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Mosquito control by plankton management: the potential of indigestible green algae

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Summary

Most kinds of phytoplankton are good food for mosquito larvae. However, *Culex, Aedes* and *Anopheles* larvae fail to develop successfully in water where certain species of closely related green algae in the order Chlorococcales are the main source of food; apparently because the larvae are unable to digest them. Many species of *Scenedesmus, Kirchneriella, Dactylococcus, Elakotothrix, Tetrallantos, Coelastrum, Selenastrum* and *Tetradesmus* have this effect. These algae may offer a practical possibility for mosquito control when introduced into mosquito breeding habitats. Introduction of these algae could be assisted by simultaneous introduction of select filter-feeding zooplankton such as *Daphnia*.

Introduction

It is a well-known ecological principle that particular communities of animals are associated with particular communities of plants. It is therefore reasonable to expect that the suitability of an aquatic ecosystem as a mosquito breeding habitat should depend, among other things, on the kinds of plants in the ecosystem. Microscopic plants (i.e. phytoplankton) should be particularly significant because they are a major part of the flora in many mosquito breeding habitats and mosquito larvae are known to feed extensively upon them (Howland 1928; Senior-White 1928*a*; Kachroo 1959; Lozovei & Luz 1976; Scorza *et al.* 1977).

During the 1920s there were field surveys of the phytoplankton in mosquito breeding habitats (Coggeshall 1926; Senior-White 1926; Boyd & Foot 1928; Hamlyn-Harris 1928; Matheson & Hinman 1930; Bradley 1932). They were part of an attempt to explain the striking variation in abundance of mosquito larvae in different places that appeared superficially to be equally suitable. In general, the surveys were inconclusive. Virtually all kinds of algae were found where mosquitoes were breeding, though diatoms, desmids, and certain kinds of green algae (e.g. *Spirogyra*) often were particularly common where mosquito larvae were most abundant (Rudolfs & Lackey 1929; Sen 1938; 1941). Blooms of certain blue–green algae were noticed to be associated with the absence of mosquito larvae from rice fields in California (Purdy 1924).

Senior-White (1928*a*,*b*) concluded that no further progress could be expected until pure cultures of algae could be tested with mosquito larvae under controlled laboratory conditions. In fact, laboratory studies (Metz 1919; Barber 1927; 1928; Hinman 1930) found that mosquito larvae could grow and pupate successfully in pure cultures of algae, but the results were not consistent. Interpretation of results was complicated by possible toxicity in culture media, a suspicion that colloidal particles in the media were of nutritive value to mosquito larvae (Hinman 1930) and identification of algae only to genus, despite the possibility that different species might have different effects on mosquito larvae.

The matter was dropped for many years, but it was recently shown in Hawaii that the green alga *Kirchneriella irregularis* could kill *Aedes albopictus* larvae in container breeding habitats (Marten 1984). Whereas toxicity was the explanation for all previously documented cases of algae killing mosquito larvae (Gerhardt 1961; Dhillon & Mulla 1981), *Ae. albopictus* appeared

to die of starvation because they were unable to digest *Kirchneriella*. It was not necessary for the *Kirchneriella* to be highly abundant to kill the larvae; they only had to be abundant enough to predominate in larval guts to the exclusion of other food. Moreover, when a small quantity of water containing *Kirchneriella* was added to water in containers where *Ae. albopictus* was breeding successfully, the *Kirchneriella* often displaced other phytoplankton within a few weeks, making the water unsuitable for *Ae. albopictus* larvae.

This raised two questions: (1) whether there are more species of algae that have the same effect as *Kirchneriella irregularis* and (2) whether the effect applies to other species of mosquitoes in addition to *Ae. albopictus*. To answer these questions, a broad spectrum of algae species were screened for their effect on six species of mosquito larvae. Particular attention was given as to whether larvae digested the algae. Also investigated were possible methods for replacing algae in water where mosquitoes are breeding with algae that are indigestible for mosquitoes.

Materials and methods

LARVAL SURVIVAL

Pure cultures of algae were obtained from a collection at the University of Texas (Starr 1978) and cultured in sterile 'Woods Hole MBL' medium (Stein 1971). There was no evidence of nutritional or toxic effects of the culture medium on mosquito larvae. Mosquito eggs were collected from wild populations and hatched in autoclaved tap water to which no food for mosquito larvae was added. Larvae were pipetted into test jars within 24 h of hatching.

Each species of alga was tested in two ways. In the first, 'pure culture', three newly hatched *Aedes albopictus* or *Culex quinquefasciatus* larvae were placed in a jar containing 200 ml of a pure culture of the algae to be tested, and the jar was covered with an inverted petri dish. There was continuous fluorescent illumination and room temperature was maintained at 25°C. Algal densities were in the range of 5000 to 50 000 cells/ml, sufficient for the larvae to consume the algae rapidly (only three larvae were used in each replicate to ensure that larval grazing did not reduce algal densities).

The second kind of test was 'pond water mixtures' intended to provide a closer approximation to natural conditions than pure cultures. In 'pond water mixtures', the algae in water that normally supported larval development were replaced by a particular species of alga to be tested. This was accomplished by mixing 100 ml of pure algae culture with an equal quantity of filtered water from a prawn aquaculture pond in Hawaii where *Cylindrospermum* was the dominant alga. The pond water was passed through a 0.45 μm Millipore filter, which removed all algae, preventing contamination of the mixture with algae other than those to be tested. Although the filtration process also removed a substantial number of bacteria, it allowed enough through to simulate a pond environment. All other procedures were the same as in 'pure culture' tests.

The number of larvae in each jar, as well as their instars, was recorded twice a week until all larvae either died or developed into adults. If all three larvae in a particular test grew to the adult stage, the test was considered conclusive and was not repeated. However, whenever any larvae died, the test was repeated and considered conclusive only if the same result occurred the second time. If the second result was not the same, the test was repeated until a pattern emerged. Approximately 1000 tests were made.

Screening of algae began by testing several dozen species of algae over a broad taxonomic range. Whenever a particular species of algae was observed to have a negative impact on the mosquito larvae, cultures of other species in that genus as well as cultures from related genera of algae were obtained for testing. A total of 107 species were tested with *Cx. quinquefasciatus* and *Ae. albopictus*.

Nine species of algae that proved particularly favourable or harmful to *Cx. quinquefasciatus* and *Ae. albopictus* were tested with *Cx. tarsalis*, *Anopheles freeborni*, *An. quadrimaculatus* and *An. albimanus*. (The nine species are labelled in Tables 1, 2, and 4). The *Cx. tarsalis* and

Anopheles larvae came from laboratory colonies, and test procedures were the same as for *Cx. quinquefasciatus* and *Ae. albopictus*.

ALGAL DIGESTIBILITY

Thirty-one species of alga were tested for digestion by *Ae. albopictus* larvae. Digestibility was evaluated by microscopic examination of the gut contents of third-instar larvae that had been feeding in a pure culture of algae for 1 h, using visible cell damage (broken cells or intrusion of India ink into the cell) and reduction in chlorophyll fluorescence as indicators of digestion (chlorophyll fluoresces red under ultraviolet light but ceases to do so when broken down by digestion). In addition, larval faeces were cultured to evaluate the viability of algae after their cells had passed through larval digestive tracts.

The nutritional value of algae was evaluated with carbon radiotracer. Third-instar *Cx. quinquefasciatus* larvae were placed in pure cultures of algae labelled by introducing ^{14}C-bicarbonate 24 h earlier. Larvae were allowed to remain in the culture and feed on labelled algae for periods ranging from 15 min to 24 h. Upon removal from the labelled culture, larvae were placed in an unlabelled algae culture for 1 h to clear their guts of labelled algae before scintillation counting. The difference in carbon-14 counts, with and without clearing the gut, for larvae that were in the ^{14}C-labelled algae for 30 min was considered to be an estimate of the amount of carbon-14 in a gut full of algae. An 'assimilation index' was calculated to be the amount of carbon-14 in the larvae after clearing their guts, divided by the estimate for carbon-14 in a gut full of algae. The assimilation index increased through time as larvae fed on ^{14}C-labelled algae and accumulated carbon-14 in their tissues.

ALGAL INTRODUCTIONS

The possibility for establishing mosquito-killing algae in water where mosquitoes are breeding was tested by mixing 14 pure cultures of different species of algae listed in Table 4 and introducing 10 ml of the mixture into one-gallon jars containing 2 litres of water from a stabilization pond for pig farm wastes in Hawaii. There were 10 replicates. The pig farm water initially contained a high density of *Chlorella* and was ideal for *Cx. quinquefasciatus* larvae. After algae introductions the jars were held for 3 months under outdoor conditions, and their algae populations were monitored.

To see if filter-feeding zooplankton can graze down the algae in mosquito breeding habitats sufficiently for introduced mosquito-killing algae to have a better opportunity to take over, *Daphnia* were introduced to 21 samples from a variety of ponds in Hawaii. *Daphnia* also were introduced into 500 ml samples of pure cultures of 40 different kinds of algae. The populations of *Daphnia* and algae were then monitored for 1 month. In addition, the possible use of filter-feeding zooplankton such as *Daphnia* to facilitate the substitution of one kind of algae for another was examined by introducing algae to pig farm water (as described previously) with and without the simultaneous introduction of *Daphnia*.

Results

LARVAL SURVIVAL

With the exception of algae listed in Table 2, the tests were highly replicatable. Occasionally a larva died during the first day, regardless of the species of algae being tested, possibly because of a handling injury. However, all larvae that survived the first day generally performed in the same way with a given species of alga. Either they all survived to the adult stage or they all died as larvae. Death in the pupal stage was rare.

The larvae of both *Ae. albopictus* and *Cx. quinquefasciatus* all survived, grew rapidly, and developed normally to the adult stage in both pure cultures and pond water mixtures of the 57 species of alga listed in Table 1. This list includes all major groups of freshwater algae: diatoms, blue–green algae and green algae (including desmids). Growth was most rapid (approximately 1 week to reach the pupal stage), and larvae and adult mosquitoes attained the largest size, with colonial green algae in the

Table 1. *Cx. quinquefasciatus* and *Ae. albopictus* larvae consistently developed to the adult state with the following algae

Green algae
*Actinastrum hantzschii**
Ankistrodesmus falcatus§
*Ankistrodesmus spiralis**
*Aphanochaete elegans**
Chlamydomonas sp. (U. Texas No.796)*
Chlorella ellipsoidea§
Chlorella pyrenoidosa
*Chlorella variegata**‡
*Chlorococcum hypnosporum**
Chodatella brevispina
*Closterium acerosum**
*Closteriopsis acicularis**
Coccochloris peniocystis§
*Crucigenia lauterbornii**
Crucigenia tetrapedia
*Coronastrum ellipsoideum**§
*Cosmarium botrytis**
Desmidium swartzii
*Eudorina elegans**‡
*Gloeocystis gigas**
*Golenkinia minutissima**
*Gonium multicoccum**
*Nannochloris oculata**
Oocystis marssonii
*Oocystis minuta**
*Oocystis pusilla**
*Palmella texensis**†
*Pandorina morum**‡¶
*Paulschulzia pseudovolvox**
Pediastrum clathratum
Pediastrum duplex
Pediastrum simplex
*Planktosphaeria gelatinosa**‡
*Polyedriopsis spinulosa**
*Pseudococcomyxa adhaerans**
*Quadrigula closterioides**
*Radiococcus nimbatus**
Scenedesmus basiliensis
*Spirogyra pratensis**
*Staurastrum gladiosum**
*Tetraedron bitridens**
*Trochiscia hystrix**

Blue–green algae
Anabaena catenula
*Anabaena spiroides**
*Chroococcus turgidus**§
Cylindrospermum licheniforme
Eucapsis sp. (U. Texas No.1519)*
Lyngbya spiralis
*Microcystis aeruginosa**¶
*Nodularia spumigena**
Nostoc linckia
Oscillatoria lutea§
Phormidium faveolarum
*Spirulina platensis**

Other
Compsopogon coeruleus
*Cryptomonas ovata**†
*Navicula pelliculosa**†

*Larvae reached the pupal stage in less than 2 weeks.
†Algae in larval guts showed conspicuous loss of chlorophyll fluorescence and/or destruction of cell structure.
‡Slight signs of digestion in terms of loss of chlorophyll fluorescence or destruction of cell structure.
§No visible sign of digestion.
¶Tested with *Cx. tarsalis*, *An. albimanus*, *An. freeborni* and *An. quadrimaculatus* in addition to *Cx. quinquefasciatus* and *Ae. albopictus*.

orders Volvoccales and Tetrasporales. Growth was slowest (more than 2 weeks to reach the pupal stage) with pure cultures of green algae in the order Chlorococcales and blue–green algae in the order Oscillatoriales. Growth in pond water mixtures was generally more rapid than in pure cultures.

Cx. quinquefasciatus and/or *Ae. albopictus* larvae sometimes died when tested with the algae listed in Table 2. All the green algae in Table 2 are in the order Chlorococcales. Most larvae, particularly *Cx. quinquefasciatus*, died in pure cultures of these green algae. The typical response was slow growth to the fourth instar, with most larvae failing to pupate. Growth and survival were generally greater in a pond water mixture.

Growth and mortality with the blue–green algae in Table 2, all in the genus *Anabaena* and other genera in the family Nostocaceae, were quite different from growth and mortality with the green algae. With the blue–green algae, larvae died in some replicates but not others; they generally died in an early instar. Larvae that survived the early instar usually developed normally to the adult stage. Survival in pond water mixtures was better than in pure cultures, but there was no difference in growth

Table 2. *Cx. quinquefasciatus* and *Ae. albopictus* larvae sometimes died with the following algae

Green algae	Blue–green algae
Asterococcus superbus	*Anabaena cylindrica*
Crucigeniella apiculata	*Anabaena flos-aquae*†¶
Dictyosphaerium planctonicum§	*Anabaena sphaerica*‡
Dimorphococcus lunatus§	*Gloeotrichia echinulata*
Eremosphaera gigas	*Plectonema boryanum*§
Oocystis apiculata	
Oocystis polymorpha	
Pectodictyon cubicum§	
Selenastrum bibraianum	Other
Selenastrum minutum§	*Glaucocystis nostochinearum*

†Algae in larval guts showed conspicuous loss of chlorophyll fluorescence and/or destruction of cell structure.
‡Slight signs of digestion in terms of loss of chlorophyll fluorescence or destruction of cell structure.
§No visible sign of digestion.
¶Tested with *Cx. tarsalis, An. albimanus, An. freeborni* and *An. quadrimaculatus* in addition to *Cx. quinquefasciatus* and *Ae. albopictus.*

Table 3. *Cx. quinquefasciatus* larvae died with the following green algae, but *Ae. albopictus* larvae sometimes developed to the adult stage

*Botryococcus braunii**§	*Nephrocytium alantoideum*§
*Coelastrum microporum**§	*Scenedemus acuminatus**
Franceia amphitricha	*Scenedesmus acutiformis**
*Keratococcus bicaudatus**	*Scenedesmus acutus**
*Kirchneriella lunaris**§	*Scenedesmus obliquus*
Kirchneriella subcapitata	*Scenedesmus pannonicus*
Nephrochlamys rotunda	*Scotiellopsis oocystiformis*
Nephrochlamys subsolitaria	

**Cx. quinquefasciatus* sometimes reached the adult stage in pond water mixtures.
§No visible sign of digestion.

rates between pond water mixtures and pure cultures.

Table 3 lists algae with which *Cx. quinquefasciatus* larvae always died in pure culture, and except where noted, *Cx. quinquefasciatus* larvae always died in pond water mixtures as well. *Ae. albopictus* larvae were sometimes able to reach the adult stage with these algae in pure cultures, but growth was slow. *Ae. albopictus* larvae more frequently reached the adult stage in pond water mixtures, and growth was more rapid. The algae in Table 3 are all in the order Chlorococcales and are closely related to the algae listed in Table 4.

Pure cultures of the algae in Table 4 consistently killed both *Cx. quinquefasciatus* and *Ae. albopictus* larvae. The larvae showed virtually no growth and usually died within a few days in the first or second instar. They occasionally reached the fourth instar, but usually in an emaciated condition. Growth and survival of *Cx. quinquefasciatus* larvae in pond water mixtures of algae from Table 4 was equally poor. The same was usually true for *Ae. albopictus,* but *Ae. albopictus* sometimes survived to reach the adult stage after 1 or 2 months in a pond water mixture.

The results from testing *Cx. tarsalis* with the nine species of algae (¶ in the tables) were the same as described for *Cx. quinquefasciatus. Cx. tarsalis* developed normally in pure cultures and pond water mixtures of algae from Table 1 and Table 2 and died in pure cultures and pond water mixtures of algae from Table 4. Similarly, *Anopheles albimanus, An. freeborni* and *An. quadrimaculatus* developed normally

Table 4. *Cx. quinquefasciatus* and *Ae. albopictus* larvae died with the following green algae

Coelastrum reticulatum¶	*Scenedesmus dimorphus**§
Dactylococcus dissociatus§	*Scenedesmus dispar*
*Dictyosphaerium pulchellum**	*Scenedesmus longus*
Elakotothrix viridis¶	*Scenedesmus parisiensis*§
Kirchneriella contorta	*Scenedesmus quadricauda*§¶
Kirchneriella cornuta	*Selenastrum capricornutum**
Kirchneriella irregularis§¶	*Selenastrum gracile**¶
Scenedesmus abundans	*Tetradesmus cumbricus*
Scenedesmus bijugatus¶	*Tetrallantos lagerheimii**§

**Ae. albopictus* sometimes reached the adult stage in pond water mixtures.
§No visible sign of digestion.
¶Tested with *Cx. tarsalis, An. albimanus, An. freeborni* and *An. quadrimaculatus* in addition to *Cx. quinquefasciatus* and *Ae. albopictus*.

with green algae from Table 1 but died in pure cultures and pond water mixtures of the green algae from Table 4. Unlike *Culex*, the *Anopheles* also died in pure cultures of the blue–green algae that were tested (i.e. *Anabaena* and *Microcystis*), though they developed successfully in pond water mixtures of those algae.

ALGAL DIGESTIBILITY

Mosquito larvae that died with the green algae from Tables 3 and 4 appeared to do so because of starvation. Larval growth was slow or nil, and the larvae died in an emaciated condition despite feeding continuously on these algae. This effect was completely reversed (i.e. the larvae developed normally) when yeast was added to the water so that the larvae fed upon yeast as well as algae. The larvae also developed normally when algae from Table 1 were added to the water in equal abundance with algae from Table 4. The fact that the larvae survived in these mixtures, even when passing a significant quantity of mosquito-killing green algae through their guts, suggests that toxicity was not a significant factor.

The growth and survival of mosquito larvae tended to be better in pond water mixtures, presumably because of additional food in the mixtures. *Ae. albopictus* fared particularly well in pond water, probably becuase *Ae. albopictus* were not only filter feeding but also grazing along the glass surfaces of the jars, where they could consume food in addition to the planktonic algae being tested. In contrast, *Cx. quinquefasciatus* larvae, which engaged more exclusively in filter feeding, fared little better in pond water mixtures than in pure cultures. When *Ae. albopictus* was tested with algae from Table 4 in jars also containing wooden sticks covered with an abundant growth of fungi, the larvae grazed on the fungi and developed normally. *Cx. quinquefasciatus* larvae starved to death under the same conditions because consuming fungi was not part of their feeding repertoire.

Measurements of algal digestibility were correlated highly with how well the larvae grew and survived. When algae in the larval fore-gut and hind-gut were compared under a microscope with ultraviolet illumination, all species fluoresced brightly in the fore-gut, but most species fluoresced only dimly or not at all in the hind-gut (indicating destruction of their chlorophyll). The cell walls of many of these species were visibly broken up in the hind-gut. All tested species of algae in Table 1, on which *Ae. albopictus* grew most rapidly (as well as blue–green algae in Table 2), showed these signs of digestion. In contrast, none of the green algae from Tables 2, 3 and 4 (all Chlorococcales) lost their chlorophyll fluorescence, nor did they show any other detectable signs of digestion.

Attempts to culture the faeces of larvae feeding on *Palmella texensis* (a species associated with conspicuous digestion and rapid growth) were unsuccessful, indicating that no *Palmella* cells survived the digestion process. However, *Kirchneriella irregularis* quickly bloomed when

cultured from larval faeces, indicating that digestion was not killing the *Kirchneriella*.

In the radiotracer experiments, the assimilation index was approximately unity after *Ae. albopictus* larvae fed on ^{14}C-labelled *Pandorina morum* for 1 h; the assimilation index reached approximately 80 after they fed on the ^{14}C-labelled *Pandorina* for 24 h. This result confirms the high nutritional value of algae species on which larvae grow successfully. In contrast, the assimilation index for *Kirchneriella irregularis* was barely measurable even after larvae fed on the algae for 24 h, indicating that the nutritional value of algae in Table 4 was extremely low.

ALGAL INTRODUCTIONS

When a mixture of 14 algae species from Table 4 was introduced to pig farm water, one or more of the introduced algae replaced the *Chlorella* within 4 weeks in approximately 30% of the replicates. In some instances the introduced algae remained dominant during the 3-month period of observation, but in others the introduced algae were subsequently replaced by algae that apparently originated from the surrounding environment. Successfully introduced algae were always ones cultured from local water samples. Although culture-collection algae (which originated from other areas) sometimes became temporarily abundant, they never remained so for a long period.

When *Daphnia* were placed in pure cultures of algae from Table 1 (e.g., *Chlorella* or *Chlamydomonas*), the *Daphnia* increased in numbers and their grazing virtually eliminated the algae. In contrast, when *Daphnia* were placed in pure cultures of species from Table 4, the algae remained abundant, even though the *Daphnia* fed upon them continuously. This suggested that the grazing activities of particular filter-feeding zooplankton like *Daphnia* could put digestible algae at a disadvantage compared to less digestible species like those in Table 4.

When *Daphnia* were introduced to pig farm water, the *Daphnia* increased in numbers, and the *Chlorella* were quickly grazed down and replaced within 3 weeks by other algae (e.g., *Nannochloris*) available naturally from the surrounding environment. The same happened when *Daphnia* were introduced to other kinds of pond water. When the mixture of algae from Table 4 was introduced to the pig farm water along with *Daphnia,* introduced algae took over the phytoplankton in approximately 70% of the replicates, often as a mixture of two to five species. The algae usually persisted along with a low *Daphnia* population for the 3-month period of observation. When this happened, introduced first instar larvae of *Cx. quinquefasciatus* failed to develop, whereas the larvae developed normally when placed in controls containing *Chlorella* without *Daphnia* and introduced algae. Of paricular note was the successful establishment of culture-collection algae that were unable to establish themselves when introduced without *Daphnia*.

Discussion

LARVAL SURVIVAL AND ALGAL DIGESTIBILITY

Most species of algae—including diatoms, all green algae outside the order Chlorococcales, and most species of blue–green algae—are highly beneficial for mosquito larvae. Some species of blue–green algae in the family Nostocaceae (Table 2) kill mosquito larvae, most likely because of toxicity, but the effect is not consistent enough, particularly in pond water mixtures, to suggest a potential for mosquito control.

In contrast, some species of green algae in the order Chlorococcales (Philipose 1967) appear to have substantial potential for mosquito control. The species in Table 4—all in the families Selenastraceae, Scenedesmaceae and related families—consistently killed mosquito larvae in both pure cultures and pond water mixtures. It appears very likely that many of the approximately 200 untested species of algae in these same families, as well as possibly some closely related families, may also have the same effect on mosquito larvae.

All available evidence in this study suggests that Chlorococcales algae that kill mosquito larvae do so because they are indigestible to the larvae; no information from the scientific literature contradicts this conclusion. Howland

(1930) observed a loss of cell contents by diatoms, desmids, and filamentous green algae (e.g., *Spirogyra*) in the guts of *Aedes argenteus*, but there was no noticeable loss of cell contents from *Scenedesmus quadricauda*. The data of Kachroo (1959) reveal that diatoms, desmids, cladophorans, filamentous green algae, and planktonic green algae other than Chlorococcales were conspicuously digested by a variety of *Anopheles* species, but most species of Chlorococcales were undigested or only partially digested. Algae that do/do not support the growth of mosquito larvae correlate highly with algal digestibility documented in livestock feeding experiments (Hedenskog *et al.* 1969; Gacek *et al.* 1974).

The low digestibility of many Chlorococcales algae seems to be due to sporopollenin, a carotenoid polymer impervious to all digestive enzymes (Atkinson *et al.* 1972). Many genera in the Chlorococcales (including *Kirchneriella*, *Scenedesmus* and *Coelastrum*) have sporopollenin in their cell walls, but the amount can vary from genus to genus and species to species (Pickett-Heaps 1970; Atkinson *et al.* 1972; Marchant 1977; Hegewald & Schnepf 1979). Algae with sporopollenin typically have a digestibility of 0–10%, but their digestibility increases to 75–90% once the cell wall is broken mechanically (Pabst 1978). Mosquito larvae have no capacity in their digestive tract for mechanically breaking the cell walls of algae.

Indigestible algae can suppress the development of mosquito larvae in the field whenever the larvae consume such algae to the exclusion of more nutritious food. Because mosquito larvae consume different kinds of planktonic algae more or less in proportion to the abundance of the algae (Senior-White 1928; Kachroo 1959; Lozovei & Luz 1976; Scorza *et al.* 1977), larvae will be killed by indigestible Chlorococcales algae only when those algae are more abundant than other phytoplankton. *Cx. quinquefasciatus* died in water samples dominated by *Kirchneriella* or *Scenedesmus* that were collected during this study from ponds or roadside ditches in Hawaii, whereas they developed normally in water samples containing other kinds of algae.

There appear to be no records in the scientific literature reporting mosquito larvae living in water with an abundance of algae from the genera represented in Table 4, even though there are numerous records of larvae in water with an abundance of diatoms, euglenids, desmids, and green algae listed in Table 1; nor have larvae been collected from nature with significant quantities of algae from Table 4 in their guts, despite the fact that they have often been collected with other kinds of algae in their guts (Coggeshall 1926; Senior-White 1926; Boyd & Foot 1928; Senior-White 1928*a*; Matheson & Hinman 1930; Kachroo 1959; Lozovei & Luz 1976; Theivendirarajah & Jeyaseelan 1977; Ameen & Iverson 1978). Blue–green algae are also generally lacking in the guts of field-collected mosquito larvae.

Although the algae in Table 4 are indigestible to mosquito larvae, they are known to be highly digestible to many other kinds of animals (Mills & Wyatt 1974; Mironova 1975). Pond water in Hawaii that is dominated by *Scenedesmus*, *Kirchneriella* or *Coelastrum* typically has an abundant and diverse fauna even though there are no mosquito larvae. This fauna includes crustaceans such as ostracods and cyclopoid copepods, the latter being predators of mosquito larvae (Marten 1984).

ALGAL INTRODUCTIONS

The practical success of phytoplankton management for mosquito control will depend on whether human interventions can establish mosquito-indigestible algae in sufficient abundance, and for long enough periods, to have an impact on the mosquitoes. Mosquito-toxic algae (*Anabaena unispora* and *Chlorella ellipsoidea*) have been introduced experimentally to breeding habitats and sometimes have taken over and persisted for as long as several months, but they have not been reliable enough to be practical for mosquito control (Griffin 1956; Dhillon & Mulla 1982). Because a given aquatic habitat will be suitable only for some algal species and not others, the best control strategy may be to introduce a mixture of mosquito-indigestible species. A mixture should increase the prospect that at least one species will be competitive. Several of the

introduced species may be able to dominate the phytoplankton collectively under conditions where no single species of algae can predominate.

Filter-feeding zooplankton such as *Daphnia* can be significant competitors with mosquito larvae by grazing down digestible algae (Weed 1924). Although *Daphnia* can gain sufficient nutrients from at least some species of algae in Table 4 to maintain their population (e.g., *Scenedesmus*; Lampert 1977), they are also known to pass many of the algae in Table 4 through their guts without killing the algae (Porter 1973). The results in this study from introducing *Daphnia* simultaneously with algae from Table 4 suggest that it may be possible to employ the differential grazing pressure of filter-feeding zooplankton to facilitate the establishment of mosquito-indigestible algae even under conditions where those algae are not highly competitive. The same grazing pressure may also facilitate the persistence of mosquito-indigestible algae in the aquatic habitat. The potential of mosquito control by plankton management may lie in a stable, mutually reinforcing community of mosquito-indigestible phytoplankton, mosquito competitors (e.g., filter-feeding zooplankton), mosquito predators and possibly mosquito-toxic plants (Angerilli & Beirne 1974; Dhillon *et al.* 1982) that together form a habitat unsuitable for mosquito larvae.

A major question remains: In which habitats will the successful substitution of mosquito-indigestible phytoplankton actually kill mosquito larvae? Indigestible phytoplankton should have a particularly significant impact on mosquito species whose larvae are restricted to filter feeding. Indigestible phytoplankton may not have such an impact on species with broader feeding repertoires, particularly in habitats where feeding opportunities other than planktonic algae are present in abundance. Answers can be obtained with certainty only by substituting algae into different habitats and observing the consequences. Other unanswered questions include how long introduced phytoplankton will retain sufficient dominance under field conditions to kill mosquito larvae and whether implementation costs are competitive with other methods of mosquito control. Small-scale field studies would appear to be the next step in answering these questions and evaluating of the practical potential of indigestible phytoplankton for mosquito control.

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