MFR PAPER 1148

A Brief Review of the Involvements of *Lagenidium*, an Aquatic Fungus Parasite, with Arthropods

CLYDE J. UMPHLETT and E.M. McCRAY, JR.

ABSTRACT—Several species of the genus Lagenidium, *an aquatic planomycetous fungus, have been reported as parasites of arthropods including crabs, barnacles, and mosquito larvae.* Lagenidium callinectes, *a superficial parasite of egg masses of the blue crab, has on some occasions been found on as many as 40 percent of the egg masses collected from Chesapeake Bay waters. The same fungus species has been found as a parasite in the ova of the barnacle* Chelonibia patula *in waters off the North Carolina coast. A second species,* Lagenidium chthamalophilum, *has been observed in 34 percent of the gill lamellae of the barnacle* Chthamalus fragilis. *A* Lagenidium *sp. has been observed in laboratory-reared brown shrimp,* Penaeus aztecus, *and white shrimp,* Penaeus setiferus. Lagenidium giganteum *has been shown to be a virulent pathogen of larvae of several species of culicine mosquitoes including* Aedes aegypti, *with over 90 percent of test larvae in laboratory experiments killed consistently.* Lagenidium giganteum *has been shown to be an effective larval pathogen under field conditions also, but does not appear to be as effective against anophelines as against culicines.*

Interest in environmental conservation in recent years has prompted a search for means of controlling populations of noxious organisms with other than chemical pesticides, and there is interest of long standing regarding diseases occurring in populations of desirable and profitable organisms such as certain crustaceans. Out of these two interest areas has arisen a small literature dealing with certain aquatic fungi among which is the genus *Lagenidium*. This aquatic fungus has a long history of parasitism, and several species have been recorded from a variety of hosts including algae, other fungi, certain insects, and some of the lower and higher crustaceans (Sparrow, 1960). Species of *Lagenidium* have been found in hosts from both freshwater and marine habitats.

Clyde J. Umphlett is with the Botany Department, Clemson University, Clemson, SC 29631 and E.M. McCray, Jr. is with Technical Development Laboratories, Center for Disease Control, Savannah, GA 31402.

CRUSTACEAN INFECTIONS

Lagenidium callinectes was described by Couch (1942) as being parasitic in ova of the blue crab, *Callinectes sapidus*. Johnson and Bonner (1960) reported the occurrence of the same fungus on lamellae of the barnacle, *Chelonibia patula*. Couch described the fungus mycelium as being intracellular in the crab ova, whereas Johnson and Bonner found that the preponderance of fungal hyphae in the barnacle was extramatrical. They concluded, however, since the fungus transferred readily from barnacle to blue crab eggs in cross inoculation experiments that the fungus on the barnacle was indeed *Lagenidium callinectes*. In a comprehensive study of the disease caused in blue crabs by *L. callinectes*, Rogers-Talbert (1948) described the fungus as a peripheral parasite of the egg masses. She noted that the eggs were susceptible to infection in all stages of development. The spread of the fungus over the sponge was rapid, but it usually appeared to penetrate no deeper than three millimeters. Development of the eggs at the interior of the sponge was not retarded by the infection. Heavily diseased sponges were infected to the extent that about 25 percent of the eggs in the mass contained the fungus, and in a given sample of experimental crabs some 80 to 90 percent exhibited some degree of infection. Development of the fungus was rapid at salinities between 5 and 30 ppt, but abnormal development was noted in fresh pond water. Rogers-Talbert observed also that eggs of the oyster and mud crab were attacked in the laboratory under conditions favoring very rapid transmission of the infection. Scott (1962), in a survey of the phycomycetous fungi of marine and brackish waters in the vicinity of Gloucester Point, Va., reported that 40 percent of the blue crab egg masses collected were infected with *Lagenidium callinectes*. Bland and Amerson (1973) surveyed over 2,000 ovigerous crabs during the summer of 1971 and obtained isolates of *L. callinectes* with which they performed a detailed morphological study, but did not report the extent of the fungus in the crab population.

Another marine species has been described by Johnson (1958). *L. chthamalophilum* in the barnacle *Chthamalus fragilis* was reported in 34 percent of all host lamellae inspected. This percentage of infection was based on hosts collected from piling and mooring stakes, since 86 barnacles of the same species collected from salt marsh cord grass exhibited only three infections with *L. chthamalophilum*. Attempts to infect the barnacle, *Balanus amphitrite*, with fungus material from *C. fragilis* were unsuccessful.

Lightner and Fontaine (1973) recently observed that a *Lagenidium* sp. was infective to larval white shrimp, *Penaeus setiferus*, and a brown shrimp, *Penaeus aztecus*, reared under laboratory conditions. Natural mortality occurred in 12.4 percent of the shrimp after the fungal mycelium had invaded and replaced nearly all the internal tissues, while 20.0 percent of the larval shrimp died after experimental exposure to the fungus.

INFECTIONS IN OTHER ARTHROPODS

Couch (1935) described in North Carolina the only species of

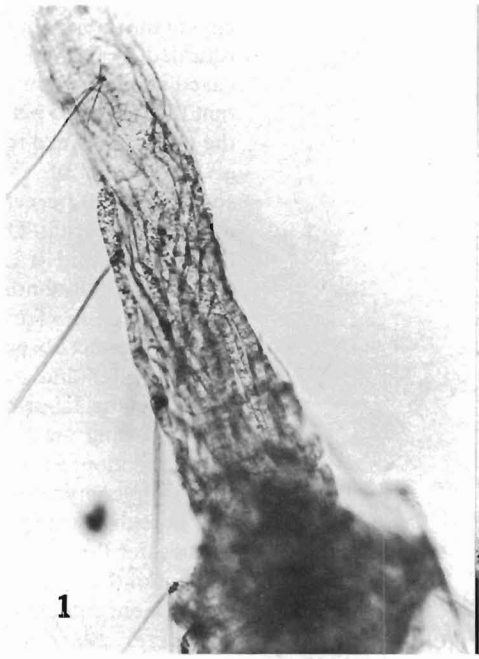

Figure 1.—(*Lagenidium giganteum* parasitizing *Culex restuans*): Non-septate hyphae growing in abdominal hemicoele. 125×.

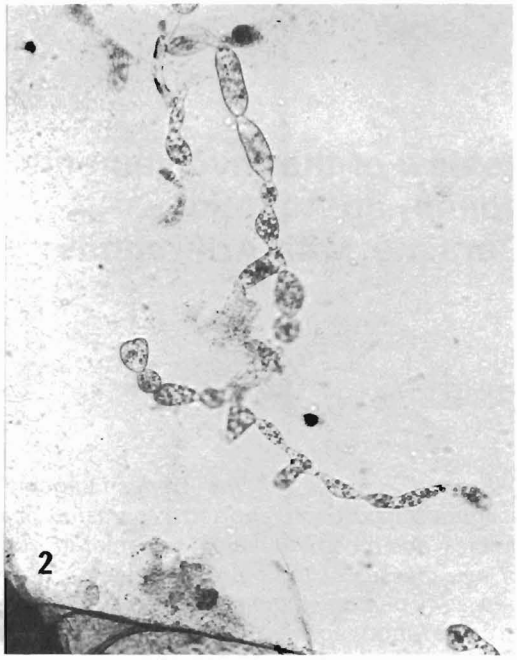

Figure 2.—(*Lagenidium giganteum* parasitizing *Culex restuans*): Septate hyphae dissected from host; each cell is potentially a sporangium, 125×.

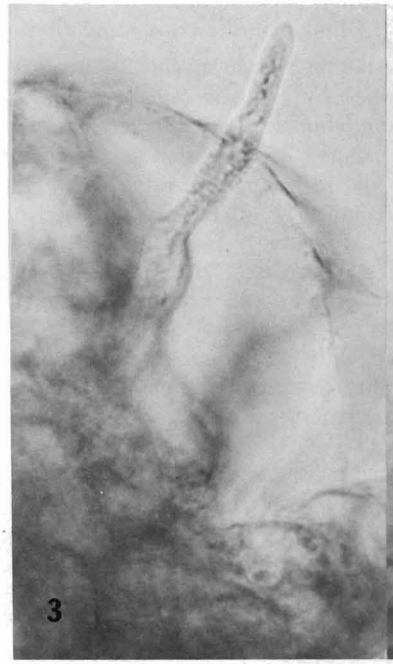

Figure 3.—(*Lagenidium giganteum* parasitizing *Culex restuans*): Discharge tube forming from sporangium and penetrating exoskeleton of host cadaver. 600×.

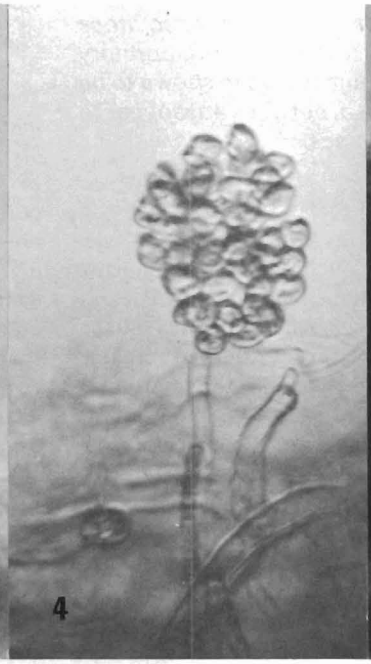

Figure 4.—(*Lagenidium giganteum* parasitizing *Culex restuans*): Biflagellate zoospores swarming in vesicle just prior to release. 600×.

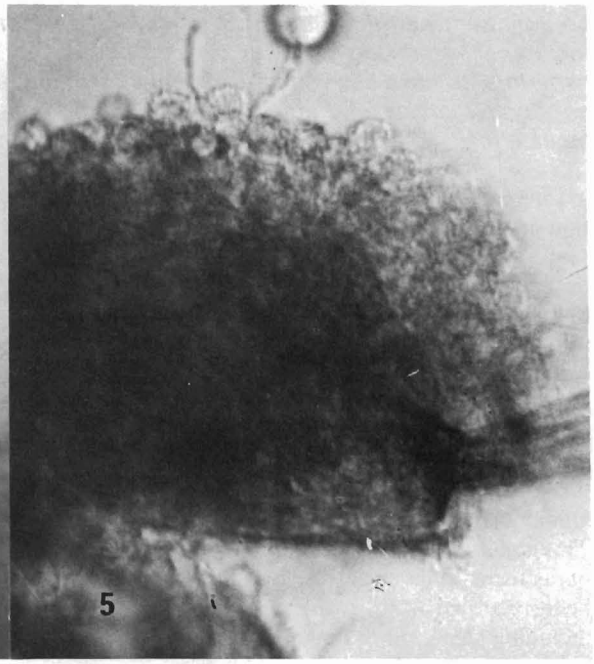

Figure 5.—(*Lagenidium giganteum* parasitizing *Culex restuans*): Cluster of encysted zoospores on anal segment; infection initiated here. 600×.

Lagenidium reported thus far to occur as a parasite in mosquito larvae. *L. giganteum* was described as a saprophyte which could function as a weak, facultative parasite of culicine mosquitoes. Willoughby (1969) reported on the nutrition of a saprophytic strain of this species which he had isolated on termite wings from a stream in England, but he did not cite any parasitic relationship of his isolate with mosquito larvae. One of the authors (CJU) isolated into pure culture two strains of what was apparently *L. giganteum* in 1963. One of the strains originated from a parasitized culicine larva, the other from a parasitized anopheline larva. Both were from Orange County, N.C. A very brief and unreported test of the fungus strain from the culicine larva at that time indicated that the fungus could

infect larvae of *Aedes aegypti*, but this line of work was not pursued until 1969 when an isolate of *L. giganteum* was obtained from a culicine larva from one of the original habitats.

An infection of a mosquito by *L. giganteum* results in the development of mycelium consisting of narrow, branching hyphae (Fig. 1) which soon increase in diameter and become septate. The hyphal segments resulting from the septations swell, thereby producing hyphae that are constricted at the septa (Fig. 2). Within 72 h after infection has occurred, the coelomic cavity of the larva is about filled with mycelial growth, and in many instances hyphae can be seen growing in the aorta of the insect. Death of the larva occurs at this time. About 24 h after an infected larva is dead, zoospore production is initiated by the fungus. The hyphal segments produce thin discharge tubes that penetrate the exoskeleton of the dead insect (Fig. 3). Through these tubes the cytoplasm contained in the segments is discharged to the outside where it is retained for a few minutes in a membranous vesicle. Cleavage of the cytoplasm occurs in the vesicle, and the biflagellate zoospores formed there escape when the vesicle breaks down (Fig. 4). The zoospore is the infectious agent (Fig. 5).

In the first report of experimentation with *L. giganteum* against mosquito larvae, Umphlett and Huang (1972) noted that this isolate behaved as a virulent parasite of *C. restuans* in laboratory tests. They found that the level of infection in larval populations varied with the amount of inoculum which was supplied as zoospores. Over 90 percent of 4-day-old larvae subjected to ca. 0.5 million zoospores (3 units) per larval culture were killed within 72 h after inoculation, whereas 10-day-old larvae with the same quantity of inoculum were stricken only at a 5 percent level (Fig. 6). However, in tests using ca. 1.5 million zoospores (9 units) per larval culture over 90 percent of larvae at all ages tested up to 10 days were killed (Fig. 6). It was noted also that when the host population was doubled and held in the same size container, larval mortality was three times that of the control when 0.5 (3 units) or 1.0 million zoospores (6 units) were utilized. When 1.5 million zoospores (9 units) were applied, mortality above 90 percent prevailed in all tests regardless of host density or larval

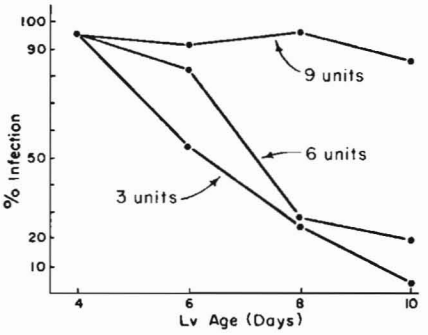

Figure 6.—Effect of larval (Lv) age in *Culex restuans* on the quantity of *Lagenidium giganteum* inoculum required to kill larvae. Note that 1.0 million zoospores equals 6 units. From Umphlett and Huang, 1972.

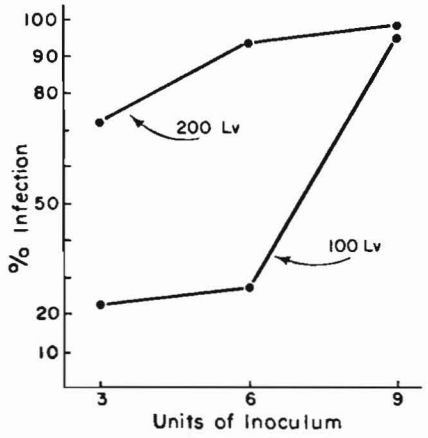

Figure 7.—Effect of larval (Lv) population density (*Culex restuans*) on the level of infection by *Lagenidium giganteum* at various concentrations of inoculum. Note that 1.0 million zoospores equals 6 units. From Umphlett and Huang, 1972.

age (Fig. 7). In a short preliminary field test, Umphlett and Huang (1972) reported that 43 percent of the larvae of *C. restuans* collected from the experimental pool three days after the introduction of inoculum were infected, 8 percent of the *Anopheles* sp. larvae in the same collection were infected, and 4 percent of the larvae of *Psorophora* sp. were infected with the test fungus. Larvae of *Anopheles* sp. occurred in three subsequent collections, but *C. restuans* larvae did not appear in any sample after the first. Umphlett and Huang (1972) suggested that *L. giganteum* was not strikingly effective against *Anopheles* spp. McCray, Umphlett, and Fay, (1973) subsequently corroborated this by reporting no mortality in *Anopheles* spp. tested. However, Giebel and Domnas (In press) reported that they were able to obtain up to 85 percent mortality of *Anopheles quadrimaculatus* in some tests, but remarked that in some experimental series no infection or only a low rate, from 5 to 10 percent, was obtained. McCray, Umphlett, and Fay, (1973) extended the known host range of *L. giganteum* to include *Aedes aegypti*, *Ae. mediovittatus*, *Ae. taeniorhynchus*, *Ae. triseriatus*, *Ae. sollicitans*, *Culex quinquefasciatus*, *Cu. tarsalis*, *Cu. fatigans*, and *Cu. nigripalpus*.

In a recent small field test with *L. giganteum* (McCray, Womeldorf, et al., 1973), two distinctly different habitats in California were utilized. The site near Hanford was intermittently dry and flooded irrigated pasture land in which *Aedes nigromaculis* was the principal mosquito species present. The fungus was applied in the test area by spraying sporangia into the water from a back-pack sprayer. To every square foot of water surface a number of sporangia approximating the number produced by the fungus in one infected fourth instar larva was applied, a potential of about 250,000 zoospores per square foot. Infection of the natural populations of *Ae. nigromaculis* in the test areas did occur, and all infected specimens died. Field populations were dramatically reduced within three days after treatment (Table 1). At this same test site, larvae of *C. tarsalis* appeared in the treated areas subsequent to the test. These larvae became infected by the fungus, and all animals collected were found to be infected and subsequently died.

The second study site, near Colusa, Calif., was in the vicinity of rice fields and associated drainage ditches. The test sites were not in the rice fields proper, but rather were isolated ditches nearby. *C. tarsalis* was the target organism in this area and three experimental sites were chosen. The water in Site No. 1 contained a high level of dissolved solids and had a pH of 10.0, while Site No. 2 had a pH of approximately 8.0, and water qualities here resembled those of the rice fields and drainage

Table 1.—The number of living *Aedes nigromaculis* larvae collected and found infected following introduction of *Lagenidium giganteum* at the site near Hanford, Calif., 1972. From McCray, Womeldorf, et al., 1973.

Sampling plot	Day of treatment	Post-treatment days	
		3	4
Test 1	411	0	0
Test 2	321	3[1]	0
Test 3	309	0	0
Control 1	367	75	24

[1]All three larvae died and were infected with *Lagenidium*.

ditches. At Site No. 3 the chloride ion concentration was about 25 times that of the normal habitat in which *C. tarsalis* breeds. Table 2 shows the number of living larvae of *C. tarsalis* collected and found infected following the introduction of *L. giganteum* in Sites 1, 2, and 3. It can be seen that in Site 2, which most nearly resembled the normal breeding habitat of the mosquito, a single introduction of the fungus infected and eliminated the natural population of *C. tarsalis*. The effect of the fungus on mosquito larvae was reduced, though, in Sites 1 and 3 in which water analyses had revealed conditions known to be detrimental to the fungus. Table 3 shows the mean daily pre- and post-treatment collections of living *C. tarsalis* larvae and pupae from Site 2 inoculated with *L. giganteum*. It should be noted that on the fifth post-treatment day no living larvae or pupae were collected, and none appeared as late as the seventeenth post-treatment day when the test was terminated.

During these studies more than 1,400 aquatic non-target organisms (small crustaceans and insects) from the treated sites were examined. No infection was observed in any of these specimens. Results of recent pathogenicity tests using *L. giganteum* at the Center for Disease Control, Atlanta, Ga.,[1] indicate that the fungus is not pathogenic to small mammals.

Umphlett and Huang (1972) offered the opinion that there is sufficient promise to dictate that further studies aimed at realization of the full potential of *L. giganteum* as an agent for the biological control of mosquitoes are feasible and desirable. McCray, Womeldorf, et al. (1973) stated that their studies revealed that the Umphlett strain of *L. giganteum* is an excellent candidate for further evaluation as a biological control agent, and that more definitive tests are in order.

[1]Ajello, L. Chief, Medical Mycology Section, Center for Disease Control, Atlanta, Ga. Pers. commun.

Table 2.—The number of living *Culex tarsalis* larvae collected and found infected following introduction of *Lagenidium giganteum* at sites near Colusa, Calif., 1972. From McCray, Womeldorf, et al., 1973.

Day after treatment	2	3	4	5	Total
Site #1					
Larvae collected	388	399	206	198	1,191
Larvae infected	0	100	3	2	105
Percent infected	0	25.5	1.4	1.0	8.8
Site #2					
Larvae collected	146	101	8	0	255
Larvae infected	146	101	8	-	255
Percent infected	100	100	100	-	100
Site #3					
Larvae collected	114	81	45	46	286
Larvae infected	0	15	3	4	22
Percent infected	0	18.5	6.7	8.7	7.6

Table 3.—Mean daily pre-and post-treatment collections of living *Culex tarsalis* larvae and pupae from Colusa site #2 inoculated with *Lagenidium giganteum*. From McCray, Womeldorf, et al., 1973.

Day	−4	−3	−2	−1	0[1]	+1	+2	+3	+4	+5	+17
Control[2]	110	122	102	125	88	72	93	80	123	112	111
Test[3]	96	96	78	93	89	88	51	36	5	0	0

[1]Day of inoculation.
[2]All instars from two plots combined.
[3]All instars from three plots combined.

LITERATURE CITED

Bland, C. E., and H. V. Amerson. 1973. Observations on Lagenidium callinectes: Isolation and sporangial development. Mycologia 65:310-320.

Couch, J. N. 1935. A new saprophytic species of *Lagenidium*, with notes on other forms. Mycologia 27:376-387.

_____. 1942. A new fungus on crab eggs. J. Elisha Mitchell Sci. Soc. 58:158-162.

Giebel, P. E., and A. J. Domnas. In press. Infection of *Anopheles quadrimaculatus* with *Lagenidium* sp. J. Invertebr. Pathol.

Johnson. T. W., Jr. 1958. A fungus parasite in ova of the barnacle Chthamalus fragilis denticulata. Biol. Bull. (Woods Hole) 114:205-214.

Johnson, T. W., Jr., and R. R. Bonner, Jr. 1960. *Lagenidium callinectes* Couch in barnacle ova. J. Elisha Mitchell Sci. Soc. 76:147-149.

Lightner, D. V., and C. T. Fontaine. 1973. A new fungus disease of the white shrimp *Penaeus setiferus*. J. Invertebr. Pathol. 22:94-99.

McCray, E. M., Jr., C. J. Umphlett, and R. W. Fay. 1973. Laboratory studies on a new fungal pathogen of mosquitoes. Mosquito News 33:54-60.

McCray, E. M., Jr., D. J. Womeldorf, R. C. Husbands, and D. A. Eliason. 1973. Laboratory observations and field tests with *Lagenidium* against California mosquitoes. Proc. Calif. Mosq. Control Assoc. 41:123-128.

Rogers-Talbert, R. 1948. The fungus *Lagenidium callinectes* Couch (1942) on eggs of the blue crab in Chesapeake Bay. Biol. Bull. (Woods Hole) 95:214-228.

Scott, W. W. 1962. The aquatic phycomycetous flora of marine and brackish waters in the vicinity of Gloucester Point, Virginia. Va. Inst. Mar. Sci., Spec. Sci. Rep. 36, 16 p.

Sparrow, F. K., Jr. 1960. Aquatic phycomycetes, 2nd ed. Univ. Michigan Press, Ann Arbor, 1187 p.

Umphlett, C. J., and C. S. Huang. 1972. Experimental infection of mosquito larvae by a species of the aquatic fungus *Lagenidium*. J. Invertebr. Pathol. 20:326-331.

Willoughby, L. G. 1969. Pure culture studies on the aquatic phycomycete, *Lagenidium giganteum*. Trans. Br. Mycol. Soc. 52:393-410.

MFR Paper 1148. From Marine Fisheries Review, *Vol. 37, Nos. 5-6, May-June 1975. Copies of this paper, in limited numbers, are available from D83, Technical Information Division, Environmental Science Information Center, NOAA, Washington, DC 20235. Individual copies of* Marine Fisheries Review *are available from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402 for $1.10 each.*