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IMPACT OF THE COPEPOD *MESOCYCLOPS LEUCKARTI PILOSA* AND THE GREEN ALGA *KIRCHNERIELLA IRREGULARIS* UPON LARVAL *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)[1]

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ABSTRACT: It was observed that a significant percentage of natural water samples in Hawaii will not support successful development of *Aedes albopictus* larvae. In some water samples this is because of the presence of the copepod *Mesocyclops leuckarti pilosa*, which feeds upon first instar larvae and is capable of eliminating them entirely. *Aedes albopictus* larvae can also fail to develop when specific phytoplankton such as the colonial green alga *Kirchneriella irregularis* are abundant in the water. *Aedes albopictus* larvae feed on *Kirchneriella* to the exclusion of nearly all other food when *Kirchneriella* is abundant, but the larvae appear to starve to death. Further study on the impacts of zooplankton and phytoplankton on mosquito larvae will lay a foundation for mosquito control through plankton management.

INTRODUCTION

It is a well-known ecological principle that the suitability of a habitat for a particular animal species depends upon the plant and animal communities in the habitat. With this principle in mind, Marten (1980) proposed mosquito source reduction by means of biological agents that modify the plant or animal communities in mosquito breeding habitats. Because plankton are a major portion of the biological communities in most mosquito breeding habitats, managing the species composition of plankton offers possibilities for rendering aquatic habitats unsuitable for mosquitoes. A first step toward developing plankton management as a practical tool for mosquito control is to determine the effects that plankton can have on the growth and development of mosquito larvae.

METHODS AND MATERIALS

Water samples were collected from approximately 50 sites on the Hawaiian island of Oahu. Each of the samples was placed in a gallon jar (half full), and the jars were held at a location where Aedes albopictus was abundant. The mosquitoes provided a continuous natural source of eggs laid on the inner surface of the jars just above the water line. Eggs were inundated once a week by water added to replace water lost to evaporation. The presence or absence of mosquito larvae in the jars and the success of their development to adults was observed for a period of eight months.

The following laboratory test was employed to evaluate whether a particular water sample could support the development of Ae. albopictus larvae. A plastic cup was filled to the halfway point with 60 ml of water from one of the jars, and mosquito larvae were introduced to the water by one of two methods. The first was to place into the cup a small piece of blotter paper to which were attached ten Ae. albopictus eggs that had been collected on the blotter paper in the field. The second method was to hatch the eggs in tap water and transfer three larvae into the cup within a few hours of hatching. In both cases larvae were observed for a period of two weeks.

The gut contents of Ae. albopictus larvae were evaluated by microscopic examination of whole-animal squashes of first instar larvae, by microscopic sections of larger instars or extrusion of their gut contents from the foregut or hindgut by dissection, and by collection of feces. Fluorescent illumination facilitated the observation of algae because of the red fluorescence of chlorophyll. Acridine orange stain with fluorescent illumination facilitated the observation of bacteria in the gut.

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RESULTS

Impact of Zooplankton

Most of the jars of water contained a large number of mosquito larvae within a week, with the larvae growing and pupating in a normal fashion. However, ten of the jars never had any mosquito larvae during the entire eight-month period even though numerous eggs could be seen along the insides of the jars.

Water from these jars was tested in cups. Examination of eggs placed into the cups showed they nearly always hatched, and newly hatched larvae were often seen in the cups. However, the larvae always disappeared within a day or two. When the water was passed through a 100 Nitex nylon mesh before placing it in a cup, none of the larvae disappeared and they developed normally. Because the nylon mesh removed only invertebrates from the water, this suggested that invertebrates were responsible for the disappearance of Ae. albopictus larvae. The different kinds of invertebrates in the water were pipetted species by species into separate cups for testing. Mosquito larvae disappeared only from cups containing the copepod Mesocyclops leuckarti pilosa.

When newly hatched Ae. albopictus larvae were placed in spot dishes with Mesocyclops, they were immediately seized and eaten by the copepods (Fig. 1). Adult Mesocyclops are small (about 2 mm in length), not much larger than newly hatched mosquito larvae. However, they were observed to feed immediately upon all larvae up to their own size, and with a few hours delay they fed on larvae up to twice their size. In addition, the copepods maintained populations of approximately 25-200 individuals per liter for the duration of the study in all jars where they were present.

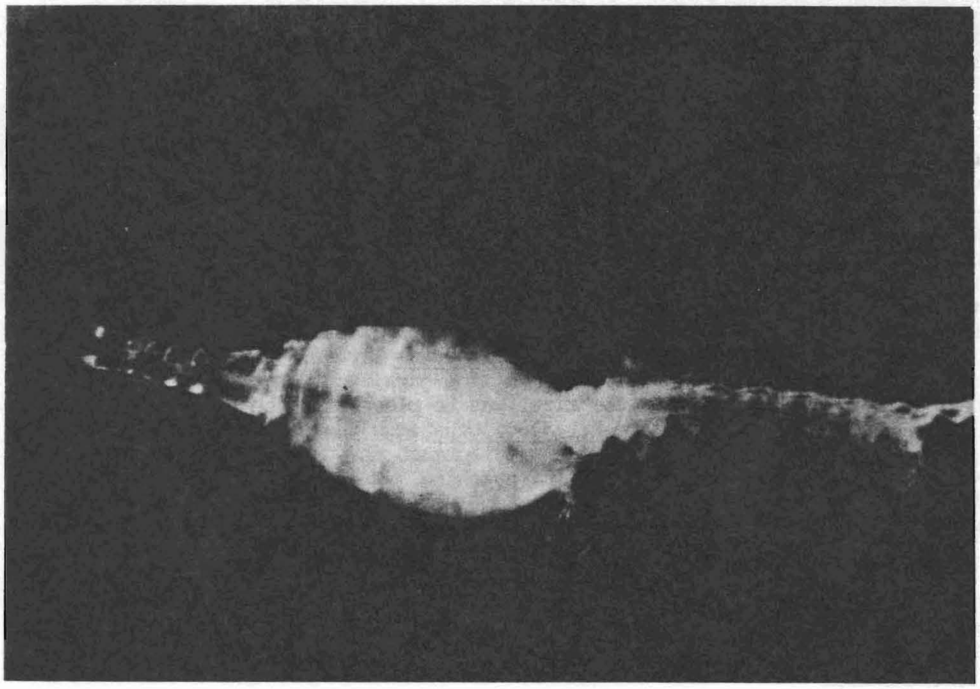

Figure 1. Mesocyclops leuckarti pilosa (lft.) eating a first instar larva of Aedes albopictus (rt.).

Impact of Phytoplankton

Many of the jars that showed a normal abundance and development of mosquito larvae during the first month ceased doing so during later months. In some cases there were no longer larvae in the water. In other cases there were larvae, but they never pupated, sometimes persisting for months in their final instar before finally dying. Both of these effects seemed to be independent of the extent of eutrophication or turbidity of the water. Aedes albopictus larvae succeeded in water samples that were as clear as tap water and failed in other water samples that were equally clear. They also both succeeded and failed in water samples that were so turbid with microorganisms that the larvae could scarcely be seen.

Of particular interest was the fact that larvae often failed in water samples that contained an abundance of microscopic life supporting a rich and abundant aquatic fauna and presumably providing ample food for mosquito larvae. Phytoplankton appear to be an important source of nutrition for *Ae. albopictus*, as their guts were observed to be packed with algae whenever algae were available in the water. Algae (e.g., *Chlorella*) appeared to be digested because they lost their chlorophyll fluorescence in the hindgut and feces. There were also bacteria in all parts of the larval guts, but it is not known to what extent the bacteria were consumed from the surrounding water and to what extent they were a resident gut flora.

The impact of algae on *Ae. albopictus* larvae was investigated in detail only for the colonial green alga *Kirchneriella irregularis* (Fig. 2), which appeared in three jars of water about three months after they were collected from aquaculture ponds. The samples originally contained a variety of phytoplankton, such as *Anabaena*, *Cryptomonas*, and *Gymnodinium* and supported normal larval development. However, once the *Kirchneriella* became abundant, the other algae virtually disappeared, the *Kirchneriella* remained abundant for the duration of the study, and no more mosquito larvae were observed in those jars (except for occasional first instar larvae), even though other aquatic fauna seemed unaffected.

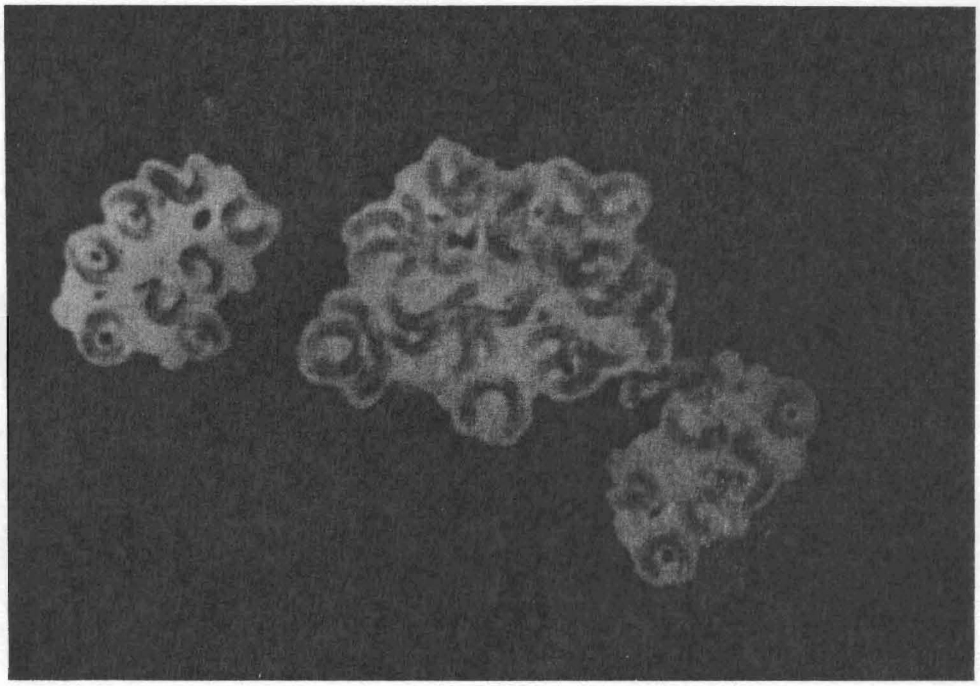

Figure 2. The colonial green alga *Kirchneriella irregularis*. The black background in the photograph is water containing India ink. The horseshoe-shaped bodies are the algal cells. The light area surrounding the algal cells is the gelatinous matrix that holds the cells together.

When water from the jars containing *Kirchneriella* was tested in cups, the *Ae. albopictus* larvae failed to grow and died within a week, still in their first or second instar. The larvae also died when the water was first passed through a 20 Nitex nylon mesh, which removed all zooplankton but did not remove algae and bacteria. However, the mosquito larvae developed normally when the water was first passed through a 5 millipore filter, which removed the *Kirchneriella* but passed smaller organisms, such as bacteria. The larvae also developed if yeast was added to the water containing *Kirchneriella* or if the water containing *Kirchneriella* was mixed in equal portions with other water that normally supported larval development.

Aedes albopictus larvae that died in water containing *Kirchneriella* fed continuously on *Kirchneriella* until their deaths, dying with their guts full of the algae. *Kirchneriella* in the hindgut and feces of dying larvae were still imbedded in their gelatinous matrix and showed no signs of having been digested.

It was possible to transfer the mosquito-suppressing effect of the water samples that contained *Kirchneriella* by introducing a small quantity of that water to water that normally supported mosquito development. The mixture supported larval development at first, but the Kirchneriella often became abundant after a month, and whenever this happened, larval development was then suppressed. The suppression varied from a lack of larval growth, accompanied by death in the first instar as had been observed in the original water samples containing Kirchneriella, to the presence of some growth but failure to pupate. The introduced Kirchneriella maintained their abundance in the water for the duration of the study, and the water retained its mosquito-suppressing qualities. Microscopic examination of the gut contents of larvae showed there was no growth when the gut was filled with Kirchneriella only. Some growth (but no pupation) occurred when a few other algae were mixed in the gut along with Kirchneriella.

Isolation of a clone of the Kirchneriella has so far resulted in a culture containing Kirchneriella and a colonial "Chlorella-like" alga that, like Kirchneriella, is imbedded in a gelatinous matrix. It has not yet been ascertained whether the "Chlorella" is an alternate form of Kirchneriella (e.g., an autospore) or a contaminant. When this culture was tested in cups, the larvae sometimes died within a week and other times developed beyond the first instar but died in emaciated condition after three or four weeks.

Algae from the Kirchneriella culture have been introduced to a variety of natural water samples after centrifuging the culture and diluting it with distilled water so the algae could be introduced without introducing a significant quantity of mineral nutrients from the culture medium. In many cases the Kirchneriella or "Chlorella" (or a mixture of the two) have taken over within a month, and cup tests have shown a range of responses from no growth and death in the first instar to retarded growth with no pupation. Controls, to which no algae were introduced, supported normal larval development.

DISCUSSION

Mesocyclops leuckarti pilosa was first reported to feed on mosquito larvae by Bonnet and Mukaida (1957). Each copepod was observed to eat 15-20 larvae per day. Riviere and Thirel (1981) introduced M. l. pilosa to natural mosquito-breeding containers in Tahiti and observed that they virtually eliminated Aedes aegypti and Aedes polynesiensis larvae from the containers, though they had little impact on Culex quinquefasciatus larvae.

Numerous predators of mosquito larvae have been identified and studied in the past, but Mesocyclops may have exceptional potential as a biological control agent because, in containers at least, normal levels of Mesocyclops abundance are sufficient to eliminate virtually any Aedes larvae that appear. Mesocyclops is very effective wherever it occurs because it feeds primarily upon ciliated protozoa and is able to sustain substantial populations whether mosquito larvae are abundant or not. However, Mesocyclops is of limited natural occurrence in Ae. albopictus container-breeding habitats in Hawaii, possibly because of poor resistance to the dessication that occurs whenever such habitats dry up. As there are few reports in the scientific literature concerning cyclopoid copepods as predators on mosquito larvae, it should be worthwhile to examine other species for their impacts on mosquito populations.

It appears that the decline in the capacity of many water samples in this study to support normal development of Ae. albopictus larvae after several months was a consequence of phytoplankton succession from species that support Ae. albopictus development to ones that do not. The succession may have been accelerated by the presence of the larvae themselves. So far the process has been documented only for Kirchneriella, but it appears Kirchneriella may be only one of many kinds of phytoplankton that are capable of suppressing larval development.

The impact of Kirchneriella is associated with consumption of the algae by the larvae, but the exact mechanism of the impact is not known. Toxicity may be involved, but it also may be that Kirchneriella is not of nutritional value to the larvae even though it is consumed instead of useful food. It is possible the gelatinous matrix of Kirchneriella inhibits digestion, as Porter (1975) has reported gelatinous algae to be indigestible to Daphnia. Toxic exudates of Anabaena unispora and Chlorella ellipsoidea have been reported to cause mortality in mosquito larvae (Gerhardt 1961, Dhillon and Mulla 1981). However, toxic exudates do not seem to be responsible in the case of Kirchneriella because the suppressive effect was eliminated when Kirchneriella was filtered from the water. Moreover, mortality due to toxins usually occurs within hours, whereas, mortality from Kirchneriella took days.

The practical potential of Mesocyclops and Kirchneriella for mosquito control is still unknown. It will depend on the environmental conditions in which Mesocyclops or Kirchneriella can establish populations large enough to have a significant impact on mosquito larvae and whether they do so in the microhabitat where larvae actually occur. It could also depend upon the extent to which human intervention can assist the establishment of those zooplankton or phytoplankton in mosquito breeding sites.

The most significant outcome of this study is the demonstration that plankton can have a decisive impact on the success of mosquito larvae. The results suggest it should be worthwhile to catalog the impact of different kinds of plankton on different kinds of mosquito larvae. In those cases where there is an impact, it will be worthwhile to determine the ecological conditions under which those plankton can be established in mosquito breeding sites.

Acknowledgments

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