

## Stages of infection and ecological effects of a fungal epidemic on the eggs of a limnetic copepod

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**SUMMARY.** Fungal infection of calanoid copepod (*Diaptomus novamexicanus*) eggs was observed in each of three years in an alpine lake (Castle Lake, California, U.S.A.). Stages in the infection process were examined by light and scanning electron microscopy and evidence was obtained that the *Lagenidium*-like fungus concerned was a virulent parasite. Fungal destruction of eggs varied in timing and severity from year to year. The maximum impact of the disease was an estimated 48.4% decrease in potential copepod recruitment in 1976 due to the onset of a severe epidemic early in the summer growing season. The minimum impact, a 5.6% decrease in potential recruitment, was recorded in 1975. In this year the proportion of infected eggs was reduced and large numbers of juveniles had been released before the fungal disease began. The 1974 epidemic was intermediate in severity. The effect of these epidemics on Castle Lake calanoid populations is discussed in relation to temperature, food availability and predation.

### Introduction

The nature and ecological consequences of fungal epidemics within aquatic invertebrate populations have been little studied despite numerous reports of fungi growing upon planktonic animals and their eggs (reviewed by Unestam, 1973). Previous records have often been confined to qualitative descriptions of declines in zooplankton abundance correlated with fungal growth on the animals concerned. The mass mortality of copepods (*Eurytemora hirundoides*) in the Northern Baltic has been attributed to the oomycete *Leptolegnia* (Vallin, 1951). Petersen (1910) observed the complete decimation of *Leptodora kindtii* populations by another species of *Leptolegnia* and briefly speculated that these epidemics may be important to other planktonic animals normally preyed upon by this cladoceran. Scott (1956) noted the

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dramatic decline of a *Daphnia* population which had been attacked by the fungus *Aphanomyces patersonii*. A related species *A. astaci* is considered responsible for the almost complete annihilation of *Astacus astacus*, the European crayfish, in the latter half of the last century (Shikora, 1903). The ecological effects of fungal parasitism may be far more complex, however, than simply the catastrophic elimination of a single species. Green (1974) has concluded from laboratory studies that parasitism may substantially reduce the reproductive capability of cladocerans. Canter & Lund (1948, 1951, 1953) have described the controlling influence fungal parasites of phytoplankton may have on the magnitude, timing and species composition of algal maxima. These classic descriptions were based on comparative observations between epidemic and disease-free years. There appear to have been no equivalent reports which similarly examine zooplankton dynamics in relation to seasons of severe versus reduced parasitism.

In this study we present microbiological and ecological observations on the fungal attack of eggs of *Diatomus novamexicanus* (Herrick), a calanoid copepod which is normally abundant in the plankton of Castle Lake, California. In order to understand the mode of association between the fungus and animal we document, by light and scanning electron microscopy, stages in the infection process. Then, to assess the consequences of the epidemic for natality, growth and abundance of the copepod, we compare population characteristics during an epidemic season with those of two summers in which the incidence of fungal infection was much lower.

### Study site

Castle Lake is a 20.1 ha, subalpine (1706 m) body of water in the Klamath Mountains of Northern California, USA. It is mesotrophic with an average Secchi depth of about 12 m, and lies in a cirque basin having a mean depth of 11.4 m and a maximum depth of c. 35 m. The zooplankton community is dominated by the cladocerans *Daphnia rosea* (Sars), *Bosmina longirostris* (Müller) and *Holopedium gibberum* (Zaddach) and by the calanoid *Diatomus novamexicanus* (Redfield & Goldman, 1978).

### Methods

#### Zooplankton sampling and enumeration

Zooplankton samples were taken with an 11-l self-acting plankton trap, equipped with an 80- $\mu$ m net. Sampling was done from a raft anchored over the deepest portion of the lake. In 1974, individual samples were drawn from 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0, and 30.0 m, while in 1975, collections were made at 2-m intervals from 1.0 to 20.0 m and replicate samples from each depth were pooled into fifteen composites. For 1976, fifteen composites were also formed, though sampling was done at 1-m intervals from 0.5 to 29.5 m and samples from adjacent depths were combined (0.5 + 1.5, 2.5 + 3.5 m, etc). The formation of composites has been estimated to result

in a negligible loss of captured animals (Redfield & Goldman, 1978), and the sampling regimes are assumed to be equally representative. Collecting was done at midday at 5-day intervals from 24 June to 22 September 1974, and from 20 June to 23 September in both 1975 and 1976. Additional sets were taken on 13 June 1976 and 20 June 1974.

Enumeration was carried out at  $\times 45$  on an open petri dish. All *Diatomus* in each sample were counted as males, females (with and without egg sacs), copepodites and nauplii. Data from stratified sampling was integrated to numbers per  $m^2$ , and densities of males and females were summed for presentation in a single adult category. The samples were then pooled by date and through subsampling and random encounter, three additional variables were monitored. The size distribution of copepodites was obtained by measuring the metasomal lengths (cephalothorax) of 50–100 adolescents to within 0.03 mm using an ocular micrometer. Brood size and proportion of eggs infected by the fungus were estimated by examining c. 100 eggs in subsamples on each date, and recording eggs per sac, total eggs and those with visible manifestations of fungal infection (zoosporangia, exit tubes, or clearing).

#### Scanning electron microscopy

For examination by scanning electron microscopy, animals with infected eggs were transferred from formaldehyde-preserved samples through an ethanol series into absolute alcohol. This material was dried using the critical point technique of Paerl & Shimp (1973) and then coated, on an SEM stub, with a 7-nm gold-palladium layer by vacuum evaporation. To minimize cellular damage due to thermal effects, 1-min evaporation and deposition periods were interspaced with 2-min intervals of cooling. The coated specimens were examined with a Cambridge Mark 2A Stereoscan scanning electron microscope.

#### Estimation of egg mortality

Fungal-related mortality was estimated as follows. The number of eggs per  $m^2$  of lake was calculated from gravid female densities and average brood size data for the two



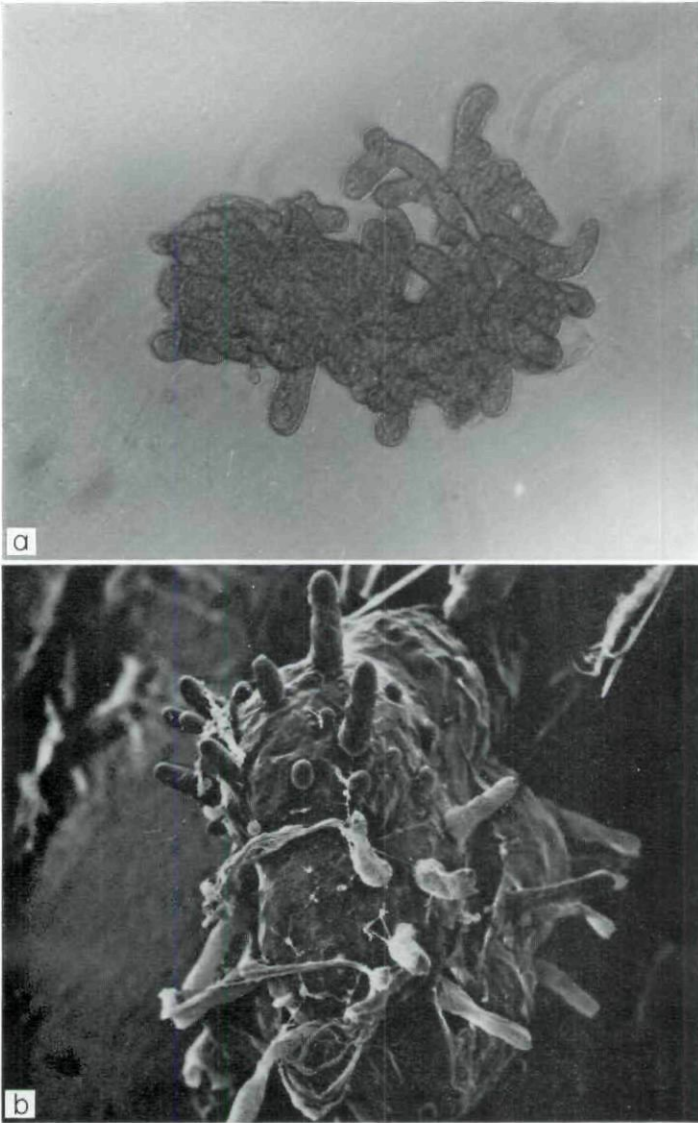


PLATE 1. (a) Interior of infected *Diaptomus* egg. The egg has been broken open to reveal the tightly packed mycelium inside. Phase contrast  $\times 670$ . (b) Exterior of infected *Diaptomus* eggs. The upper egg is in a late stage of the disease with many mature exit tubes penetrating through and beyond the egg sac membrane. The egg below is in the final stage of the disease in which all exit tubes have dehisced and the egg-sac membrane is degraded by saprophytic micro-organisms. Scanning electron micrograph  $\times 670$ . (c) Late developmental stage of a fungal exit tube. Scanning electron micrograph  $\times 6700$ . (d) Four infected zooplankton eggs in one egg sac, each egg in a different stage of infection. Phase contrast  $\times 500$ .

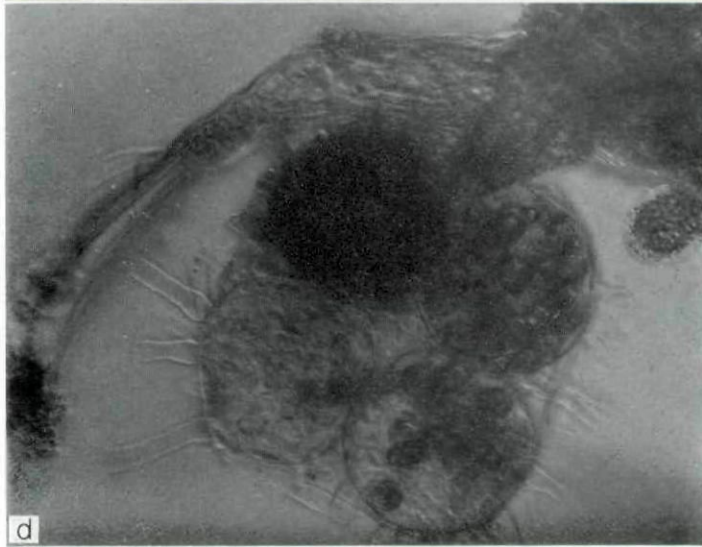
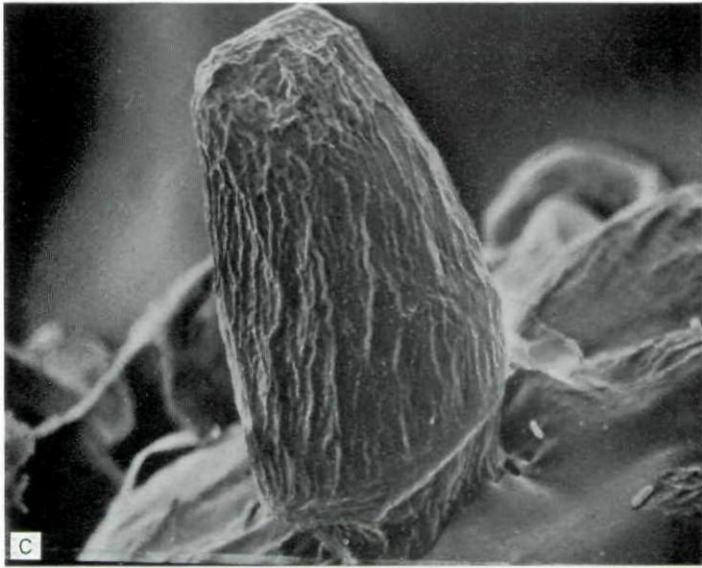


PLATE 1 *continued*

sampling days of highest infection in each summer. These figures were multiplied by the proportion of infected eggs on the appropriate day, and the resultant two estimates for each year were averaged. The values so obtained necessarily underestimate actual egg losses due to parasitism. Eggs in the early phases of the disease could not be distinguished from healthy ones and thus the estimates of the proportion attacked are conservative. Further, the estimate does not include those eggs destroyed without trace prior to the two sampling dates at the peak of the epidemic, nor those infected subsequent to the disease climax.

## Results

### *Stages of infection*

Fungal infection is first evidenced by the formation of densely packed mycelial material within the zooplankton egg. This mycelium consists of an irregularly branched and lobed, non-septate, thick (average diameter 12  $\mu\text{m}$ ), granular hypha (Plate 1a), which grows to completely consume the contents of the egg. Asexual reproduction is initiated by the breaking of this thallus into fragments which become zoosporangia. Each zoosporangium produced an exit tube, which penetrates through, and up to 60  $\mu\text{m}$  beyond, the egg sac membrane (Plate 1b,c). These extra-matrical exit tubes (up to fifteen per egg) are constricted at the point of emergence from the egg membrane (Plate 1c) and when mature, they break open at the tip releasing the contents of the zoosporangium. No encystment of zoospores at the aperture of the exit tubes was recorded. However, an occasional zoospore remained within the exit tube, and germinated to form a long (up to 25  $\mu\text{m}$ ), thin (1–2  $\mu\text{m}$  diameter) germ tube. Upon dehiscence of all sporangia the egg membrane, which prior to infection is relatively free of attached bacteria, becomes colonized by large numbers of filamentous and rod-shaped micro-organisms. The remains of the egg and fungus gradually degenerate through this final saprophytic stage.

In a small proportion of infected eggs (less than 10%), fungal development leads towards the formation of thick-walled resting spores. These spores are c. 20  $\mu\text{m}$  in diameter with

2–3  $\mu\text{m}$  thick walls and a prominent, eccentrically located droplet. Up to thirty are produced in a single egg and within such eggs production of zoosporangia is minimal, often absent and never concurrent with thick-walled spore production. No antheridia have been detected in association with these spores and therefore their sexual or asexual origin remains unclear.

*Diaptomus* females in Castle Lake normally carry three to five eggs in a single sac. Despite their proximity, each egg seems to be separately infected, presumably by a separate zoospore. Each egg develops independently through the various disease stages. It is common to find an individual sac in which each egg is at a different stage of fungal development; an example is shown in Plate 1d. Here, one egg has been completely consumed and left more or less transparent, another is in the early stage of zoosporangium and exit tube production, while thick-walled resting spores have been formed in the other two eggs. The fungal attack is always localized on the eggs and even in the final stages of infection no fungi have been found associated with the body of the adult female.

### *Taxonomy of the fungus*

Samples of infected eggs have been examined by Dr L.G. Willoughby (Freshwater Biological Association, Windermere). He has tentatively assigned the fungus concerned to the genus *Lagenidium* (Phycomycetes).

### *Extent of the disease within the Diaptomus population*

The fungal infection was present in each season, though the proportion of infected eggs differed between years (Fig. 1). On one to four dates in each summer, more than 50% of the eggs were diseased; the most severe attack was observed on 13 June 1976 when over 80% were infected. The mean percentages of eggs with signs of infection for the seasons were, consecutively 56, 45 and 73% (these averages are from data in Fig. 1 and include only those dates when more than forty eggs were seen in samples and when the fungus was present). With the exception of five infected eggs in a total of twenty samples of 19 August 1976, no fungi were seen during



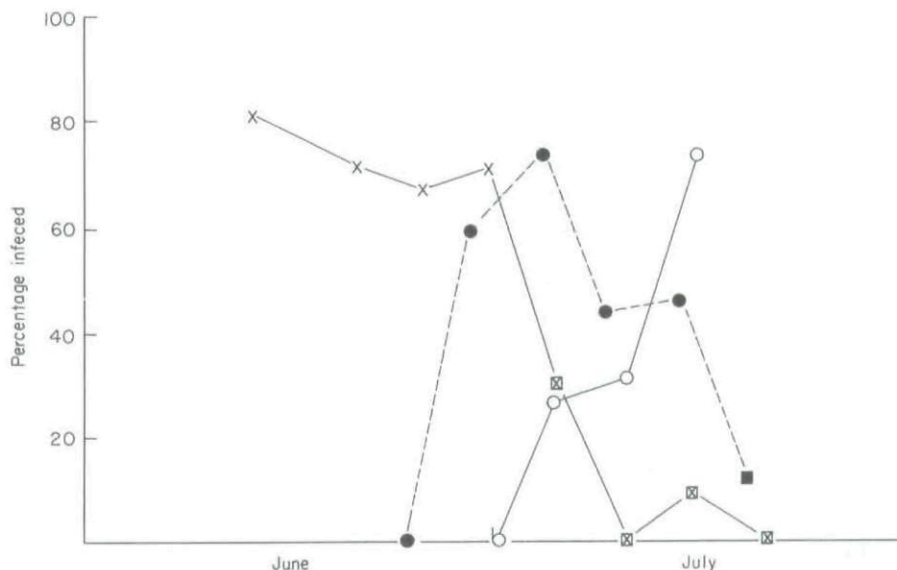


FIG. 1. Percentage of infected eggs of *Diaptomus novamexicanus* in the 1974 (●), 1975 (○), and 1976 (X) seasons of egg production in Castle Lake. Each point is based on the examination of an average of 100 eggs with the exception of four dates of sampling, indicated with squares, on which less than forty eggs were examined.

a second period of egg production which extended from about 20 July to September.

#### *Fungal-related mortality in relation to Diaptomus population dynamics*

The overall effects of reduced natality due to the epidemic varied considerably from year to year and was strongly related to the timing of the fungal infection. In 1974 and 1975 large numbers of eggs had already hatched prior to the onset of infection. Consequently, at the climax of the disease in each of these two years neonata were abundant (over 300% of concurrent adult densities, Fig. 2) and the effects of egg destruction on total population densities of *Diaptomus* were reduced. In 1976, however, the epidemic occurred very early in the season during the first phase of egg production and naupliar densities remained extremely low (even lower than adult densities) by comparison with the previous two years. The density of adults in 1976 was as high as in the earlier summers (Fig. 2) and the modest reduction in average brood size (2.9 cf 4.5 and 3.4 eggs per sac, 1974 and 1975) does not account for the large drop in the concentrations of young. Rather, the losses of 1976

appear to have resulted from the early onset and sustained high intensity of the epidemic.

These conclusions are further reinforced by a comparison of actual recruitment into the *Diaptomus* population each year with potential rates of recruitment in the absence of fungal-related egg mortality (Table 1). The reduction of potential recruitment was greatest in 1976 (48.4%) when large numbers of eggs were attacked and low concentrations of live young had been released. This estimate may be more conservative than those for 1974 and 1975 since fungal infection in 1976 began, and possibly achieved its peak density, prior to the start of sampling on 13 June. In 1975, egg densities were low when the epidemic gained momentum, average proportion infected was lower than in the other years, and most importantly, high numbers of juveniles had been released before the fungal attack began (Table 1). As a consequence, the overall impact of the egg infection on potential recruitment in 1975 was low. The effects of the disease in 1974 appear to have been intermediate. Approximately four times as many eggs were infected in this year compared to 1975; however, unlike 1976, the large numbers of eggs killed were partly

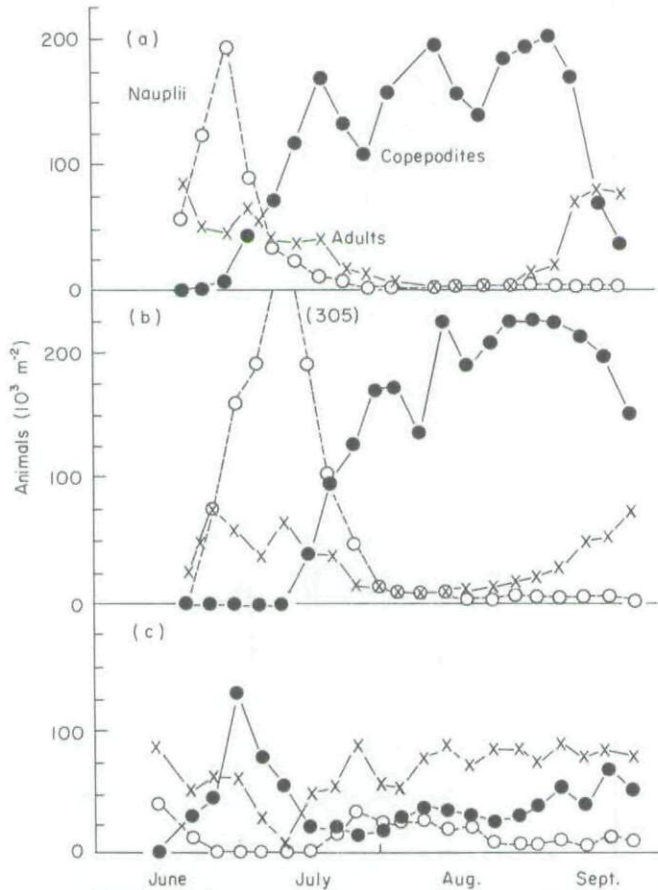


FIG. 2. Variation in the abundance of *Diaptomus* during 1974 (a), 1975 (b) and 1976 (c). Each point is the integrated total from 13–15 stratified samples. Animals were enumerated as adults (X), copepodites (●) and nauplii (○).

TABLE 1. Estimated reduction in potential recruitment of *Diaptomus novamexicanus* due to fungal destruction of eggs

Year	Date	Total eggs		Infected eggs		Neonata	Potential recruits	Potential recruits killed (%)
		( $10^3 \text{ m}^{-2}$ )	(%)	( $10^3 \text{ m}^{-2}$ )	( $10^3 \text{ m}^{-2}$ )			
1974	29 June	104.9	59	61.9	200.0	304.9		
	4 July	85.4	73	62.3	132.7	218.1		
	Average			62.1	166.4	261.5	23.7	
1975	10 July	59.7	33	19.7	306.7	366.4		
	15 July	15.1	74	11.2	246.6	261.7		
	Average			15.5	276.7	314.1	5.6	
1976	13 June	85.9	81	69.6	40.3	126.2		
	20 June	46.2	72	33.3	40.6	86.8		
	Average			51.5	40.5	106.5	48.4	

offset by the substantial quantity of young released prior to the climax of the infestation.

Growth and maturation characteristics of the *Diaptomus* populations were similar in

1974 and 1975 but changed dramatically in the last year of observation (Fig. 2). The numbers of nauplii and copepodites were sharply reduced in 1976. However, the rate

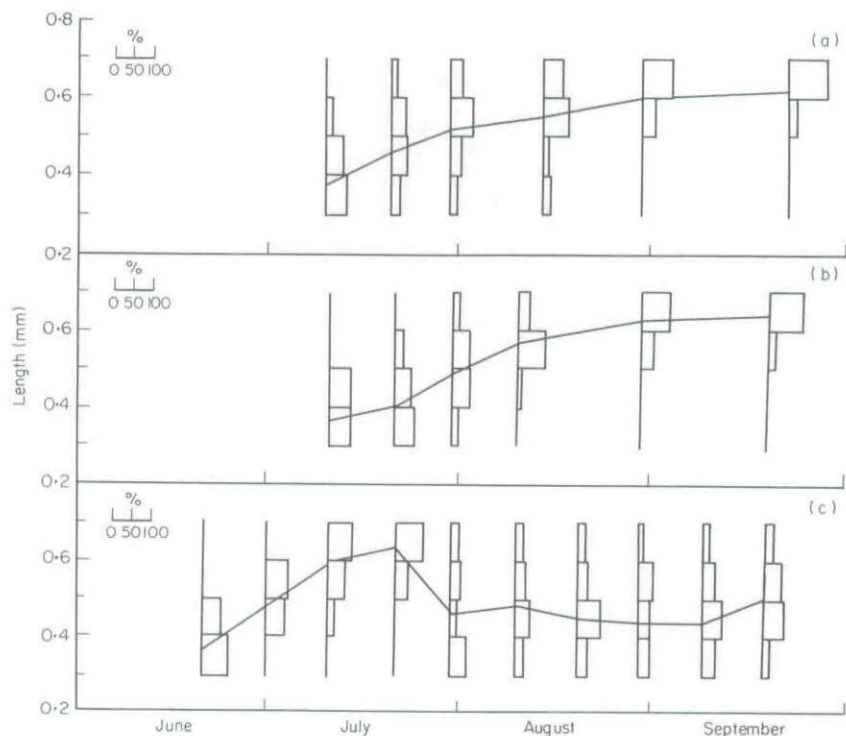


FIG. 3. Temporal changes in the size distribution and average length of *Diaptomus* copepodites in Castle Lake in 1974 (a), 1975 (b) and 1976 (c). Distributional data are percentages falling into the 0.3–0.4, 0.4–0.5, 0.5–0.6 and 0.6–0.7 mm length classes, and the means are weighted averages from the original measurements on each date.

of growth of the copepodites, as reflected by mean length within the category (Fig. 3), was much higher early in 1976 than in the previous summers. As a result the first crop of young matured in about 1 month in 1976; this process took nearly 2 months in the preceding two summers (see Fig. 3). Adult population densities rose from a minimum in mid-July 1976 to a maximum *c.* 2 weeks later (Fig. 2). This period of increase was greatly compressed by comparison with the late summer development of adult populations in 1974 and 1975. In contrast with previous years, the resultant mid-summer adult population of 1976 started to reproduce shortly after maturity, as is reflected by the increase in nauplii in late July (Fig. 2). The reduction of natality caused by the fungal epidemic of 1976 was therefore associated with major alterations of the *Diaptomus* population; a typically univoltine population acquired characteristics of a bi- or possibly tri-voltine one. However, factors independent

of the disease, such as food levels, predation and temperature, may have contributed to these shifts. These will be discussed below.

## Discussion

A question of key relevance to the interpretation of any fungal association is whether the micro-organism concerned is saprophytic or parasitic. Although most members of the genus *Lagenidium* are virulent pathogens, at least one species is saprophytic (Karling, 1947), while another is only weakly parasitic and can be cultured as a saprophyte (Couch, 1935). Complete proof of parasitism ultimately rests with the successful re-inoculation of this *Lagenidium*-like fungus on healthy eggs; however, several lines of evidence strongly support parasitism as the observed mode of association. Each egg is attacked separately and fungal development is contained within



the egg until exit tubes are formed. With parasite-like specificity, only the eggs are attacked and there is never any association of the fungus with the adult female's body. Finally, the outer membrane of healthy eggs is completely free of attached micro-organisms. It is only in the very final stages of exit tube formation and dehiscence that saprophytic micro-organisms begin to colonize this membrane in large numbers.

Similar cases of parasitic attacks limited to the eggs of invertebrate hosts have been frequently recorded. Parasitism by *Aphanomyces ovidestruens* is restricted to the egg sacs of *Diaptomus gracilis* where it produces a highly specialized vegetative mycelium (Gicklhorn, 1923). Karling (1944) and Sparrow (1939) reported species of *Lagenidium* which parasitize the eggs of rotifers. Another virulent pathogen of this genus, *L. callinectes*, attacks the eggs of the blue crab *Callinectes sapidus* (Couch, 1942). Certain chytrids are known to infect the eggs of rotifers (e.g. Karling, 1946) and helminths (Buckley & Clapham, 1929).

Major differences in the population dynamics of *Diaptomus* are associated with the severe epidemic of 1976. These include a low crop of nauplii from the overwintering generation (Fig. 2); rapid growth (Fig. 3) and maturation of the adolescents produced during the initial period of egg production (note the increase in adults in July, Fig. 2); and a second reproductive interval from mid-July into September (note nauplii, Fig. 2). It must therefore be asked, was the fungal epidemic solely responsible for the drop in recruitment, and can the changes in growth and reproduction of the population be reasonably ascribed to the resultant reduction in standing crop of copepodites. These two questions will be discussed in relation to summers of 1975 and 1976 for which more supplementary information is available.

To answer the first question, causes of the low numbers of young early in the summer of 1976, other than the fungal infection, must be evaluated. The cladoceran *Polyphemus pediculus* (L.) which can prey on calanoid nauplii was present in very low densities during June and therefore large-scale naupliar losses cannot be attributed to this species. The other invertebrate predator on zooplankton in Castle Lake, *Macrocyclus albidus* (Jurine), is not con-

sidered important because it typically inhabits depths greater than 20 m in the limnetic region, well below depths occupied by the vast majority of nauplii. Predatory species such as *Leptodora*, *Bythotrephes* and *Chaoborus* are absent from Castle Lake. Predation of nauplii by advanced copepodites of *D. novamexicanus* is also highly unlikely. This species is herbivorous and extremely small (adults attain a maximum size of 0.75 mm) by comparison with those zooplankters which are known to eat their own nauplii. Further, the advanced copepodite instars of *Diaptomus* are typically well separated in time from the period of maximum naupliar densities. Algal biomass levels in Castle Lake were high in June 1976 relative to past years (see below) and therefore decreased survivorship of nauplii due to decreased food availability seems most unlikely. Neither is the slight decrease in brood size adequate to explain the dramatic decline. We conclude that the fungal attack was the primary cause of the reduction in naupliar densities in early 1976.

With reference to the second question, the unique pattern of growth, maturation and reproduction in 1976 can be largely explained by greater food resources and higher temperatures relative to previous years. The copepodites of 1976 were found primarily in the epilimnion which averaged 3°C warmer than in 1975. The young of 1975 tended to occur at greater depths of correspondingly lower temperature. As a result, adolescents were probably exposed to temperatures at least 5°C warmer in 1976 than in 1975. Concentrations of grazable algae over the period of rapid copepodite growth in 1976 (20 June–20 July) were about 21% higher than over the comparable interval (10 July–14 August) in 1975. This difference is magnified when the fungal-related reduction in copepodite recruitment is considered and algal biomass levels are expressed as an average per adolescent over the periods of growth. Copepodite densities averaged  $51.0 \times 10^3$  individuals  $m^{-2}$  in 1976 and  $119.1 \times 10^3$  individuals  $m^{-2}$  in 1975. The resultant indices of algal food availability are 303  $\mu g$  per animal (1976) compared with 109  $\mu g$  per animal (1975), a difference of 178%. It is therefore probable that the increased food abundance and higher temperatures in 1976 promoted the rapid maturation and subsequent

reproduction of *Diaptomus*. The reduction in natality due to the fungal epidemic may have indirectly facilitated the shift to a bivoltine pattern as a result of increased availability of algae per individual copepodite, and possibly decreased intraspecific competition for food as a consequence.

Egg mortality due to the epidemics substantially affected the abundance of *Diaptomus* in two of the three years investigated. It is possible that in this system parasitism was more influential than predation. As noted, densities of carnivorous zooplankton which could prey upon *Diaptomus* are greatly reduced in the region of the lake occupied by this copepod. Furthermore, work on the salmonids of Castle Lake suggests that *Diaptomus* is subject to far less predation pressure from trout than other components of the zooplankton community (Wurtsbaugh, Brocksen & Goldman, 1975).

#### Acknowledgments

This work was supported by a New Zealand National Research Advisory Council Fellowship to Warwick Vincent and National Science Foundation Grants GB 19052 and DEB 76-19524 to Charles R. Goldman. The authors wish to thank C.R. Goldman for use of unpublished data; L.G. Willoughby for helpful discussion and taxonomic assistance; the Facility for Advanced Instrumentation; University of California, Davis, for the use of equipment; the Freshwater Biological Association, Ambleside, Cumbria, England, for use of laboratory and library facilities; Connie L. Vincent for assistance with manuscript preparation; and J. Simmiss for typing the final draft.

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(Manuscript accepted 13 November 1978)

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