unwillingness to spend more money to support less hazardous control methods. However, the bias of the people filling out the questionnaire sent to each town may have influenced these responses; no town in Massachusetts has actually polled their citizens on these questions. In fact, some towns within projects have taken advantage of new provisions which allow towns to collect additional money for MC activities which projects can then only spend in these towns.

Among towns that actually withdrew from MC programs, monetary constraints played a role in 73% of these decisions, followed by environmental concerns in 32% and concern over effectiveness in 27% of these cases. Towns that contemplated withdrawing but did not do so, indicated that monetary and effectiveness considerations dominated their concern.

The number of towns in MCPs declined in the late eighties. Economic factors, not environmental concerns, were the dominant reason given for withdrawal. This trend has reversed itself significantly in the last several years. The 1990 EEE problem is probably one reason, coupled with the fact that several coastal programs tried the no-control option and found mosquito numbers rose quickly.

Many towns in the Berkshire County and South Shore projects withdrew in 1981 mainly in response to Proposition 2-1/2 monetary constraints. The remaining 8 towns in Berkshire County subsequently chose to continue as a multi-town Project (Note: the town of Lanesborough rejoined in 1986, the city of Pittsfield rejoined in 1988, and other towns are contemplating reentry). South Shore actually disbanded as a project but by 1988 most towns had joined neighboring MC projects in Norfolk and Plymouth Counties. These actions suggest that these communities were not content with the no control option that resulted from the disbanding of their former project. Towns that voluntarily withdrew from MC projects but then later rejoined a project, did so for a variety of reasons. These were in order of their importance: increased public support for MC, increased mosquito annoyance, alleviation of monetary constraints, threat of EEE, improved methods and effectiveness of the project.

The Environmental Impact Statement (Sjogren 1977) prepared for the Metropolitan Mosquito Control District in Minnesota attempted to quantify the no control option for their community. Any attempt to develop similar estimates for Massachusetts would be meaningless given the lack of appropriate baseline data.