THE MICROSCOPIC FLORA AND FAUNA OF TREE HOLES*

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There are few if any natural environments on this earth without their inhabitants. This applies either to mammals or protistan organisms; the snowfields and the hot springs have algal populations, and the most acid streams may support a rich microscopic life.

In 1936, the writer was investigating the breeding of mosquitoes in North Alabama. In an old limestone sink whose shallow central pond was occupied by a grove of Tupelo gum (*Nyssa aquatica*) some twenty tree or stump holes were found in an area of about an acre. Most of these contained water, and various ones of them were breeding places for six species of mosquitoes. Because one of these mosquitoes, *Aedes thibaulti* Dyar and Knab, is relatively rare, and practically nothing was known of its breeding habits, a careful study was made of the flora and fauna of these tree and stump holes, (1). Since then, additional tree holes in Ohio have been investigated and their interesting inhabitants studied.

Tree holes that contain water are rare. In the spring, or after a long rainy spell, many will be partly filled with water, but most frequently they will either be filled with dirt and debris, or else the wood is too porous to hold water. Occasionally one will be found which is in effect a permanent pool. With reference to their contained water they may be grouped roughly as those in the tops of living stumps, those in crotches or in the tops of protruding limbs or knots, and those in the sides of trees or limbs. The first two types may catch considerable rain water, but the last usually gets only small amounts of water which runs down the side of the tree.

Holes so far studied, with few exceptions, have contained rain water, that is, have been free from contamination by surface ground water. But their fluid is rarely clear; either enough

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extracted material is picked up in running down the tree trunk, or else the debris and rotting wood in the hole provides enough so that a brown to black color obtains. Suspended materials are rare; the few determinations made of dissolved oxygen have shown variable amounts, but usually a high percentage of saturation. Light fluctuates with the size of the hole opening. Chemical analyses have not been made, beyond determinations for tannates and hydrogen ion concentration. Often a rather "sour" smell has been noted, and it was anticipated from this and because of suspected tannic acid that the pH would be on the acid side. However, hydrogen ion concentrations lower than pH 5.8 were not found, and in a few cases slight alkalinities obtained. For these latter, the strong odors indicated the presence of putrefactive processes yielding amines. Temperatures tended toward uniformity, that is, warmer in winter than surface ground waters and decidedly lower in summer. No macroscopic animals except insect larvae and mites have been found in any of the holes so far examined. In a few, weeds had sprouted and some contained moss.

This report covers observations on 26 tree holes, all in living trees and distributed as follows: Tupelo gum, 13; Black gum, 1; American elm, 3; Maple, 3; White oak, 2; Blackjack oak, 1; Red oak, 1; Sycamore, 1; and Sweetgum, 1. In addition, four tree holes containing water were examined and no living protozoa were found. In Table No. 1 are tabulated the data observed. Material from one of these latter subsequently developed a thriving culture of *Polytoma uvella* Ehrenberg. No observations were made on material from the remaining three. Three of the tree holes were periodically flooded by water from the pond in which the Tupelo gums were growing, and it is probable that when the Little Miami River is excessively high, the hole in the Sycamore is flooded.

Entrances to the holes varied from about eight inches in diameter to about one-half inch. One hole which contained water whenever visited, was about 20 inches deep, and two inches in internal diameter, with an opening about ¾ inch in diameter. It usually had about 8 inches of water and an inch of debris on the bottom.

About 140 species of algae and protozoa were recovered from these 30 holes. Since most of the holes were visited but once and since cultures from them usually developed additional species under laboratory conditions, it seems probable that a much larger list of organisms could be compiled. The hole which gave the greatest number of species was Number 3, which showed 63 species. This hole was periodically flooded, but its organisms were more abundant than in the pond, and many of them were not common to the pond. On one occasion its water was deep brown, due to enormous numbers of *Trachelomonas reticulata* Klebs. It contained such species as *Chrysococcus rufescens* Klebs, *Cryptochrysis commutata* Pascher, *Chroomonas acuta* Utermohl, *Euglena mutabilis* Schmitz, *Trachelomonas rugulosa* Stein, *Phacus hispidula* (Eichw.) Lemm., *Cryptoglena pigra* Ehrenb., *Astasia Klebsii*, Lemm., *Menoidium tortuosum* Stokes, and *Trigonomonas compressa* Klebs. These are forms which the writer has not found to be widespread in natural bodies of water. There was also a species of Trachelomonas and one of Menoidium not referable to described species. On the other hand, there were forms which were very common to decaying submerged vegetation, as *Chilomonas paramecium* Ehrenberg, and such cosmopolitan forms as *Cyclidium glaucoma* O. F. M.

The large species list for this particular hole might be accounted for by the chances for frequent entry of water from the pond. But the development therein, in large numbers, of forms not common to, or not observed in the pond at all, argues for the existence of specific microclimatic conditions in the tree hole favorable for such organisms. That this is the case is further borne out by other tree holes. Holes Numbers 10 and 12 had 16 and 34 species respectively, and were so high that only rain water could trickle in. Hole Number 10 was the breeding place for four species of mosquitoes, so it is surprising that its list of organisms comprises even 16 species. Its organisms were not unusual, but most of the Chlamydomonas and Chlorogonium in it were colorless or nearly so, and in a large population of *Blepharisma undulans* Stein, the pink color was either completely gone or partly so. Hole Number 12, with 34 listed species showed some unusual forms as *Acinetactis mirabilis* Stokes, about whose validity Pascher (2) is perhaps dubious, and *Dactylochlamys pisciformis* Lauterborn. Green flagellates were scarce in this hole, but it contained *Phacus triqueter* (Ehrenb) Dujardin—and they were all colorless or nearly so! The existence of these colorless, but apparently thriving forms of normally green flagellates is further evidence for specific microclimatic factors.

A comparison of the organisms in all holes shows a strong tendency to recur. It has been stated elsewhere (3) that only very few protozoa or flagellates might be reasonably expected in a random sample of a natural water, as a stagnant pool, or quiet spot close to a river bank. If we take the entire 30 holes examined for this study, some of the organisms occur with a relatively high frequency. This tendency is shown in Table I. Table II lists all the protozoa and algae found in the 26 holes containing living organisms. While the percentage of occurrence

TABLE I

THE LOCATION OF 26 TREE HOLES INVESTIGATED FOR MICROSCOPIC LIFE, SHOWING LOCATION, KIND OF TREE, HYDROGEN ION CONCENTRATION WHEN OBSERVED, AND LISTING THOSE SPECIES WHICH OCCURRED IN FOUR OR MORE OF THE HOLES

Hole No.	Location	Kind of Tree	pH	No. Species	*Oxytricha* sp.	*Halteria grandinella*	*Metopus* sp.	*Vorticella* sp.	*Distigma proteus*	*Menoidium incurvum*	*Colpoda aspera*	*Oicomonas* sp.	*Menoidium* sp.	*Chilodonella uncinatus*	*Cinetochilum margaritaceum*	*Cyclidium glaucoma*	*Astasia inflata*	*Chlamydomonas* sp.	*Bodo globosus*	*Hexamitus crassa*	*Polytoma uvella*	*Trachelomonas volvocina*
1	Alabama	White Oak		11	x		x	x	x								x					
2	"	Blackjack Oak		2								x		x								
3	"	Tupelo Gum	6.0	61	x	x	x	x	x	x		x	x	x		x	x	x		x		x
4	"	" "		3																	x	
5	"	" "		1								x										
6	"	" "	6.2	13	x	x		x							x						x	
7	"	" "	6.0	6				x							x		x					
8	"	" "		2						x												
9	"	" "		10			x		x	x										x		
10	"	Black Gum	6.1	16	x	x					x											
11	"	Tupelo Gum		4											x			x				
12	"	" "	6.0	35	x	x	x	x	x	x	x	x	x	x		x			x	x		x
13	"	" "	5.8	8			x	x														x
14	"	" "		12	x					x						x				x		x
15	"	" "	6.0	6		x		x		x			x									
16	"	Sweet Gum		35	x																	
17	"	Tupelo Gum	6.1	8		x			x									x	x			
18	"	White Oak		2					x				x	x	x	x	x	x	x		x	
19	Ohio	Maple	7.0	9					x		x											
20	"	Maple		4				x			x											
21	"	American Elm	7.2	1							x	x										
22	"	" "	7.2	6															x		x	
23	Kentucky	Red Oak		6																		
24	Ohio	American Elm	7.1	1																		
25	"	Sycamore	7.1	27			x	x														
26	"	Maple	7.1	1																		

in samples is not strikingly large, it is nevertheless decided, and is further corroboration of some sameness in these habitats.

The water in beech holes (Fagus sylvatica) was studied by von Brandt (4) and his findings indicate that the environment might be restrictive. He listed only six protozoa from tree holes, but Mayer (5) in a "large number" of beech holes found 34 additional species, 14 of which are included in our list. His list and ours contain many organisms characteristic of waters rich in organic contaminants.

It is interesting to speculate on how these forms first entered tree holes which now have no connection with ground water. Upward growth of the hole may be postulated in a few cases. Upward migration of some forms in the film of water along the bark in wet seasons may also be taken into account. Some of the forms are those which Unger (6) and others have shown to be found on vegetation in the form of cysts. But for others, cysts are as yet unknown, and these could hardly be transported to the tree holes by winds or animals. Perhaps a combination of methods is the easiest way to account for entrance. A few high tree holes were investigated but none had any water in them.

It is worth noting that algae, exclusive of the flagellated forms, were largely absent from these situations. Pleurococcus was probably the most frequent, but despite the diffuse light present in some of the situations, Protococcales were almost wholly lacking, only a few diatoms were seen, and no blue green algae were recorded. It may be inferred that there was too much organic matter present for the development of most algae, since several species of algae were found in the Tupelo gum pond, and at least some of the holes admitted enough light for chlorophyll bearers.

Altogether the collection of organisms studied herein is interesting because it exhibits a tendency to be an environmental group; because of its unusual forms; because of the tendency for some of the green flagellates to lose their chlorophyll and assume a saprophytic existence; and because of the evidence which it may offer for microclimatic factors in these small environmental niches.

TABLE II

LIST OF ALL ORGANISMS IDENTIFIED IN TWENTY-SIX TREE HOLES

BACILLARIEAE
- Pennales
  - Naviculineae
    - Navicula sp.
- Chrysophyceae
  - *Chrysococcus rufescens*

CRYPTOPHYCEAE
- *Chilomonas oblonga*
- *Chilomonas paramecium*
- *Chroomonas acuta*
- *Cryptochrysis commutata*
- *Cryptomonas erosa*
- *Cryptomonas ovata*
- *Cyathomonas truncata*

CHLOROPHYCEAE
- Volvocales
  - *Chlamydomonas* sp. 1
  - *Chlamydomonas* sp. 2
  - *Chlamydomonas* sp. 3 colorless
  - *Chlorogonium euchlora*
  - *Chlorogonium elongatum*
  - *Chlorogonium* sp. colorless
  - *Polytoma uvella*
  - *Polytomella citri*
- Ulrotrichales
  - *Protococcus viridis*
  - *Sphaeroplea* sp.
  - *Ulothrix zonata*
  - Unidentified algal filaments

EUGLENOPHYCEAE
- Euglenaceae
  - *Cryptoglena pigra*
  - *Euglena acutissimum*
  - *Euglena gracilis*
  - *Euglena gracilis* (?) colorless
  - *Euglena mutabilis*
  - *Euglena pisciformis*
  - *Euglena polymorpha*
  - *Euglena tripteris*
  - *Euglena viridis*
  - *Lepocinclis ovum*
  - *Phacus hispidula*
  - *Phacus longicauda*
  - *Phacus pyrum*
  - *Phacus Stokesii*
  - *Phacus triqueter*
  - *Phacus* sp., colorless
  - *Trachelomonas euchlora*
  - *Trachelomonas hispida*
  - *Trachelomonas intermedia*
  - *Trachelomonas reticulata*
  - *Trachelomonas rugulosa*
  - *Trachelomonas verrucosa*
  - *Trachelomonas volvocina*
  - *Trachelomonas* sp.
- Astasiaceae
  - *Astasia Dangeardi*
  - *Astasia inflata*
  - *Astasia Klebsii*
  - *Astasia* sp.
  - *Distigma proteus*
  - *Menoidium incurvum*
  - *Menoidium tortuosum*
  - *Menoidium* sp.
- Peranemaceae
  - *Anisonema ovale*
  - *Entosiphon ovatum*
  - *Entosiphon sulcatum*
  - *Heteronema acus*
  - *Notosolenus orbicularis*
  - *Peranema granulifera*
  - *Peranema ovalis*
  - *Peranema trichophorum*
  - *Petalomonas Steinii*
  - *Scytomonas pusilla*
  - Unidentified flagellates—several species

MASTIGOPHORA
- Pantostomatinae
  - *Acinetactis mirabilis*
  - *Bodopsis* sp.
  - *Cercobodo crassicauda*
  - *Cercobodo longicauda*
  - *Mastigamoeba reptans*

PROTOMASTIGINAE
- Oicomonadaceae
  - *Oicomonas obliqua*
  - *Oicomonas ocellata*
  - *Oicomonas sociabilis*
  - *Oicomonas Steinii*
  - *Oicomonas* sp.
- Craspedomonadaceae
  - *Monosiga ovata*
- Monadaceae
  - *Monas minima*
  - *Monas vivipara*
  - *Monas vulgaris*
- Bodonaceae
  - *Bodo angustus*
  - *Bodo globosus*
  - *Bodo lens*
  - *Pleuromonas jaculans*
  - Tetramitaceae
    - *Tetramitus pyriformis*
- Distomatinae
  - *Hexamitus crassus*
  - *Trepomonas agilis*
  - *Trepomonas rotans*
  - *Trigonomonas compressa*

SARCODINA
- Actinopoda
  - *Acanthocystis aculeata*
  - *Actinophrys sol*
  - *Heterophrys myriapoda*

TABLE II (*Continued*)

Rhizopoda
  Proteomyxa
    Nuclearia simplex
  Amoebaea
    Arcella vulgaris
    Amoeba radiosa
    Amoeba tachypodia (?)
    Amoeba sp. 1
    Amoeba sp. 2
    Centropyxis aculeata
    Cochliopodium bilimbosum
    Difflugia globosa
    Difflugia pyriformis
    Euglypha alveolata
    Hartmanella hyalina
    Trinema lineare
    Vahlkampfia albida
    Vahlkampfia guttula
    Vahlkampfia limax
INFUSORIA
  Ciliata
    Holotrichida
      Chilodonella uncinatus
      Cinetochilum margaritaceum
      Colpidium colpoda
      Colpoda aspera
      Cyclidium sp.
      Cyrtolophosis mucicola
      Dactylochlamys pisciformis
      Drepanomonas revoluta
      Drepanomonas sphagni
      Frontonia acuminata
      Holophrya discolor
      Lagnus simplex
      Lionotus fasciola
      Microthorax sulcatus
      Nassula aurea
      Spathidium spathula
    Heterotrichida
      Blepharisma undulans
      Metopus sigmoides
      Metopus sp.
      Saprodinium sp.
    Oligotrichida
      Halteria grandinella
      Strombidium sp.
    Hypotrichida
  Aspidisca costata
      Holosticha sp.
      Oxytricha fallax
      Oxytricha sp.
      Stylonichia pustulata
      Uroleptus sp.
    Peritrichida
      Opercularia sp.
      Pyxidium sp.
      Vorticella spp.
  Unidentified ciliates, several species

SUMMARY SHOWING DISTRIBUTION

Bacillarieae	1	Infusoria	
Chrysophyceae	1	Holotrichida	16
Cryptophyceae	71	Heterotrichida	4
Chlorophyceae		Oligotrichida	2
Volvocales	8	Hypotrichida	6
Ulotrichales	5	Peritrichida	3
Euglenophyceae	42	Flagellata	81
Pantastomatinae	5	Ciliata	31
Protomastiginae	14	Sarcodina	19
Distomatinae	4	Others	6
Sarcodina			
Actinopoda	3		
Rhizopoda	16		137

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