

## Fungal Infection of *Eudiaptomus gracilis* (Copepoda, Crustacea) in Lake Mondsee

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With 5 Figures and 3 Tables

Key words: Fungal infection, *Aphanomyces*, *Eudiaptomus*

### Abstract

An *Aphanomyces* sp. (Oomycetes) was found to be an important egg parasite of *Eudiaptomus gracilis* (Copepoda) in Lake Mondsee (Austria). The infection is evidenced by densely packed infection hyphae, attached with holdfasts and wrapped around the female's abdomen. The infection hyphae give rise to hyphae which penetrate the egg sac, and subsequently kill all eggs. The infection obviously has no pathogenetic effects on the egg-carrying female. Infected females appear during summer and fall, mainly in warm water layers. Infected egg clutches are found at all seasons of the year, indicating considerable birth control of the *Eudiaptomus* population by parasite infection. It seems possible that the actual *Aphanomyces* sp. is not the only parasite involved.

### Introduction

While phytoplankton fungal parasites have been investigated comprehensively (see e.g. SPARROW 1960; KARLING 1981) only few studies have taken up fungal parasitism in zooplankton. Since *Leptolegnia caudata* first was described as a cladoceran fungal parasite, apparently no more than 12 papers dealing with nine species of fungal parasites of planktonic crustaceans have been published (Table 1). Records have mainly been confined to descriptions of declines in zooplankton abundance related to fungal growth in the animals concerned. PETERSEN (1910) observed the complete extinction of *Leptodora kindti* populations by *Leptolegnia caudata*, and concluded that this epidemic probably was of great importance to the plankton development in Danish lakes since a pronounced predator of other planktonic crustaceans was eliminated. VALLIN (1951) attributed the mass mortality of *Eurytemora hirundoides* in the Northern Baltic to *Leptolegnia*. SCOTT (1956, 1961) has given accounts of zooplankton heavily infected with *Aphanomyces patersoni* and

*A. bosminae*, and noted a dramatic decline of *Daphnia* and *Bosmina* populations. A related species *Aphanomyces* sp., which kills the eggs and increases the mortality of the females of *Boeckella dilatata* caused up to 14.3% depression of birth rates in the population (BURNS 1985a). A *Lagenidium*-like fungus was also found to be a virulent parasite in eggs of the calanoid copepod *Diatomus novamexicanus* (REDFIELD & VINCENT 1979). The maximum impact of the disease was an estimated 48.4% decrease in potential copepod recruitment during a severe epidemic season in Castle Lake, California. It was concluded that occasionally parasitism may be more influential than predation in regulating copepod populations.

In the deep prealpine oligo-mesotrophic Lake Mondsee, Austria, regular zooplankton monitoring has been going on from 1987 to 1996. The zooplankton of the lake is dominated by the cladocerans *Daphnia hyalina* and *Daphnia cucullata*, the cyclopoids *Cyclops abyssorum praealpinus* and *Mesocyclops leuckarti*, and the calanoid *Eudiaptomus gracilis* (NAUWERCK 1991, 1993; WINDHAGER 1995). Fungi growing on or in planktonic animals, or their eggs, were frequently observed during these studies. Population breakdowns because of fungal infection evidently happened at times. However, cladocerans seldom suffered from infections, and the fungi found on the copepods seemed not to lead to mortality in the animals themselves. An *Aphanomyces* species was frequently found to be a parasite of *Eudiaptomus*, while another fungus, probably a *Lagenidium* sp., to a lesser degree attacked the eggs of *Cyclops*.

The present paper deals with *Aphanomyces* in *Eudiaptomus*. Morphological characteristics, life cycle and specificity of the parasite as well as its frequency during three years in the pelagic population of the copepod are described. Sur-

**Table 1.** Reported fungal parasitism of zooplankton crustaceans.

Fungal parasite	Host	Part infected	Reference
<i>Leptolegnia caudata</i>	<i>Leptodora kindti</i>	body and eggs	PETERSEN (1910)
<i>Pythium daphnidarum</i>	<i>Daphnia hyalina</i>	body and eggs	PETERSEN (1910)
<i>Pythium daphnidarum</i>	<i>Daphnia cucullata</i>	body and eggs	PETERSEN (1910)
<i>Pythium daphnidarum</i>	<i>Eubosmina coregoni</i>	body and eggs	PETERSEN (1910)
<i>Aphanomyces ovidestruens</i>	<i>Eudiaptomus gracilis</i>	eggs	GICKLHORN (1923)
<i>Leptolegnia</i> sp.	<i>Eurytemora hirundoides</i>	body	VALLIN (1951)
<i>Aphanomyces</i> sp.	<i>Daphnia hyalina</i>	body	PROWSE (1954)
<i>Aphanomyces patersoni</i>	<i>Daphnia</i> sp.	?	SCOTT (1956)
<i>Aphanomyces bosminae</i>	<i>Bosmina</i> sp.	?	SCOTT (1961)
<i>Lagenidium</i> sp.	<i>Diaptomus novamexicanus</i>	eggs	REDFIELD & VINCENT (1979)
<i>Aphanomyces</i> sp.	<i>Boeckella dilatata</i>	eggs	BURNS (1980)
<i>Aphanomyces</i> sp.	<i>Boeckella triarticulata</i>	eggs	BURNS et al. (1984)
<i>Aphanomyces</i> sp.	<i>Daphnia magna</i> (lab.)	body	SEYMOUR et al. (1984)
<i>Aphanomyces</i> sp.	<i>Boeckella hamata</i>	eggs	BURNS (1989)

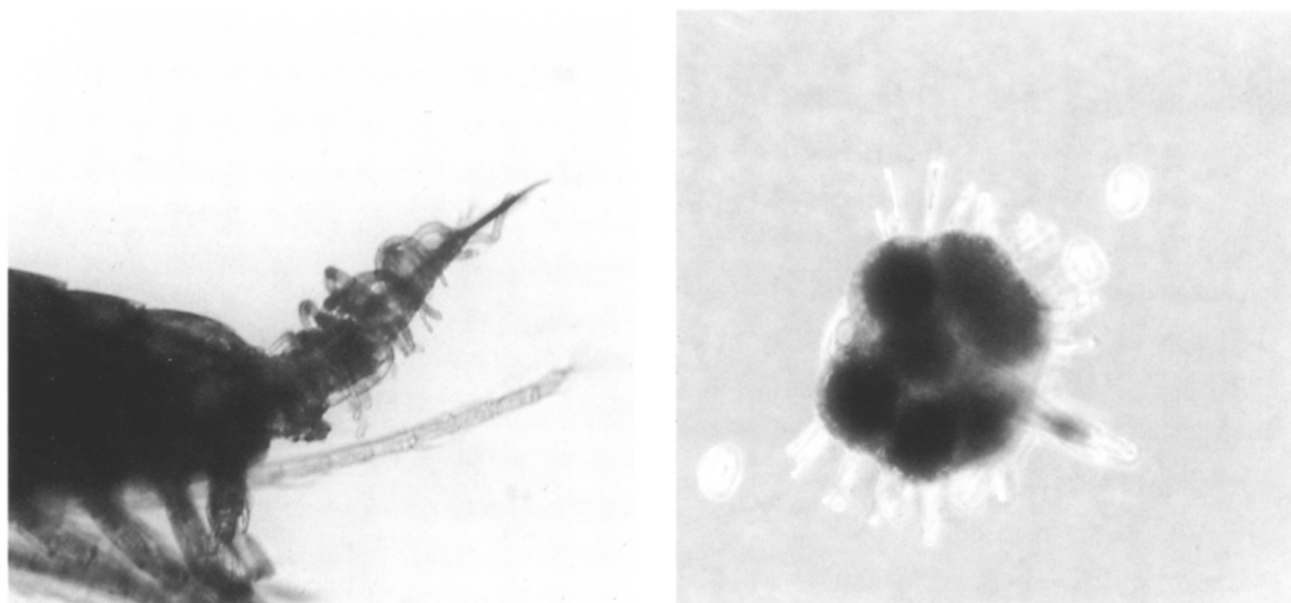
vival rates, fecundity, clutch sizes, clutch necrosis and the body length of infected and non infected *Eudiaptomus* are compared. The purpose of the study was to stress the impact of fungi on the copepod as a production controlling factor.

## Material and Methods

For detailed analysis, quantitative and qualitative zooplankton samples were collected from Lake Mondsee in September 1996. For quantitative sampling, a 10 l Schindler trap was used, and samples were taken at 2 m intervals from 0–20 m and at 30 and 40 m depth. These samples were used for identification of relative infection rates in different depths. Qualitative samples were taken by a large tow net, 100 µm mesh size, which was hauled vertically from about 30 m depth

to the surface. From the obtained zooplankton sample, living fungal parasitized and non-parasitized female *Eudiaptomus gracilis* were separated and maintained in two different containers at an average density of about 700 ind. l<sup>-1</sup>. The animals were fed with concentrated phytoplankton from the lake, and the survival rate was determined daily during one week. Quantitative samples and a portion of the qualitative samples were preserved in 4% formalin solution. Neutral red was used to stain the parasites for detailed morphological characteristics.

In total, more than 1300 specimens of *Eudiaptomus gracilis* (35% female adults, 15% male adults, and 50% copepodites) caught in September 1996 were examined for the presence of parasites. The annual distribution of infected females and damaged eggs was calculated on biweekly quantitative regular zooplankton counts from Lake Mondsee for the years 1994–1996, including some 1300 samples of 10 liters each, collected from surface to 42 m depth, and more than 1000 adult females altogether.



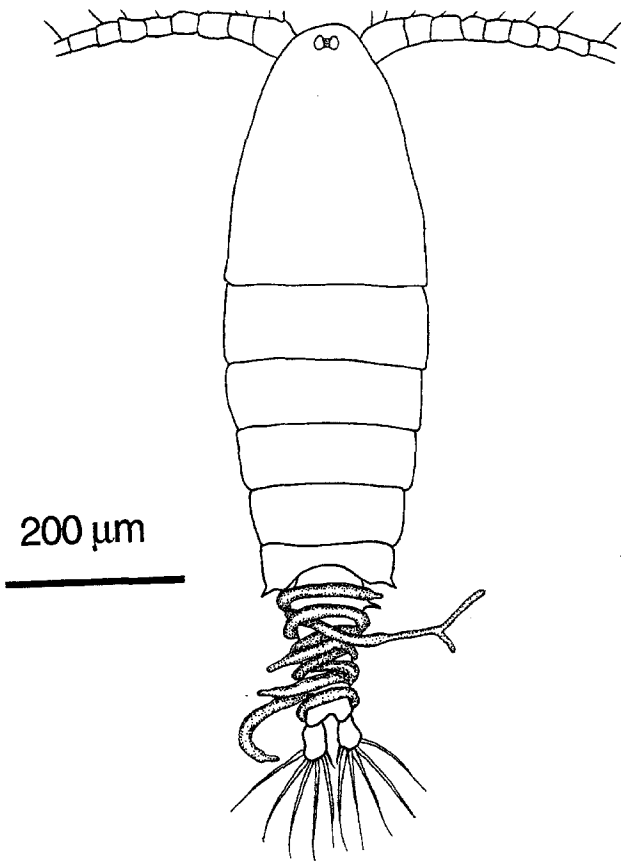
**Fig. 1.** Infection by the fungal parasite *Aphanomyces* sp. (Oomycetes). Left: Female abdomen. Right: Egg sac.

## Results

### Fungal parasite *Aphanomyces* sp. (Oomycetes)

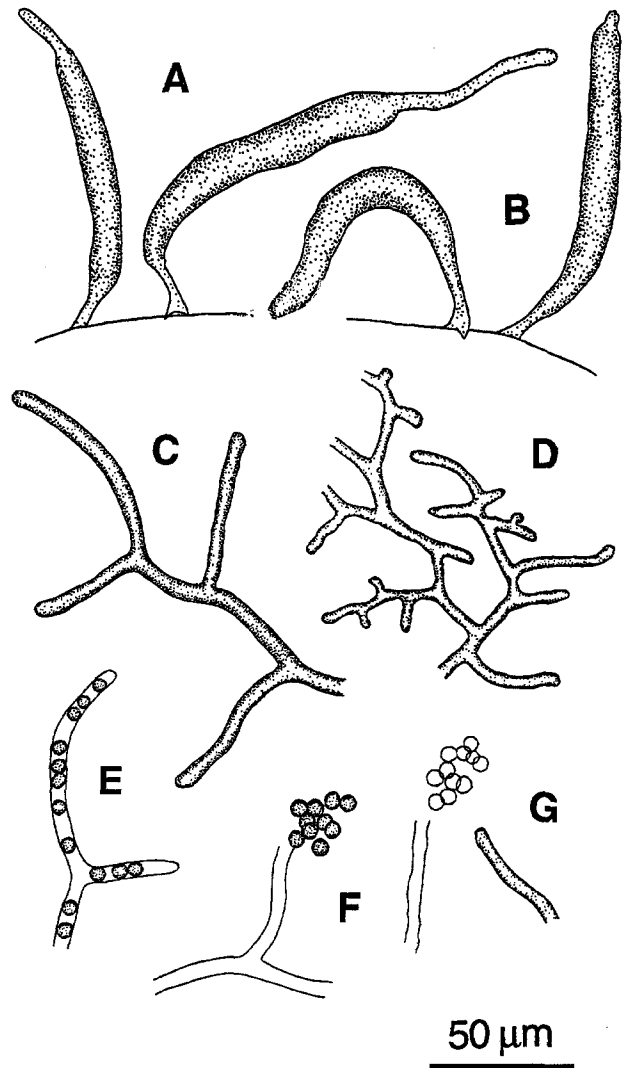
Only mature females of the *Eudiaptomus* were found to be infected. The fungal incidence was about 36%. Fungal infection was evidenced by the existence of densely packed infection hyphae which attached to the female's abdomen by holdfasts and wrapped around the abdomen (Figs. 1 and 2). Distally, these specialized infection hyphae which were about 10–15  $\mu\text{m}$  in diameter and 100–125  $\mu\text{m}$  long, gave rise to hyphae which penetrated the host's eggs or the outside of the thin cover of the clutch. The fungal attack was always localized to the eggs, and even in the final stages of infection, no fungi were found associated with the body of the adult females. It should be mentioned, however, that this could quickly become the case in dead animals.

Parasitized egg sacs appeared to be pale yellow in colour, and usually enlarged by protruding fungal hyphae. The fungal hyphae within the eggs of *Eudiaptomus gracilis* were highly branched, contorted, irregular, non-septate, and averaged 4.8  $\mu\text{m}$  in diameter (Fig. 3A, B). They were found to spread into the yolk, in this way filling the entire clutch and



**Fig. 2.** Fungal infection in female *Eudiaptomus gracilis*. The infection hyphae are wrapped around the abdomen.

finally killing it. The hyphae are of brownish-green colour and have a granular cytoplasm. The extramatrical hyphae were usually less branched and stronger (Fig. 3C). Usually, the complete hyphae were transformed into zoosporangia once the eggs had been nearly consumed by the fungi (Fig. 3D). The zoospores formed a single row in the zoosporangium, and encysted at the mouth of the zoosporangium immediately after emergence (Fig. 3E, F). Each cyst was about 5  $\mu\text{m}$  in diameter, giving rise to a reniform zoospore which emerged through a pore in the wall of the cyst. Only the empty cyst was left near the mouth of the zoosporangium (Fig. 3G).



**Fig. 3.** A, B. The infection hyphae of *Aphanomyces* sp. which attach with tapering holdfasts to the abdomen of *Eudiaptomus* and give rise to hyphae penetrating the egg sac. – C. Less branched and stronger extramatrical hyphae. – D. Highly branched, contorted, and non-septate fungal hyphae within the eggs. – E. Zoospores forming inside the sporangium. – F. Encysted zoospores. – G. Empty cysts.

## The effect of *Aphanomyces* sp. on the vitality of *Eudiaptomus gracilis*

The survival curves (Fig. 4) during 7 days of parasitized and non-parasitized *Eudiaptomus gracilis* females show the same slope. There is no significant difference between the curves. Accordingly, the size of infected females does not differ between infected and non-infected individuals (Table 2). Thus, the vitality of the female seems not to be influenced by the parasite.

A significant difference has been noted between clutch sizes of infected and of non-infected females (Table 2). If the vitality of the female is not effected by the parasite, this should not be expected. However, it could have been that one size class, e.g. senescent adults, were more easily attacked by the fungus. Seasonal comparisons in fact indicate that the age of the animals plays a role in infectability, be it because of physiological reasons or simply because of time. Thus, the observed smaller clutches in infected females, even if they are significant, do not necessarily need to be a result of the fungus infection itself.

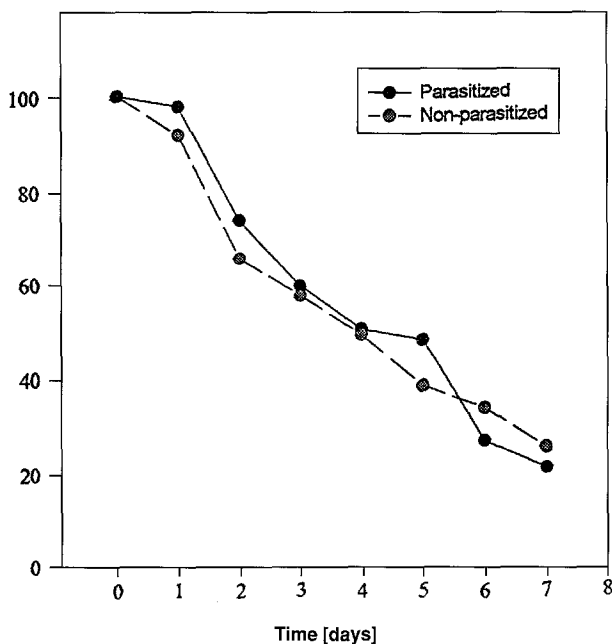


Fig. 4. The effect of *Aphanomyces* sp. on the survival rate of *Eudiaptomus gracilis* females.

Table 2. Body length and clutch sizes of fungal parasitized and non parasitized *Eudiaptomus gracilis*.

	Parasitized			Non-parasitized			P
	Mean	SD	N	Mean	SD	N	
Body length (mm)	0.895	0.017	50	0.897	0.010	55	>0.5
Clutches (egg number)	5.6	1.13	30	6.7	1.50	47	<0.05

Table 3. Non-necrotic and necrotic clutches in fungal parasitized and non-parasitized *Eudiaptomus gracilis*.

	Parasitized		Non parasitized		P
	%	N	%	N	
Non-necrotic clutch	37.5	136	34.5	180	>0.05
Necrotic clutch	96.1	62	8.1	51	<0.005

Not surprisingly, the percentage of necrotic eggs is much higher in infected females than in non-infected (Table 3). Probably, a good deal of the necrotic eggs in fact were fungi-infected but did not show typical signs. The same may be true for necrotic eggs in virtually non-infected females.

## Spatio-temporal variation of fungal infections in *Eudiaptomus gracilis*

During the September study 1996, no infected females were found in depths greater than 15 m. This may point at the importance of water temperature for the development of the fungus. Depths between 10 m and 15 m are the main daytime residential water layers of adult *Eudiaptomus gracilis* at this time of the year, while nocturnal migration brings them up to warm epilimnic layers (NAUWERCK 1993).

Annual variability of relative amounts of infected females and necrotic eggs reveals repeating basic patterns (Fig. 5). Infected females with typical twisted hyphae around the abdomen regularly appear from July and up to the end of the year. Maximal numbers are found in October/November. Nevertheless, necrotic eggs appear in reasonable numbers almost any time of the year. Low numbers are found during winter, but sometimes even in late spring and in the middle of summer. Clearly detectable fungus infections are by far not always apparent. There is some correlation between numbers of necrotic eggs and numbers of infected females, but numbers of necrotic eggs do not increase dramatically with the appearance of such females, and eggs sacs with typical sporangia can be found even when no infected females are observed.

Instead, the succession of necrotic egg's minima and maxima shows similar patterns from one year to the other, and seems to be more or less correlated with *Eudiaptomus* cohorts reaching adult stage or old age respectively.

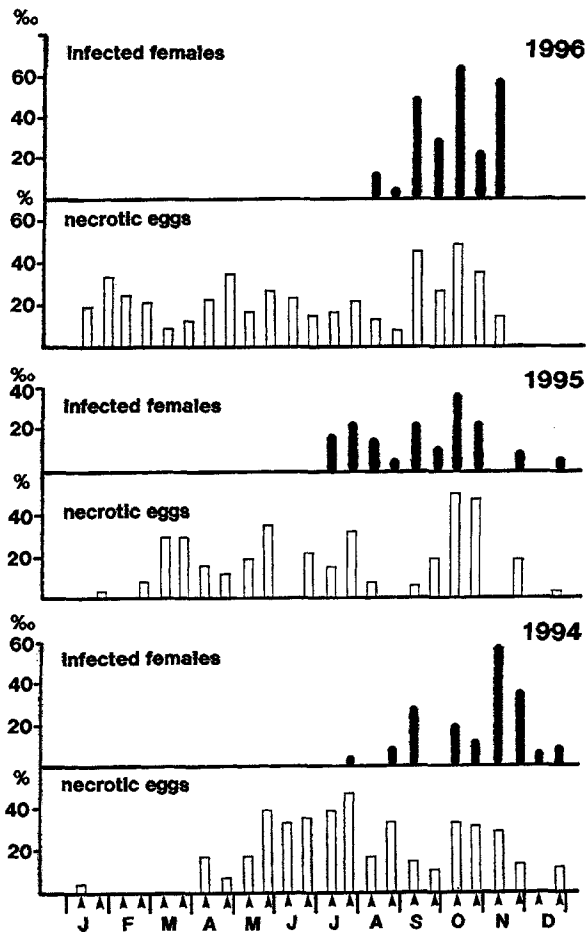


Fig. 5. Lake Mondsee 1994–1996. Fungal infection in *Eudiaptomus gracilis*.

## Discussion

There seems to be no doubt that the parasite of *Eudiaptomus gracilis* described above should be placed in the genus *Aphanomyces* because a single row of zoospores is encysting at the mouth of the zoosporangium after emergence, which is a characteristic of this genus. A fungal parasite of *Eudiaptomus gracilis*, *Aphanomyces ovidestruens* GICKLHORN, was described by GICKLHORN (1923) from several small lakes in Germany, and has not been reported since (SCOTT 1961). GICKLHORN noted that parasitized *Eudiaptomus gracilis* was orange-red or cadmium-red in colour. In the present study, no significant colour difference was observed between parasitized and non-parasitized animals. The infection hyphae in this study, unlike those described by GICKLHORN, are smaller in diameter and did not swell distally into „bulbous appressoria“. Instead, they tapered before branching into fine hyphae in the eggs of the host (Fig. 3).

BURNS (1980) reported one fungal parasite in *Boeckella dilatata* under the name of *Aphanomyces ovidestruens* from

two eutrophic lakes in New Zealand. Later, this fungus was considered to be a new species because of some morphological and ecological particularities (BURNS 1985a). The infection hyphae of fungi parasites in *Boeckella dilatata* also did not swell into “bulbous appressoria”. But the diameter of the infection hyphae is wider (16–25 µm) than that of the fungi in the present study. The morphological features may be varied over fairly wide ranges, probably in response to nutritional and other environmental factors (BURNS 1980). Therefore, more detailed morphological and ecological information is needed in order to identify the present *Aphanomyces* to a species level.

According to our results, there is no effect of the fungal parasite on the survival rate, body length and productivity of the infected *Eudiaptomus gracilis*. This agrees with the observation that *Aphanomyces* does not enter the living adult female’s body, nor the male, but only the eggs. Similar cases of parasitic attacks limited to the eggs of copepods have been reported (GICKLHORN 1923; REDFIELD & VINCENT 1979). Both parasitic *Aphanomyces ovidestruens* and *Lagenidium* sp. are restricted to the eggs of *Eudiaptomus gracilis* and of *Diaptomus novamexicanus* respectively. It has also been found that *Aphanomyces* sp. of *Boeckella dilatata* is an egg-parasite (BURNS 1985b). But in this case, the mortality was higher in parasitized females (41%) than in non-parasitized females (18%).

The actual observations show very high necrosis rates in eggs of parasitized females (96%). It is to be expected that in fact all attacked female’s eggs are condemned to be destroyed. No parasitized clutches could ever be observed where single eggs had survived, but it was always the whole clutch which was penetrated by the fungus hyphae.

It may be worth mentioning that eggs of *Cyclops abyssorum praealpinus* and *Mesocyclops leuckarti* from Lake Mondsee showed a much lesser degree of parasitization than the eggs of *Eudiaptomus*, and, in contrast to *Eudiaptomus*, only single eggs within a clutch were attacked, and only about 5 or 6 sporangia were formed in each infected egg.

From Fig. 5 it is evident, that at an average, about 20% of all *Eudiaptomus* eggs were virtually necrotic. Maxima, reaching more than 40% usually occurred together with maximal female fungi infection. Real infection rates certainly were higher. Without any doubt, fungal parasitism can be considered as a major population (birth rate) controlling factor. However, there exists a discrepancy between virtually infected females and virtually infected eggs. Assuming an average clutch size of 10 eggs, proportions between infected female numbers and infected clutch numbers remain about 1 : 10.

Another contradiction seems to be that in the September 1996 study 36% of all females were infected, while in 1994–1996 field observations 6% were not exceeded. Certainly, warm temperature quickly increases the infection of females. The September study was carried out with animals from warm water layers only, kept in room temperature at very

high concentrations. This may partly explain the differences. Yet, it remains an open question whether necrotic eggs found during the absence of virtually infected females are the result of an *Aphanomyces* infection alone, or if other or additional factors are involved. The fact that GICKLHORN's "bulbous appressoria" actually could be observed on infected egg clutches during winter, when no infected females appeared, could point to broad variability of our *Aphanomyces* sp. morphology and reproduction behaviour, or perhaps, to the participation of more than one fungus in the puzzle.

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