dimer of the 57- and 30-kD polypeptides (Fig. 3B). The catalytic properties of purified component I were examined by ^{31}P nuclear magnetic resonance and reversed-phase HPLC. In the presence of Mg^{2+}, CoA, and 4-CBA, component I catalyzes the cleavage of ATP to AMP and PP_i coupled with the formation of the 4-HBA:CoA adduct (the partial reactions 1, 2, and 3 of Scheme 3). Catalysis of ATP cleavage (partial reaction 1, Scheme 3) did not occur in the absence of CoA. Component I, in combination with component II, Mg^{2+}, and CoA, gave complete conversion of 4-CBA and ATP to 4-HBA, AMP, and PP_i.

Component II was purified from the Pst I–Pst I–pT7.6 *E. coli* subclone (Fig. 1A). Fractionation of the 40 to 70% ammonium sulfate protein cut on a DEAE-cellulose column, followed by gel filtration on a calibrated FPLC Superose 12 column, provided pure component II (Fig. 3B). Component II is a ~65-kD protein (α_4 tetramer) that catalyzes the hydrolysis of synthetic 4-HBA:CoA thioester (*26*) to 4-HBA and CoA.

The 4-CBA dehalogenase activity is thus a sum of the activities of a 4-CBA:CoA ligase, a 4-CBA:CoA dehalogenase, and a 4-HBA:CoA thioesterase. We note the role that the dehalogenase sequence data played in the isolation of the active enzyme; even the short motif shown in Fig. 2 allowed us to infer the cosubstrate and cofactor for this reaction. We expect that, as protein sequence databases expand, discoveries of this type could become routine and the characterization of new sequences could be facilitated.

REFERENCES AND NOTES

1. D. A. Abramowicz, *Crit. Rev. Biotechnol.* **10**, 241 (1990); L. C. M. Commandeur and J. R. Parsons, *Biodegradation* **1**, 207 (1990).
2. U. Klages and F. Lingens, *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1: Orig. Reihe C* **1**, 215 (1980).
3. _____, *FEMS Microbiol. Lett.* **6**, 201 (1979).
4. W. J. J. van den Tweel, N. ter Burg, J. B. Kolz, J. A. M. duBont, *Appl. Microbiol. Biotechnol.* **25**, 289 (1986).
5. R. Müller, R. H. Oltmanns, F. Lingens, *Biol. Chem. Hoppe-Seyler* **369**, 567 (1988); T. S. Marks, A. R. W. Smith, A. V. Quirk, *Appl. Environ. Microbiol.* **48**, 1020 (1984).
6. R. Müller, J. Thiele, U. Klages, F. Lingens, *Biochem. Biophys. Res. Commun.* **124**, 178 (1984).
7. T. S. Marks, R. Wait, A. R. W. Smith, A. V. Quirk, *ibid.*, p. 669.
8. J. Thiele, R. Müller, F. Lingens, *FEMS Microbiol. Lett.* **41**, 115 (1987); A. Elsner, F. Löffler, K. Miyashita, R. Müller, F. Lingens, *Appl. Environ. Microbiol.* **57**, 324 (1991).
9. P. Savard, L. Péloquin, M. Sylvestre, *J. Bacteriol.* **168**, 81 (1986).
10. H. Charest and M. M. Sylvestre, unpublished data.
11. S. Tabor and C. C. Richardson, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1074 (1985).
12. The 4.5-kb insert was sequenced as two separate fragments. A Sal I–Sal I 3.0-kb fragment was cloned into pUC19. Sequencing clones were generated from this plasmid by the method of nested deletions (*13*) (Amersham kit) and analyzed by restriction digest and agarose gel electrophoresis. We performed oligonucleotide sequencing by using the dideoxy chain termination method with the modified form of T7 DNA polymerase (*14*) and [^{35}S]deoxyadenosine triphosphate (dATP) (Sequenase kit of U.S. Biochemical Corp.). Universal and synthetic oligonucleotides were used as primers. The 1.5-kb Sma I–Sal I fragment was cut with restriction endonucleases into four overlapping fragments. The fragments were cloned into M13 mp18 or M13 mp19. We sequenced the oligonucleotides by using the dideoxy chain termination method (Amersham M13 sequencing kit) with [^{35}S]dATP. The nucleotide positions assigned to ORF I (30-kD polypeptide) and ORF II (57-kD polypeptide) have been confirmed by NH_2-terminal peptide sequencing of the purified translation products. The nucleotide positions of ORF III (19-kD polypeptide) await confirmation or adjustment based on the NH_2-terminal sequence of the observed 16-kD translation product.
13. S. Henikoff, *Gene (Amsterdam)* **28**, 351 (1984).
14. S. Tabor and C. C. Richardson, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4767 (1987).
15. K. Hori *et al.*, *J. Biochem. (Tokyo)* **106**, 639 (1989).
16. J. R. deWet, K. V. Wood, M. DeLuca, D. Helinski, S. Subramani, *Mol. Cell. Biol.* **7**, 725 (1987).
17. E. Lozoya *et al.*, *Eur. J. Biochem.* **176**, 661 (1988).
18. R. Weckermann, R. Fürbass, M. A. Marahie, *Nucleic Acids Res.* **16**, 11841 (1988).
19. J. F. Staab, M. F. Elkins, C. F. Earhart, *FEMS Microbiol. Lett.* **59**, 15 (1989).
20. D. H. Farrell, P. Mikesell, L. A. Actis, J. H. Crosa, *Gene (Amsterdam)* **86**, 45 (1990).
21. K.-H. Knobloch and K. Hahlbrock, *Arch. Biochem. Biophys.* **184**, 237 (1977).
22. G. C. Woodrow, I. G. Young, F. Gibson, *Biochim. Biophys. Acta* **582**, 145 (1979); G. F. Bryce and N. Brot, *Biochemistry* **11**, 1708 (1972); K. T. Greenwood and R. K. J. Luke, *Biochim. Biophys. Acta* **454**, 285 (1976); F. Rusnak, W. S. Faraci, C. T. Walsh, *Biochemistry* **28**, 6827 (1989).
23. M. Kanda, K. Hori, T. Kurotsu, S. Miura, Y. Saito, *J. Biochem. (Tokyo)* **105**, 653 (1989).
24. R. Roskoski, W. Gevers, H. Kleinkauf, F. Lipman, *Biochemistry* **9**, 4839 (1970); *ibid.*, p. 4846.
25. M. DeLuca and W. D. McElroy *Methods Enzymol.* **57**, 3 (1978).
26. S. M. Merkel, A. E. Eberhard, J. Gibson, C. S. Harwood, *J. Bacteriol.* **171**, 1 (1989).
27. C. B. Lawrence and D. A. Goldman, *Comput. Appl. Biosci.* **4**, 25 (1988); W. R. Pearson and D. J. Lipman, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2444 (1988).

28. We thank S. Tabor for providing the T7 expression system and P. Mariano, D. Julin, J. Kozarich, and J. Gerlt for comments regarding the manuscript. Supported by the National Sciences and Engineering Research Council of Canada, by the fonds pour la Formation de Chercheurs l'Aide à la Reserche du Quebec, and by the NIH (grant GM 28688).

22 February 1991; accepted 2 May 1991

Regulatory Role of Parasites: Impact on Host Population Shifts with Resource Availability

JAN O. WASHBURN, DAVID R. MERCER, JOHN R. ANDERSON

Effects of infections by the ciliate *Lambornella clarki* on larval populations of its mosquito host *Aedes sierrensis* were examined in laboratory and field studies. When host populations developed with sufficient food, mortality from parasites was additive and reduced the number of emerging mosquitoes. For food-limited populations, mortality was compensatory or depensatory; emerging adults were as or more abundant with higher average fitness than those from uninfected control populations. When nutrients were scarce, parasitic infections relaxed larval competition and increased per capita food by reducing host abundance. Food limitation altered larval feeding behavior, reducing horizontal transmission and subsequent mortality from parasitism.

DESPITE THE WIDESPREAD OCCURrence of parasites and the diseases they cause, few quantitative data are available on how these organisms affect host abundance in nature (*1*). Assessing whether parasites regulate host populations is challenging because their impact cannot be inferred from incidence rates alone and because comparative evidence from infected and non-infected populations is extraordinarily difficult to obtain; consequently, models describing the effects of parasites on host population dynamics have relied heavily on laboratory studies (*1*). Theoretically, parasitism can result in a reduction, no change, or even an increase in host abundance; such host mortality effects are termed additive, compensatory, and depensatory, respectively. Determining the frequency and importance of these host population responses in nature and their underlying mechanisms are critical and formidable tasks for both theoretical and applied ecologists (*1*). Moreover, understanding these mortality patterns is crucial for implementing effective biological control strategies.

Despite these difficulties, by creating host and parasite populations in laboratory microcosms and manipulated natural habitats, we have demonstrated that the impact of fatal infections by the parasitic protozoan, *Lambornella clarki* (Ciliophora: Tetrahymenidae), inflicted on populations of its natural mosquito host, *Aedes sierrensis* (Diptera: Culicidae), changes with different resource regimes. Interactions between the effects of food limitation and parasitism as

Department of Entomology, University of California, Berkeley, CA 94720.

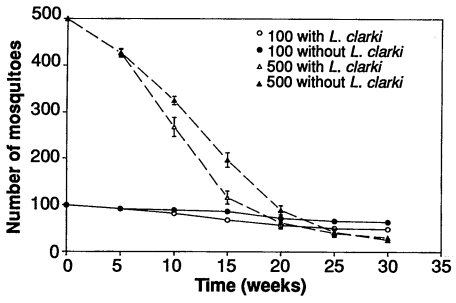

Fig. 1. Numbers of surviving *A. sierrensis* in high- and low-density populations developing in the presence and absence of *L. clarki* infections. Points represent means (± 1 SEM) of 10 high-density or 12 low-density populations. Survivors include the cumulative number of pupae removed plus surviving larvae in each population. The average numbers of surviving mosquitoes in high-density populations with *L. clarki* were significantly lower than in control populations at 10 weeks [ANOVA, $F(1,22) = 6.38$, $P < 0.02$] and 15 weeks [ANOVA, $F(1,22) = 14.68$, $P < 0.001$].

regulating forces in host population dynamics resulted in a shift from additive to compensatory or depensatory parasite mortality. The differential impact of parasitism suggests that *L. clarki* modulates the outcome of intraspecific competition for food during the prolonged period of larval development.

Lambornella clarki infects larvae of *A. sierrensis* in water-filled treeholes of western North America during the winter rainy season (*2, 3*). In nature, mosquito development is protracted and can require 7 months during which larval populations may experience mortality from both resource limitation (*4, 5*) and multiple parasite cycles of *L. clarki* (*6, 7*). Shortly after treeholes accumulate rainwater, the first cycle begins when free-living trophont forms of *L. clarki* transform into host-seeking theronts that attach to first instar larvae, penetrate the cuticle, and enter the hosts' hemocoels (*6*). Endoparasitic ciliates multiply and produce infections that are fatal to hosts in about 3 to 4 weeks. Horizontal transmission results from trophonts that escape from moribund hosts, transform into theronts, and attack surviving larvae. Successful horizontal transmission is critical for *L. clarki* because, in addition to being hosts, *A. sierrensis* larvae are also predators of *L. clarki*; all larval stages except newly hatched first instars consume free-swimming ciliates during filter feeding (*3*). Although *L. clarki* epizootics eliminate host populations in some treeholes, seasonal incidence levels in most habitats are low (*7*), and determining whether mortality from ciliate infections regulates *A. sierrensis* populations is a complex problem.

Table 1. Winglengths of *A. sierrensis* adults that emerged from larval populations developing in the presence and absence of *L. clarki* infections. Values are means (± 1 SEM) of 10 high-density or 12 low-density replicates; only 9 high-density populations without parasites produced adult females. Data were analyzed by one-way ANOVA ($P < 0.05$).

Larval density	*L. clarki*	Winglength (mm)	*P*
		Males	
Low	−	2.54 ± 0.08	0.97
Low	+	2.54 ± 0.03	
		Females	
Low	−	3.06 ± 0.10	0.23
Low	+	3.01 ± 0.11	
		Males	
High	−	2.31 ± 0.07	<0.04
High	+	2.40 ± 0.09	
		Females	
High	−	2.71 ± 0.07	<0.02
High	+	2.97 ± 0.19	

In laboratory microcosm studies with different host densities, we compared the dynamics of *A. sierrensis* larval populations that experienced *L. clarki* enzootics with those of control populations without parasites. Microcosms were maintained in an environmental chamber with natural photoperiod and temperature regimes that permitted fourth instar larval diapause and horizontal transmission of *L. clarki* (*8*). An initial population of either 100 (low density) or 500 (high density) first instar *A. sierrensis* was placed in each microcosm, and enzootics were established by the addition of small numbers of *L. clarki* (*9*). We censused populations and added food to microcosms at 5-week intervals; the total food added was the same for each microcosm and was sufficient for most individuals in low-density populations to successfully complete development but severely limiting for those at the higher density (*10*). Effects of parasitism were evaluated by comparing the number, size, and infection status of emerged adult mosquitoes (*11*).

In this microcosm study, parasite enzootics at the two population densities had strikingly different effects on successful host development. For low-density populations, significantly fewer mosquitoes emerged from microcosms with *L. clarki* compared to controls [48 versus 64%, ANOVA, $F(1, 22) = 4.93$, $P < 0.03$] indicating that the major impact of the parasite was additive host mortality. In contrast, mortality from parasitism appeared to be compensatory for high-density populations because there was no statistically significant difference in emergence success between treatment and control populations [6.1 versus 5.1%, ANOVA, $F(1, 22) = 0.70$, $P = 0.41$]. However, the average adult produced from high-density populations was significantly larger when parasites were present compared to controls, an effect we did not observe in low-density populations (Table 1).

Since larger females produce more eggs per clutch and live longer in nature (*4*), females from high-density populations with *L. clarki* had a higher average fitness than those from controls. The mechanism for this shift to larger body size appears to be relaxation of resource competition during larval development mediated by parasite-induced mortality. Adult mosquito size is a plastic phenotypic trait that is tightly correlated with larval food resources (*12*). Census data show that for several months during larval development, the average population size in high-density microcosms with parasites was lower than controls (Fig. 1). However, survivorship curves later converged, resulting in comparable rates of adult emergence. Before nutrients became severely limiting, fatal *L. clarki* infections reduced host abundance during early development and ultimately increased the per capita food for surviving larvae which developed into adults that were almost as large as those from low-density populations. In high-density microcosms without parasites, population densities and resource competition remained high, resulting in the production of

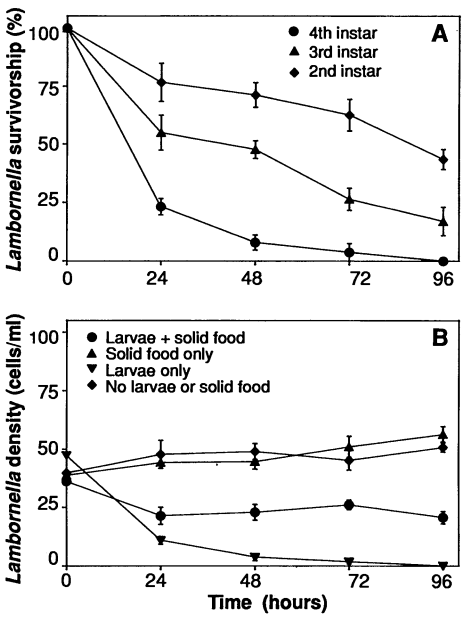

Fig. 2. (**A**) Survivorship rates for *L. clarki* trophonts introduced into microcosms with *A. sierrensis* populations comprised of different larval stages. The same number of larvae was placed in each microcosm. Each point represents the mean trophont survivorship (±1 SEM) for six replicated microcosms. Trophont densities were determined by thoroughly mixing the contents of each microcosm, removing and staining a 1.5-ml water sample, and counting the number of ciliates in a 1.0-ml subsample. Trophont densities were divided by the density at time = 0 to determine survivorship in each microcosm. (**B**) Survivorship of *L. clarki* trophonts introduced into microcosms with larvae of *A. sierrensis* in the presence and absence of a substrate food source. Each point represents the mean trophont density (±1 SEM) for six replicated microcosms, and densities were determined as above.

smaller adults. Within low-density populations, adults from both treatment and control microcosms were of similar size because food was much less limiting (Table 1).

Infection rates in adult mosquitoes suggest that horizontal transmission of *L. clarki* was lower in high-density compared to low-density populations. Infected adults are primarily produced from hosts initially parasitized as fourth instars (*13*), and 3.5 times as many adults had parasites in low-density than in high-density treatments [6.6 (n = 12) versus 1.9% (n = 10)]. Additionally, microscopic examination of water samples after emergence was complete revealed *L. clarki* trophonts in 50% of low-density microcosms but only 20% of those from high-density treatments (*14*). Both observations suggest that in later stages of host development parasite incidence was lower in high-density populations. Similarly, in most natural treeholes both trophont density and percentage of infected hosts decline when high-density populations of late instar larvae are present (*3*, 7).

Additional laboratory data suggest that larval predation reduced parasite incidence in the high-density microcosm populations. *Aedes sierrensis* larvae can feed by either browsing substrates or filtering water. When filter feeding, larvae consume free-swimming trophonts (*3*), and ingestion rates progressively increase for later instars (Fig. 2A). However, in the presence of solid food such as that provided in experimental microcosms, browsing predominates, and trophont populations persist at higher densities (Fig. 2B). Since trophonts that escape from cadavers require 48 to 72 hours for transformation into infective theronts, in the high-density experimental microcosms where food was severely limited, predation by late instar larvae would suppress populations of free-swimming ciliates and reduce rates of horizontal transmission and levels of infection. For low-density populations in microcosms having much more food per larva, filter feeding rates were presumably lower, ciliates survived longer, and rates of transmission were higher. These results suggest that the impact of *L. clarki* on host population dynamics in microcosms was significantly modulated by resource availability, intraspecific competition, and host feeding behavior.

To determine whether similar factors affect the impact of *L. clarki* on host populations in nature, we compared the production of adult *A. sierrensis* from parasitized and unparasitized larval populations developing in natural treeholes. In these natural breeding sites, organic detritus, microbial films on leaf litter and decaying wood, and microorganisms in the water column provide larvae with food (*15*). Because it was not possible to directly measure or control these resources in individual treeholes, we chose a broad range of treehole sizes for manipulations. In doing so, we assumed that small holes should contain fewer nutrient resources than large holes; hence, numerically identical larval populations would be more resource-limited in small holes than in large ones. These assumptions are reasonable because treehole size alone explains 52% of the variance in numbers of adult mosquitoes emerging from natural treeholes (*5*).

We first eliminated the biological communities from 42 treeholes that ranged in maximum volume from 0.6 to 21.1 liters. Treeholes were filled completely with water and boiled to kill the resident aquatic organisms (*16*). Communities in each habitat were restructured by the addition of 1050 or 2050 newly hatched *A. sierrensis* larvae, live or killed *L. clarki* trophonts, and a culture of microorganisms that are normal faunal elements of water-filled treeholes in California. Holes of similar volume were designated as treatment or control and manipulated at the same time; paired communities were reconstructed with the same number and source of hosts and the same parasite cultures (*17*). After 1 week, we sampled 50 larvae from each treehole and examined them microscopically for *L. clarki* infections. After sampling, experimental holes were left undisturbed for the duration of larval development (3 months) (*18*). A trap designed to retain emerging adult mosquitoes was placed over each hole when pupation commenced, and adults were removed twice weekly throughout the emergence period (4 months).

Examination of larval samples and dissection of adult mosquitoes revealed ciliate infections in only 18 of the 28 experimental treehole populations inoculated with live *L. clarki*. The average survivorship to adulthood for these populations with parasites was 28.5 ± 5.3% (mean ± 1 SEM; n = 18; range, 0.06 to 65.9%) compared to 41.0 ± 6.1% (n = 14; range, 1.1 to 68.0%) for controls; additionally, 4.6% of the adults from *L. clarki*–positive holes were infected females, which neither seek blood meals nor reproduce (*13*). Thus, the overall impact of parasite mortality on host populations from all treeholes was additive (*19*). Nonetheless, the effect of *L. clarki* enzootics on rates of adult emergence from individual habitats shifted over the size range of manipulated treeholes as predicted by the microcosm

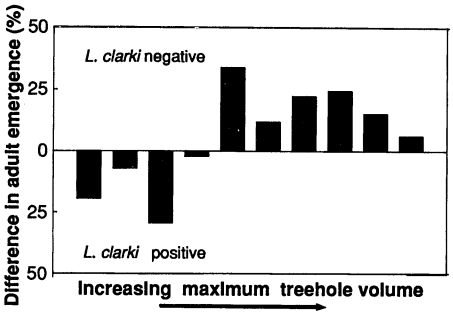

Fig. 3. Pairwise comparison of the differences between emergence success of *A. sierrensis* populations developing in natural treeholes with and without enzootics of *L. clarki*. Each bar represents the difference in survivorship between a control hole and its paired *L. clarki* positive hole. Bars above the line indicate greater survivorship of control populations whereas bars below the line indicate greater survivorship of populations with *L. clarki*. Results from ten treehole pairs ranked by increasing maximum volume (0.7 to 21.1 liters) are shown; the impact of parasitism shifted to reduced host emergence in treeholes with maximum volumes greater than 3 liters. Both treeholes within pairs were manipulated on the same date and received the same number of first instar larvae (either 1050 or 2050). Populations with ciliate epizootics were not included; only populations with enzootic levels in larval samples (mean = 9.2 ± 4.3% incidence, n = 10) were used for pairwise comparisons. Treeholes in the *L. clarki*–positive treatment group include both holes with natural *L. clarki* (n = 4) and holes inoculated with live trophonts (n = 6). For cases (n = 4) where pairwise comparisons could be made between control and either of two treatment treeholes, we always chose treatment holes with volumes most similar to controls. This selection did not bias results because in all four cases, the treatment populations that we did not choose responded the same, relative to controls, as those shown.

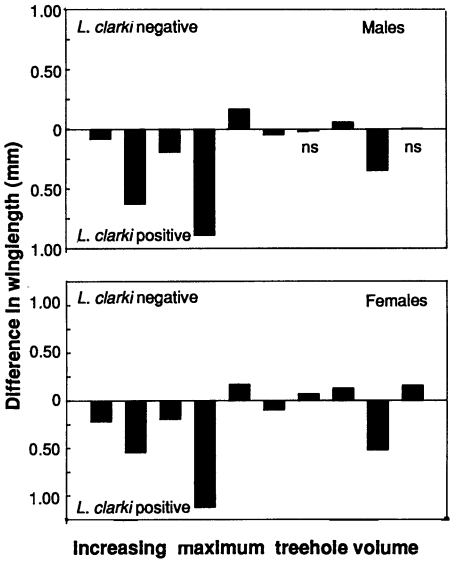

Fig. 4. Pairwise comparison of differences in average winglength measurements of male and female *A. sierrensis* adults that emerged from populations developing in natural treeholes with and without enzootics of *L. clarki*. Bars extend in the direction of the treehole in each pair that produced larger adults (see Fig. 3 legend). Average winglengths within each pair were compared by a t test ($P < 0.05$); all means are significantly different except those indicated by ns.

study. In the smallest treeholes where larval densities were highest, survivorship to adulthood in populations with ciliate infections was greater than for paired control populations without parasites; in larger holes where larval densities were lower, survivorship was greater for control populations without ciliate infections (*20*) (Fig. 3). Similarly, adults of both sexes that emerged from the four smallest treeholes with parasites were significantly larger than adults from corresponding control holes, whereas no trends in adult size were detected in comparisons of larger holes (Fig. 4). These results show that the impact of *L. clarki* infections on host mortality was depensatory in the smallest treeholes (where food was likely to be most limiting) and additive in large holes (where food was likely to be more abundant).

Data from these laboratory and field studies clearly show that although parasites can regulate host population levels, the effect of parasitic infections is not always a reduction in host abundance. Overall, 30.1% fewer mosquitoes emerged from manipulated treeholes with *L. clarki* (n = 18) compared to controls without parasites (n = 14). Because the infection levels of experimental populations were similar to those in natural treehole populations, our data suggest that even low average infection levels of *L. clarki* substantially reduce *A. sierrensis* abundance. However, the regulatory role of *L. clarki* is modified by availability of food, competition among larvae, and changes in feeding behavior. Under certain conditions (for example, resource limitation), natural enemies such as *L. clarki* may actually increase the fitness of adult mosquitoes by allowing for more or larger individuals, or both, to complete development. Because size and longevity are positively correlated, such mosquitoes possess a greater potential for com-pleting multiple gonotrophic cycles and serving as vectors for vertebrate diseases (*21*). Our results suggest that effective deployment of biological control agents is dependent on careful consideration of other ecological factors that affect target pest populations.

REFERENCES AND NOTES

1. R. M. Anderson and R. M. May, *J. Anim. Ecol.* **47**, 219 (1978); R. M. Anderson, *Nature* **279**, 150 (1979); J. C. Holmes, in *Population Biology of Infectious Diseases*, R. C. Anderson and R. M. May, Eds. (Springer-Verlag, New York, 1982), pp. 37–51; M. P. Hassell *et al.*, *ibid.*, pp. 15–35; M. E. Scott and R. M. Anderson, *Protozool. Today* **5**, 179 (1989); G. W. Esch, A. O. Bush, J. M. Aho, Ed., *Parasite Communities* (Routledge, Chapman & Hall, New York, 1990).
2. J. O. Corliss and D. W. Coats, *Trans. Am. Micros. Soc.* **95**, 725 (1976); T. B. Clark and D. G. Brandl, *J. Invert. Pathol.* **23**, 341 (1976).
3. J. O. Washburn, M. E. Gross, D. R. Mercer, J. R. Anderson, *Science* **240**, 1193 (1988).
4. W. A. Hawley, *J. Anim. Ecol.* **54**, 955 (1985); W. A. Hawley, in (*12*), pp. 167–184; J. O. Washburn and J. R. Anderson, unpublished data.
5. J. O. Washburn, J. R. Anderson, D. R. Mercer, *J. Med. Entomol.* **26**, 173 (1989).
6. ———, *J. Invert. Pathol.*, in press.
7. D. E. Egerter and J. R. Anderson, *ibid.* **46**, 296 (1985); J. O. Washburn and J. R. Anderson, *ibid.* **48**, 296 (1986).
8. Microcosms consisted of 750-ml plastic cups. A foam disk (2.5 cm thick) was cut to shape and secured to the bottom of each cup, providing a spatial refuge for free-swimming ciliates, a substrate for bacterial and fungal growth, and a surface for larvae to browse. Initially each microcosm was filled with 500 ml of autoclaved, natural treehole water diluted with deionized water; deionized water was added weekly to maintain constant volumes. All microcosms were maintained at 11°C with a 10-hour photophase; after 16 weeks the photophase was increased to 14 hours in order to terminate larval diapause and stimulate pupation. The experiment was ended after 30 weeks.
9. Parasite additions were standardized by adding identical volumes of a mixture of free-swimming ciliates that were grown in vitro (*3*) or released from moribund hosts from in vivo cultures. To remove ciliates from growth media, in vitro cultures were poured into 100-ml volumetric flasks; a layer of dilute, autoclaved treehole water was added above the media in the flask neck, and ciliates were removed by pipette after they migrated into the water. *Lambornella* density in the suspension of combined ciliates was estimated by removing five samples and counting the number of cells (*3*). Half the ciliate solution was autoclaved, and aliquots were added to control populations; the remaining half containing live ciliates was used to inoculate treatment populations. Ciliate additions yielded a final density of 4.2 ± 0.8 (mean ± 1 SEM) cells/ml in the 500-ml microcosms and were timed so that theronts began attaching to hosts when larvae were 48 to 72 hours of age. This phenology closely matches initiation of the first parasite cycle in natural treeholes following accumulation of rainwater (*6*). To quantify *L. clarki* attack rates in the first parasite cycle without destructively sampling experimental populations, we established additional sham microcosms of 100 (n = 10) and 500 (n = 5) larvae that received the same *L. clarki* inoculum as those in the experiment. We removed 25 larvae from each sham population 72 hours after the experiment was set up; larvae were stained with amide black dye and examined (×100 to ×400) for the presence of ciliates encysted on the cuticle (*3*). Incidences of *L. clarki* attached to larvae in the low- and high-density shams were 11.8 and 10.4%, respectively.
10. Microcosms received 0.1 g of ground, autoclaved Purina Rat Chow initially and at 5-week intervals for the first 25 weeks. Food levels were determined from previous laboratory experiments in which we reared populations of 100 larvae on different amounts of ground rat chow. From these experiments, a composite index which considered survivorship and adult winglength was used to determine the amount of food used in the laboratory experiment reported here.
11. We removed pupae weekly from all microcosms, and we measured the winglength and parasite status of each emerged adult by microscopic dissection (×10 to ×40).
12. L. P. Lounibos, J. R. Rey, J. H. Frank, Eds., *Ecology of Mosquitoes: Proceedings of a Workshop* (Florida Medical Entomology Laboratory, Vero Beach, FL, 1985).
13. D. E. Egerter, J. R. Anderson, J. O. Washburn, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7335 (1986).
14. Water from each microcosm was examined for trophonts in two ways. First, a 5- to 10-ml sample was placed in a small petri dish and inspected (×10 to ×40). Second, water was squeezed from foam disks in each microcosm and passed through filter paper (Whatman 1); 2 to 3 ml of the water containing the concentrated microorganisms was examined for *L. clarki* trophonts.
15. S. R. Carpenter, *Oikos* **39**, 17 (1982); *Ecology* **63**, 219 (1983).
16. For field manipulations, the standing water from each of 42 treeholes at two field sites (Marin and Mendocino counties) was repeatedly filtered to remove mosquito larvae; water was returned to holes which were then brought to their maximum volumes with deionized water. Maximum treehole volumes were determined by summing the amount of natural treehole and deionized water added. Immersion heaters powered by generators were used to bring the contents of each hole to a rolling boil. After 20 min of boiling, heaters were removed, remaining dead insect larvae were skimmed from the water surface, and treehole openings were covered with fiberglass screen. A week after boiling, a water sample was collected from each hole and examined in the laboratory for the presence of *L. clarki* trophonts.
17. Holes were ranked by maximum volume and divided into 14 groups of three holes of similar size. To ensure the establishment of ciliate infections in sufficient experimental holes for comparisons, two of each triplet were designated as *L. clarki* treatments and one was designated as control. Within triplets, treatments were randomly assigned except for 11 that still contained trophont populations despite boiling. One hole naturally positive for *L. clarki* was assigned to each of 11 triplets and received aliquots of autoclaved ciliate cultures. We released a total of 62,100 first instar larvae into the 42 experimental treeholes over a period of 15 days. Larvae were hatched from eggs collected from laboratory colonies originating from mosquitoes collected in Fresno, Marin, and Mendocino counties. We added sufficient numbers of live or autoclaved *L. clarki* trophonts to each treehole to produce a final density of one cell/ml when the holes were filled to their maximum volumes; parasites were cultured in vitro from *L. clarki* previously collected at the same field site. Before the experiment, we also cultured representative treehole microorganisms (rotifers, protozoa, bacteria, and fungi), from natural treehole water samples that did not contain *L. clarki*. Samples were pooled and cultured in 2.8-liter flasks containing dilute cerophyl media (*3*). During the reconstruction of each community, we added a volume of the media with microorganisms equal to 1% the maximum volume of the hole. After additions, each hole was filled to its maximum volume with deionized water. While larvae of nine insect species are known to develop in water-filled treeholes in California, *A. sierrensis* is the only suitable host for *L. clarki*. *Aedes sierrensis* dominates most communities, and adults make up 81% of the emerging insects [D. L. Woodward, A. E. Colwell, N. L. Anderson, *Bull. Soc. Vector Ecol.* **13**, 1 (1988)]. Because of differences in feeding ecologies and low relative abundances, it is unlikely that the exclusion of other insect species affect the outcome of host-parasite interactions.
18. Water levels in experimental holes were allowed to fluctuate naturally during larval development. During the period when adults were emerging, holes at risk of drying completely received a volume of water equal to 25% of their maximum volumes.
19. Infection levels were determined from larvae and adults collected for holes receiving live trophont additions and with naturally occurring *L. clarki*. Both epizootic and enzootic incidence levels were found in larval samples removed from experimental populations; the range (<2 to 100%), average incidence of *L. clarki* in larval samples (17.0 ± 1.8%, n = 18), and the proportion of infected adults in emerged mosquitoes (10.9 ± 3.2%, n = 18) were similar to values previously reported for naturally infected host populations (*5, 6*). No infections were detected in any control populations.
20. All results and conclusions were the same, regardless of whether infected adults were included or not in analyses. Although female *A. sierrensis* infected with *L. clarki* are parasitically castrated (*14*), it is appropriate to include such individuals in survivorship and size comparisons because they represent successful larval development and resource use. However, when assessing the biological control potential of *L. clarki*, these individuals should be removed from estimates of adult mosquito production.
21. F. Agudelo-silva and A. Spielman, *Am. J. Trop. Med. Hyg.* **33**, 1267 (1984).

22. Supported by NIH grant AI20245 and by University of California Mosquito Research Funds. We gratefully acknowledge the technical assistance of the Marin-Sonoma Mosquito Abatement District and the staff of the UC Hopland Field Station in conducting the field experiment. We thank G. Atilano, E. Hartmann, A. Norton, E. Omi, L. Tewksbury, S. van Nouhuys, and W. Yee for technical support and V. Brown, E. Hartmann, D. Woodward, and W. Yee for comments on the manuscript.

28 February 1991; accepted 11 June 1991