Abiotic Factors Influencing *Aedes* Mosquito Larvae Distribution in UNDERC Vernal Ponds

By

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ABSTRACT

Seven vernal ponds on UNDERC property were sampled for mosquito larvae determined to species. Mosquito species census data was combined with previously collected data from the same ponds. Tests were done on each pond for a variety of abiotic characteristics including canopy cover, conductivity, pH, true color, phosphate, nitrate, and ferrous iron as possible factors influencing oviposition preferences and subsequent larval species distribution patterns. There was suggestion that *A. communis* prefers high color and phosphate concentrations, *A. excrucians* prefers open canopy, and *A. tricurus* and *A. diantaeus* prefer high conductivity and neutral pH. Other species distribution patterns could not be established with the collected data.

INTRODUCTION

Mosquitoes are nuisance pests the world over as well as vectors of many lethal or debilitating disease. The depth of knowledge of specific mosquito species is often correlated to their ability to vector human diseases, and thus there is often incomplete knowledge of the biology and behavior of mosquitoes which do not vector diseases. Specifically, oviposition behavior has not been thoroughly studied in northern midwest mosquito species, although much research has been conducted on oviposition preferences for human vectors including *Aedes aegypti* (Zahiri 1998*)* and *Culex quinquefasciatus* (Rodcharoen 1997) vectoring malaria and lymphatic filariasis respectively. Although relative species abundance in a given aquatic habitat was originally thought to be determined primarily by mortality, it is now generally accepted that oviposition preferences by adult mosquitoes determines the distribution of eggs and resulting larvae. (Barr 1958) Cues that might prompt adult mosquitoes to lay eggs in a given area are varied, and include both abiotic factors, such as light and moisture, and biotic factors such as conspecific egg presence. (Zahiri 1998) It has been shown that using such cues, gravid female mosquitoes can lay eggs on the dry bed of a vernal pond in the summer that will be ideal larval habitat when the bed fills with water in the spring. (Enfield 1977)

On the UNDERC property in Land O' Lakes, Michigan, thirty-two vernal ponds sites have been studied as mosquito larvae habitat since the late 1960's. Data has been recorded as to the number of mosquito larvae collected from each pond in during the years 1968-1970, 1972-1974, and 1976-1979. (Hellenthal 2003) This census data indicates the sum of each species collected during the entire testing period, but not yearly counts. The ponds showed a wide varied of species distributions, but no experimentation had been done to determine what factors influenced the distribution patterns. This experiment's goals included verifying existing mosquito larval census data in seven selected vernal ponds by larval dipping. Secondly, it aimed to measure a variety of abiotic characteristics of these seven vernal ponds which might serve as oviposition cues for adult mosquitoes as suggested by literature sources (Zahiri 1998, Rodcharoen 1997, Gilardi 1992, McDonald 1995), including canopy cover, DO, conductivity, true color, and concentrations of phosphate, nitrate, sulfide, and ferrous iron. Finally, this study sought to correlate mosquito larvae distribution patterns to the abiotic factors. This information could be useful not only in helping to understand oviposition behavior in adult mosquitoes, but also in developing economical oviposition repellants to discourage mosquito infestation of lakes and ponds.

MATERIALS AND METHODS

Seven vernal ponds were selected on UNDERC property for a variety of reasons including interspersion, presence of standing water throughout the experiment, and the presence of a sign post giving positive identification of pond number to correlate with old mosquito larval data. At each pond six testing sites were establish with flags, one at each of the northernmost, easternmost, southernmost, and westernmost point of the pond, as well as two sites within the center of the pond, one meter north or south of the center of the north-south axis following the protocol of Morrison. (1992) At each testing site, one measurement was taken of conductivity, dissolved oxygen, temperature, and pH using respective meters. In addition, at each site a spherical densitometer was used to estimate canopy cover, and a one liter sample of water was taken for further water chemistry tests. A standard mosquito dipper (volume of 350 ml) was used to take five dips at each site, with all mosquito larvae being identified by eye and transferred to 70% ethanol by plastic pipette following the protocol of Barr for mosquito larval sampling. (1958) The one liter samples were tested for concentrations of sulfate, nitrate, reactive phosphate, and ferrous iron as well as alkalinity and true color using the Hach Chemical Analysis Kit. The Hach kits spectrophotometrically measure concentrations of desired compounds with the following errors: sulfide +/- 0.01 mg/L, nitrate +/- 0.01 mg/L, phosphorus +/- 0.01 mg/L, ferrous iron +/- 0.009 mg/L, alkalinity +/- 1 mL, and true color +/- 10 PtCO Units. All tests followed protocol from *Hach Procedures Manual.* (2000) Preserved mosquito larvae were identified to species using a dissection microscope and *Mosquitoes of Minnesota.* (Barr 1958) This mosquito data was added to existing data collected from 1968 to 1972. (Hellenthal 2003) The relative abundance, that is the total number of larvae of a given mosquito species in a given pond divided by the total number of mosquito larvae in a given pond, was determined for each of the seven most common species of mosquitoes in all seven ponds.

Statistical analysis of abiotic characteristics of the vernal ponds was conducted with SYSTAT statistical analysis software. An ANOVA test was run for each abiotic factor, and a post hoc TUKEY determined which ponds possessed significantly different factors from the others. For each abiotic factor a graph was produced ranking the seven vernal ponds in order with TUKEY lines linking statistically similar ponds. Using these graphs correlations were made between each of the seven most common mosquito species' relative abundances in seven ponds, and the abotic factors of those ponds. It was assumed that all vernal ponds were being fully utilized by mosquito larvae such that any species could have ovideposited in any pond had it "wanted to."

RESULTS

Fig. 1-6 show the results of tests and statistical analysis of abiotic factors in the seven vernal ponds. Alkalinity was determined to be zero or near zero for all of the seven ponds, suggesting a very poor buffering capacity. The sulfide concentrations were also determined to be zero or near zero for all seven ponds. This was verified by a sulfide test completed by McDonald and Thompson in 1995 on five of the seven ponds in this experiment. The dissolved oxygen levels of the ponds were not successfully measured due to a malfunctioning meter, but the assumption was made that due to the relatively shallow nature of all ponds, dissolved oxygen would be relatively high throughout. Fig. 7 shows the results of mosquito larval sampling by pond. N values were particularly low, probably due to missing the main mosquito hatch of the season, so heavy reliance was placed on old data for the remainder of the experiment. Fig. 8 shows this combined old data from Hellenthal and the experimental data from 2003. Although this data could not be analyzed statistically since the Hellenthal data represents summations of all years sampled and a consequent n of 1, it was used to suggest apparent trends in mosquito oviposition tendencies. Pond 31 was often an outlier and did not follow assumed trends. This can be explained by its many abnormal abiotic characteristics, as seen in Fig's 1-6, and less reliance was placed on Pond 31 in establishing trends. *A. communis* was seen to prefer ovideposition in waters with high true color and high concentrations of reactive phosphate. Fig's 9 and 10 show these trends. *A. excrucians* was seen to prefer ovideposition in areas of decreased canopy cover as the ponds with the highest percentage of *A. excrucians* larvae were also the three ponds with the least average canopy cover, with the exception of pond 31. *A. trichurus* shows a moderate preference for high conductivity as well as a moderate preference for more neutral pH, excepting pond 31, as seen in Fig's 11 and 12 . *A. diantaeus* dispersion was strongly correlated with high conductivity and more neutral pH as shown in Fig's 13 and 14. Nitrate levels were not correlated with any species distribution, and there were no apparent trends in the distribution of species A. punctor, A. cinereus, or *A. abserratus*.

DISCUSSION

The abiotic factors showed much more variance than was originally expected. Three ponds in particular, 5, 6 and 7, are all located very close to each other geographically (ponds 6 and 7 are located on either side of a road no more than 20 yards from each other) and yet all three often showed significant ($P < 0.05$) differences in abiotic factors. The flat nature of the UNDERC property probably explains these differences, as nearby ponds can have very different watersheds. Pond 31 was associated with the sphagnum mat of a large and relatively young bog, and thus had widely different abiotic characteristics from the other six ponds. Its water was very clear, had low conductivity, iron and nutrients, and almost neutral pH. There was also significant sphagnum growth around several of the ponds (6, 15, and 31 especially) possibly explaining the low pH of these ponds. The large percentage of rainwater in the ponds could also explain the lower pH values. (Wetzel 1983) Both *A. trichurus* and *A. diantaeus* showed an intolerance for acidic waters, preferring instead ponds with more neutral pH. Conductivity also seemed to play a role oviposition preferences supporting findings by Zahiri that salinity affects the likelihood of several species of *Aedes* mosquitoes to lay eggs in water. (1998) High conductivity was preferred by *A. trichurus* as well as *A. diantaeus.* Color varied in proportion to ferrous iron content. This is most probably a result of ferrous iron's tendency to complex with organic bases in the presence of tannic acids staining the water a tea color. (Wetzel 1983) These darkly stained waters were preferred by *A. communis.* This could possibly serve as an adaptation in allowing the larvae's dive response to be more effective as darkly stained water would quickly hide the submerging larvae. Sulfide levels were low for all ponds probably due to the oxidative nature of the ponds as sulfide is reduced to sulfate. (Wetzel 1983) In future experiments sulfate might be considered as an oviposition cue rather than sulfide. Canopy cover showed no significant trends since canopy cover varied greatly within the same pond. Pond 6 in particular had half of its area shaded and the other half exposed to the sun. However, the means of canopy cover themselves do show a positive correlation to the abundance of sun-loving mosquito species such as *A. excrucians.* (Gilardi) Phosphate and nitrate levels gave good indications of the overall productivity of the ponds as both can be limiting nutrients in aquatic habitats. (Wetzel 1983) Although pond productivity can indirectly affect larval dispersion and especially maximum larval density, only *A. communis* was seen to correlate its distribution pattern with phosphate levels. Gilardi states that *A. communis* demonstrate a preference for rapidly drying ponds in which phosphate concentrations would be rapidly increasing. (1992) This is one possible explanation for the noticed trend. Nitrate did not seem to have an effect on any species' distribution. Alkalinity was determined to be zero or very close to zero for all seven ponds indicating a very poor buffering capacity. This might be due to low calcium carbonate levels in pond water, or an associating with acidifying sphagnum moss. It leaves the ponds susceptible to large changes in pH and subsequent die-off of mosquito larvae adapted to a specific pH range.

Trends for *A. punctor, A. cinereus*, or *A. abserratus* could not be established from the existing data. It is possible additional oviposition cues than the ones tested for are responsible for these species distribution patterns, or that conspecific or interspecific competition determines their distribution. In particular, the large prevalence of *A.*

abserratus in Pond 31 remains somewhat of a mystery, especially since Gilardi claims that *A. punctor* is almost always found in equal numbers to *A. abserratus,* a trend not noticed in Pond 31. (1992) Future studies might focus on which particular factor of Pond 31's water attracts *A. abserratus* oviposition and not *A. punctor.*

The lack of mosquito larvae collection was somewhat disappointing, and no verification of old distribution patterns could be made. However, the combined mosquito larvae data provided a good indication of larval dispersion patterns in the seven ponds surveyed and enough information to make inferences on abiotic cues in oviposition behavior. However, there is a distinction between mosquito species with a single early hatch each year including *A. abserratus, A. communis, A. diantaeus, A. excrucians,* and *A. punctor,* and those with multiple generations yearly including *A. cinerus,* and *A. trichurus.* (Gilardi 1992) In future studies larval collection should take place earlier in the year and continue through the summer to adequately sample both types. These samples should also take place yearly for a number of years so that statistical differences can be determined in larval distribution between various ponds.

Future studies might look at other possible oviposition cues including total dissolved solids, dissolved oxygen, sulfide, manganese, and aluminum. Furthermore, biotic factors might be considered. It has been demonstrated that certain species of *Aedes* mosquitoes will prefer to ovideposit in water containing other *Aedes* eggs up to a critical density when additional *Aedes* eggs become inhibitory to further oviposition. (Zahiri 1998.) In addition it has been shown that certain species of copepods, which prey upon mosquito larvae, secrete chemicals which act as attractants for gravid female mosquitoes to ovideposit, thus increasing the food resources in the pond. (Torres-Estrada 2001)

Determining if either conspecific or interspecific interactions influence oviposition behavior in mosquito species at UNDERC would be a worthwhile next step.

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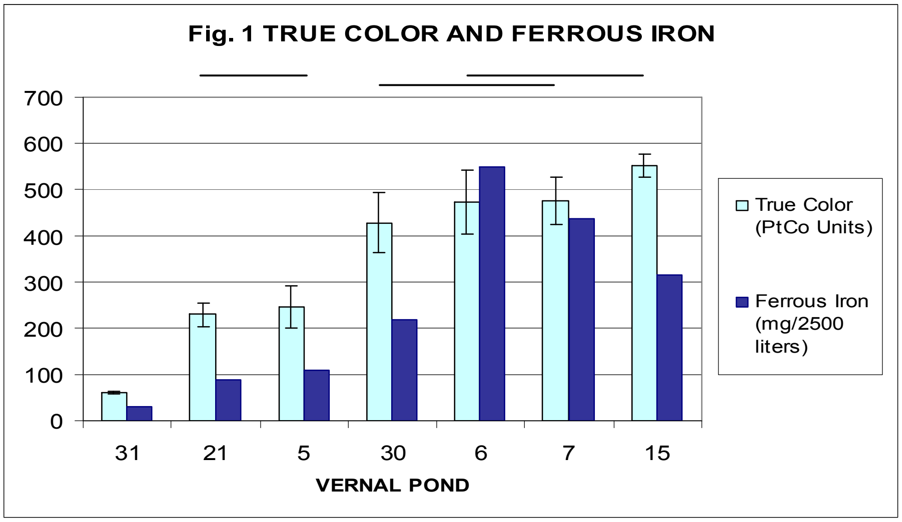

Fig. 1 shows true color in platinum cobalt units of seven vernal ponds listed on the x axis in the lighter bars. Error bars show one standard deviation. TUKEY lines above the graph link statistically similar ponds. Darker bars show Ferrous iron in mg per 2500 liters. Error bars are not shown due to significant error. All ponds were statistically similar in regards to ferrous iron content.

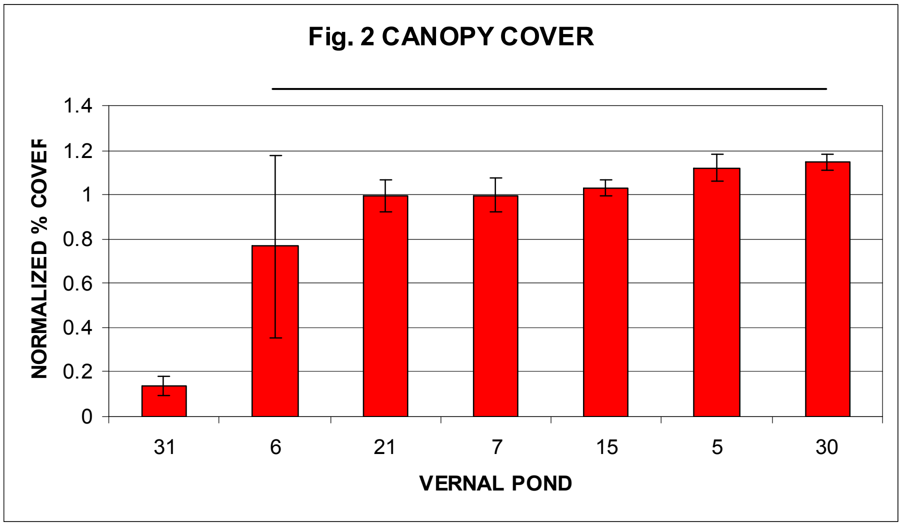

Fig. 2 shows normalized canopy cover in ARCSIN ((% Canopy Cover) ^(0.5)) of seven vernal ponds listed on the x axis. Error bars show one standard deviation. TUKEY lines above the graph link statistically similar ponds.

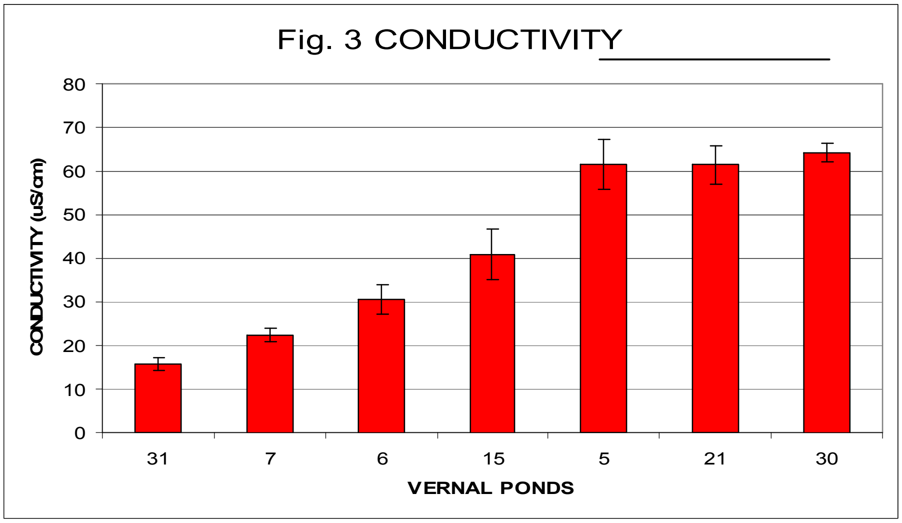

Fig. 3 shows conductivity in microseimons per centimeter of seven vernal ponds listed on the x axis. Error bars show one standard deviation. TUKEY lines above the graph link statistically similar ponds.

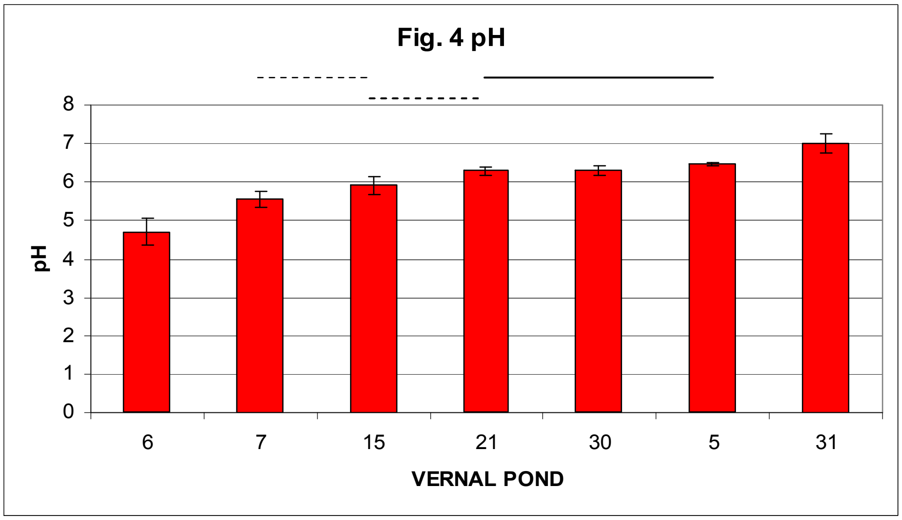

Fig. 4 shows pH of seven vernal ponds listed on the x axis. Error bars show one standard deviation. TUKEY lines above the graph link statistically similar ponds. Dotted TUKEY lines represent 0.045<P<0.055.

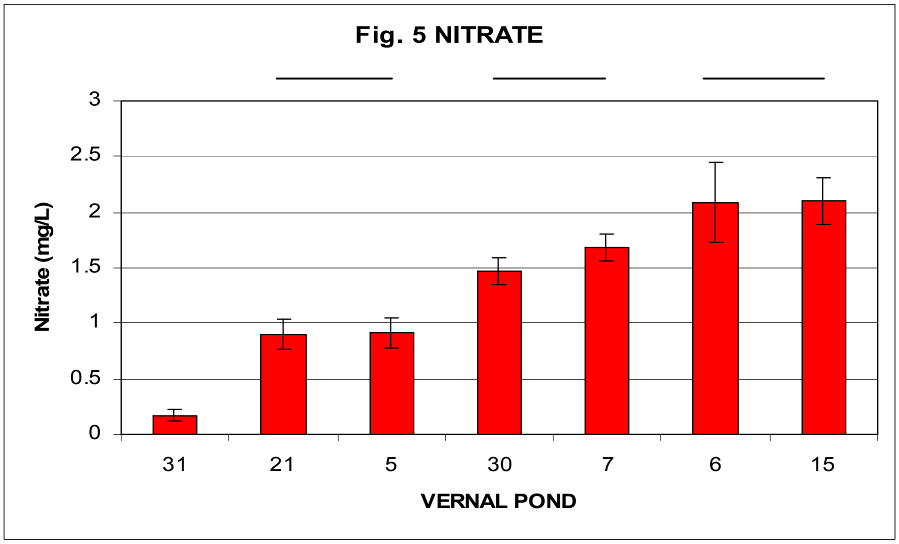

Fig. 5 shows nitrate concentrations in mg/L of seven vernal ponds listed on the x axis. Error bars show one standard deviation. TUKEY lines above the graph link statistically similar ponds.

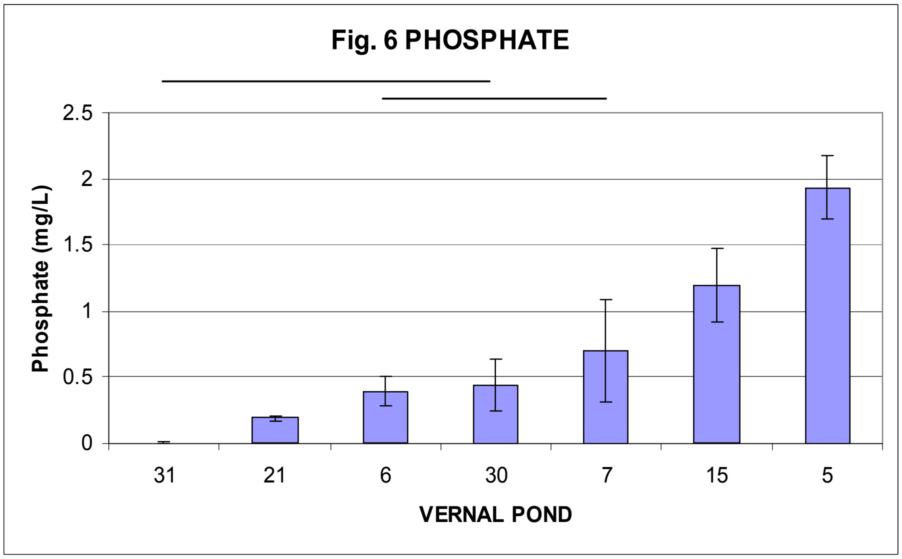

Fig. 6 shows reactive phosphate concentrations in mg/L of seven vernal ponds listed on the x axis. Error bars show one standard deviation. TUKEY lines above the graph link statistically similar ponds

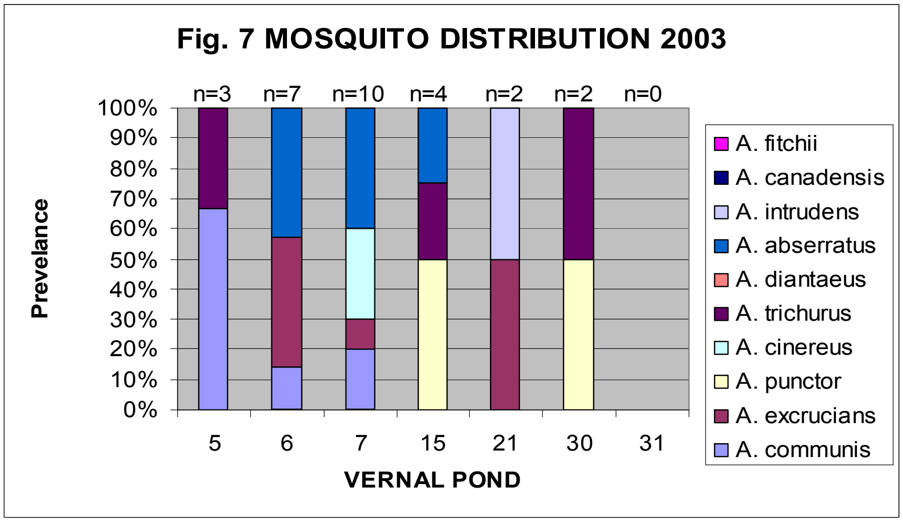

Fig 7 shows distribution of mosquito larvae collected during 2003 in seven vernal ponds listed on x axis. Graph shows relative abundance given by total larvae found of given species in each pond divided by total larvae found in each pond.

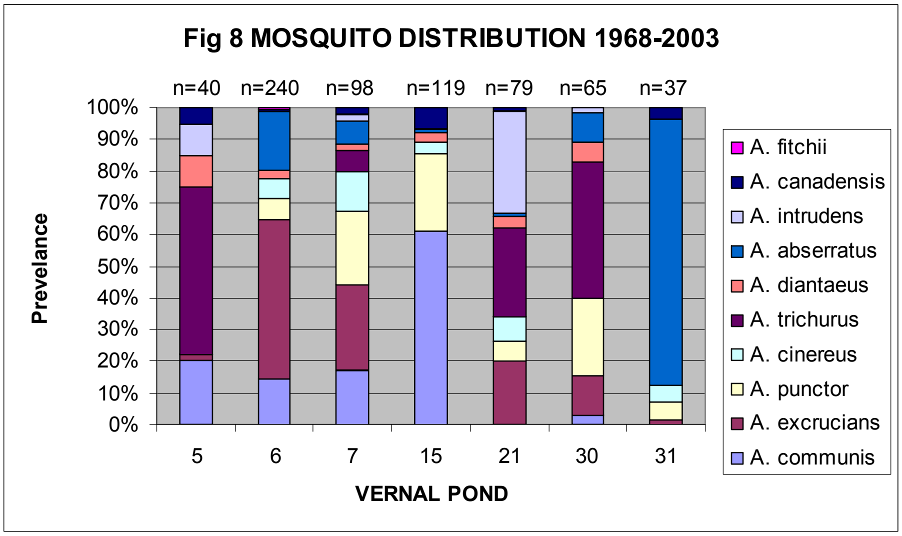

Fig 7 shows distribution of mosquito larvae collected during 1968-1970, 1972-1974, 1976-1979, and 2003 in seven vernal ponds listed on x axis. Graph shows relative abundance given by total larvae found of given species in each pond divided by total larvae found in each pond.

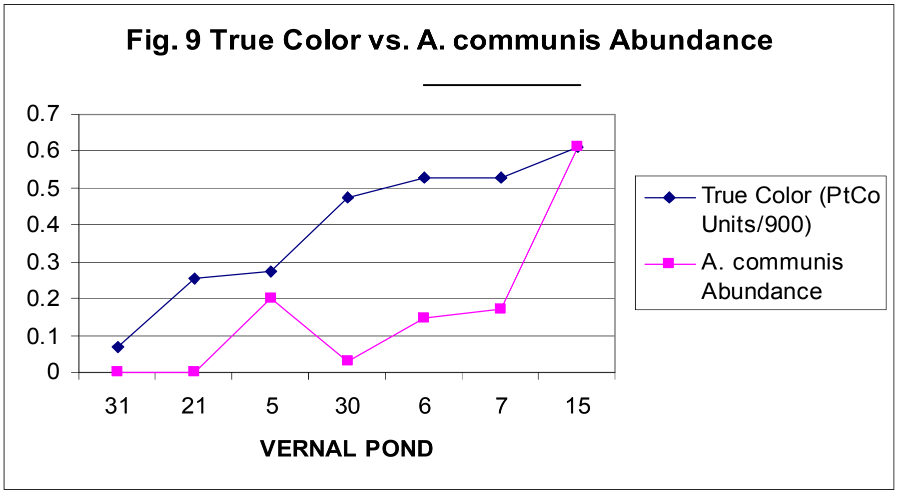

Fig 9 shows a direct relationship between true color and *A. communis* relative abundance. True color is shown in scaled units. TUKEY lines link statistically similar true color values.

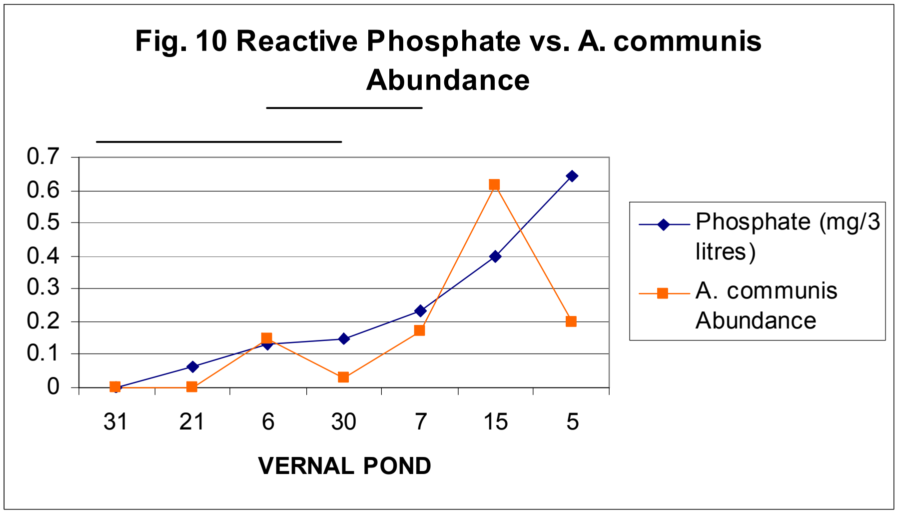

Fig 10 shows a direct relationship between reactive phosphate concentration and *A. communis* relative abundance. Phosphate is shown in scaled units. TUKEY lines link statistically similar phosphate values.

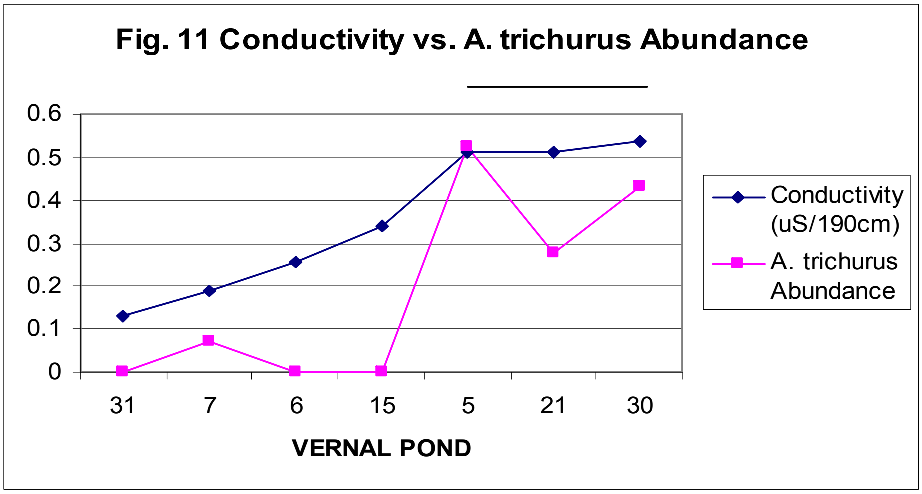

Fig 11 shows a direct relationship between conductivity and *A. trichurus* relative abundance. Conductivity is shown in scaled units. TUKEY lines link statistically similar conductivity values.

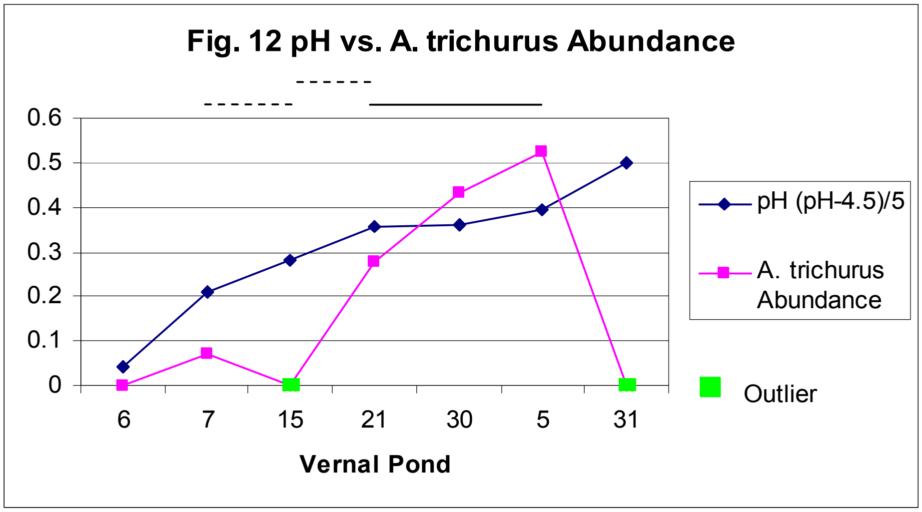

Fig 12 shows an intolerance for acidic waters in *A. trichurus* larvae. pH is shown in scaled units. TUKEY lines link statistically similar pH values. Ponds 31 and 15 are outliers in this trend, possibly due to low n values in Pond 15 and widely different other abiotic factors in Pond 31.

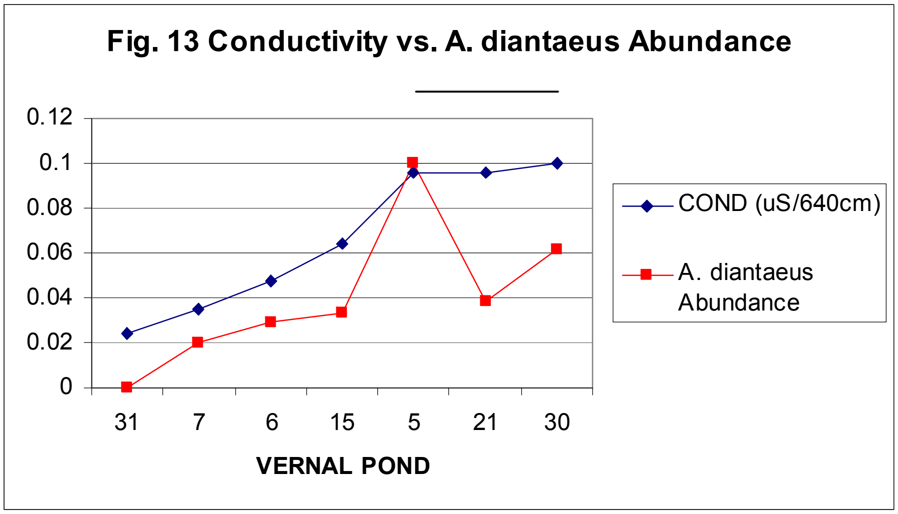

Fig 13 shows a direct relationship between conductivity and *A diantaeus* relative abundance. Conductivity is shown in scaled units. TUKEY lines link statistically similar conductivity values.

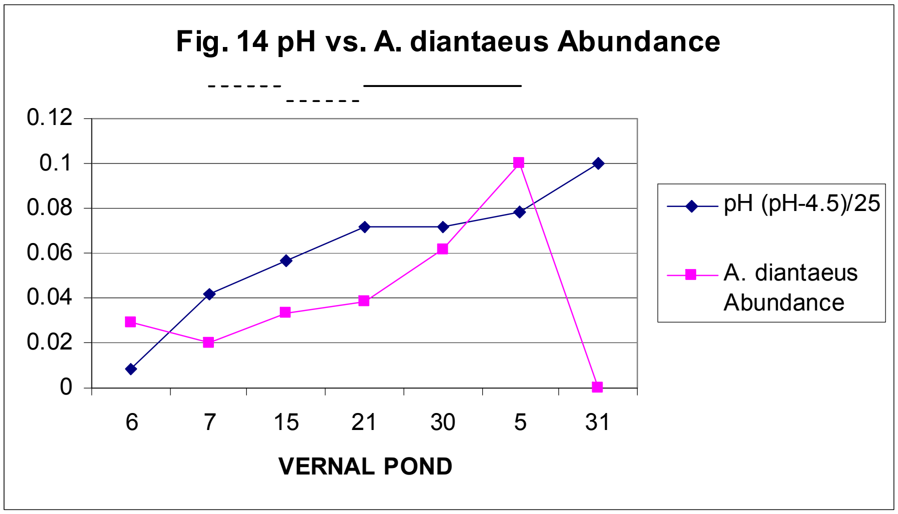

Fig 14 shows an intolerance for acidic waters in *A. diantaeus* larvae. pH is shown in scaled units. TUKEY lines link statistically similar pH values. Pond 31 is an outlier in this trend possibly due to other abiotic factors being widely different.