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Population Dynamics and Production Studies of Species of *Nebelidae* (*Testacea*, *Rhizopoda*) in an Aspen Woodland Soil

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Synopsis. Three species of *Nebelidae* represented 2% of the mean annual density, 2% of the total production numbers, 4% of the mean annual biomass, and 3% of the total annual production of all the testacean species observed in an aspen woodland soil. Mean annual biomass and total annual production for the *Nebelidae* were estimated as 0.03 and 6.4 g wet weight m^{-2} , respectively, while total annual ingestion, respiration losses and egestion losses were calculated as 42.9, 10.8 and 25.7 g wet weight m^{-2} respectively. The annual production biomass varied from 150 to 310 (mean 230) times the standing crop of *Nebelidae* in all the soil layers. The dry weight of carbon respired annually by the *Nebelidae* was estimated as 0.5 g m^{-2} , which amounted to 0.06% of the total annual carbon input.

Little is known about several aspects of the ecology of soil *Testacea*, especially annual population dynamics, secondary production, biomass turnover and impact on the organic cycle. The *Euglyphidae* have received some interest (Smith 1983, Schönborn 1975, 1977, 1978, 1982, Coûteaux 1976, 1978, Laminger 1978, Laminger et al. 1980) but other families such as the *Nebelidae* have been studied very little. Several other aspects, particularly taxonomy and geographical distribution (Deflandre 1936, Bonnet and Thomas 1960, Grospietsch 1965, Decloitre 1977, 1979, 1982), are reasonably well known. Much basic biological information is still lacking and the interpretation of much of the ecological information available is limited by this. Some ecological information, e.g., annual fluctuations in density and biomass, annual production, generation times, number of generations per year and mortality rates, is available in bits for a few species of *Nebelidae* (Lousier 1974 a, b, Schönborn 1977, 1978, Coûte-

aux 1978, Laminger et al. 1980). The objectives of this paper are to detail (1) the annual variation in density, biomass, production and turnover in each of the soil and litter layers, (2) the relationship to the total testacean community, and (3) the relative importance in the aspen woodland soil, of the *Nebelidae*. The study was carried out in the cool temperate deciduous woodland for a period of 57 weeks.

Study Site and Methods

Site Location and Description

The general study area comprised about 21 km² at the northern end of the Kananaskis Valley in the Fisher Range of the Rocky Mountains of Alberta, Canada. Detailed descriptions of the site, including additional information on the soil, vegetation, climate, litter input, litter decomposition, and litter and soil chemistry, were provided by Lousier (1974 a, 1975, 1976) and Lousier and Parkinson (1976, 1978, 1979). In summary, the study site was an aspen woodland located at 1400 m ASL on a well-drained, south-facing slope. The climate is continental, characterized by short, dry summers and relatively long, cold winters with intermittent, warm, chinook winds. The soil has been classified in the orthic gray luvisol subgroup, and has a surface organic horizon easily separated into L (whole litter), F (fragmented litter), H (humus) and Ah (black mineral) layers. (Lousier 1974 a). The canopy was dominated by trembling aspen (*Populus tremuloides* Michx.), with balsam poplar (*P. balsamifera* L.) being less frequent in occurrence. Various grasses, herbs and wild rose shrubs are the main components of the understory.

Sampling and Analysis

The arrangement of the study plots in the aspen woodland has been illustrated in Lousier and Parkinson (1979); the sample preparation techniques and sample examination procedures were outlined by Lousier and Parkinson (1981 a); and the sampling program and population analysis equations were developed in Lousier (1984 a).

Results

Three species of *Nebelidae* were observed as live cells or empty tests throughout the study period in the aspen woodland soil (Table 1). All the nebelid species could be considered constant in frequency (*senus* Couëteaux 1976). Table 1 also presents the biomass (wet weight 10⁻⁶ cells) measured for each species of *Nebelidae*. Estimates of the live weight of

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Table 1

Occurrence, distribution, biomass (mg 10^{-6} cells) and constancy of species of *Nebelidae* in the organic layers of the aspen woodland soil

Species observed	Biomass	No. of cells measured	Distribution				Constancy (%)***			
			L	F	H	Ah	L	F	H	Ah
<i>Hyalosphenia minuta</i> Cash	0.6±0.1*	100	**+/+	+/+	+/+	+/+	100	100	100	100
<i>H. subflava</i> Cash	6.2±0.5	100	+/+	+/+	+/+	-/+	100	100	100	0
<i>Heleopera petricola</i> Leidy	10.7±0.6	100	+/+	+/+	+/+	-/+	100	100	100	0

* Standard error

** Observed as living/observed as empty tests

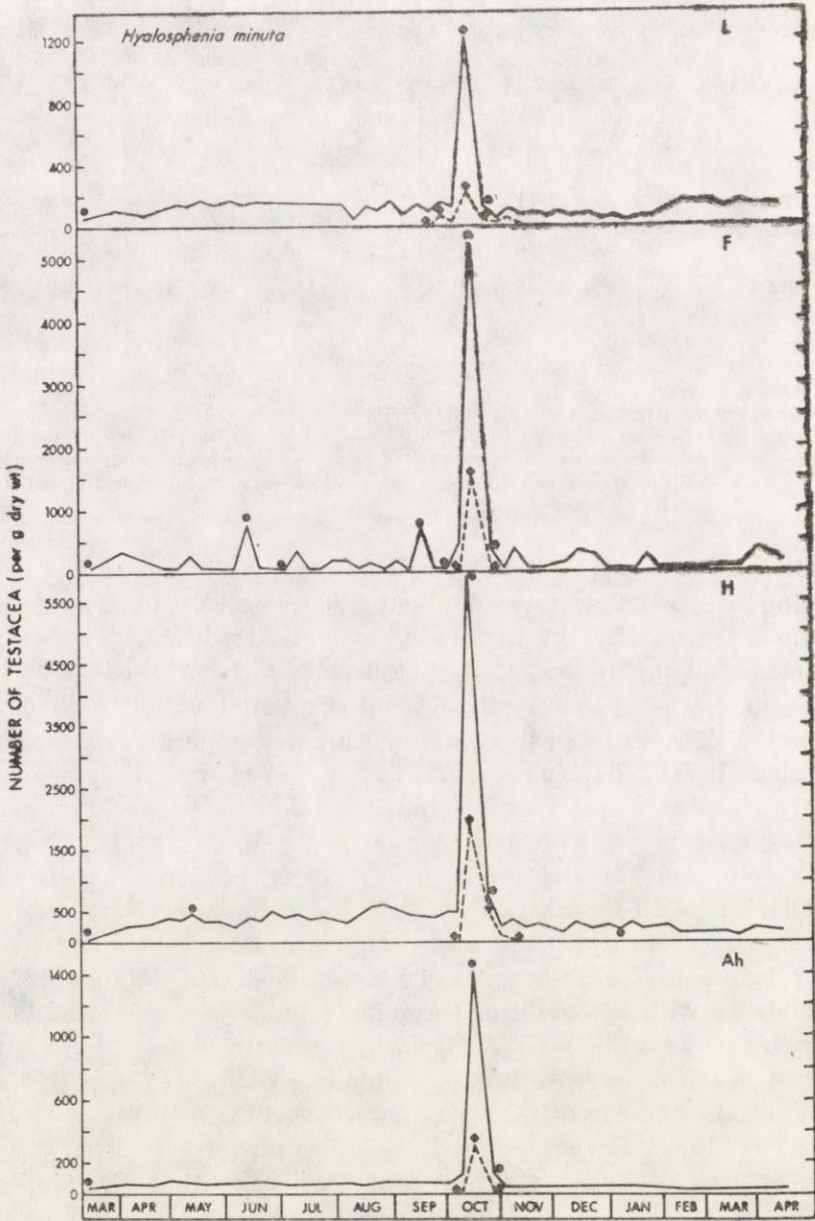
*** Constancy: Species observed in >50% of samples — constant in frequency;
Species observed in 25–50% of samples — incidental in frequency;
Species observed in <25% of samples — accidental in frequency
(C o û t e a u x 1976).

an individual cell were made by calculating the volume of the cell and assuming a specific gravity of about 1.0 (H e a l 1970). The estimates were made primarily on live individuals isolated from field samples with some augmentation with measurements from the C o û t e a u x slides. There was no observed seasonal or spatial variation in cell size or biomass. The estimates given in Table 1 compare favourably with those given in S c h ö n b o r n (1977).

Only *H. minuta* was found living in all four soil layers; *H. subflava* and *H. petricola* were restricted to the L, F, H layers. These two species colonized the decomposing leaf litter earlier than *H. minuta* (*H. petricola* — 18 months, *H. subflava* — 24 months, *H. minuta* — 36 months, L o u s i e r 1982) and may require more of the litter layer (L and F) influence for their occurrence, although *H. subflava* increased in abundance with age of litter throughout the 5 year colonization study period (L o u s i e r 1982). *H. petricola* increased in biomass to 36 months, and then began a decrease for the remainder of the study period, while *H. minuta* also increased in biomass from the time of initial colonization (L o u s i e r 1982). Given the early colonization of leaf litter by some of the species of *Euglyphidae*, it was surprising that *H. minuta* and *H. subflava*, species also of small size, with platelet tests, and occurring in the L layer, were so slow to colonize.

Hyalosphenia minuta

H. minuta had the most unique annual abundance curves of all the constant species (Fig. 1). The curve for each layer was almost identical in pattern to the curves in the other layers, with numbers per g dry



weight about equal in the L and Ah layers and in the F and H layers. All four populations were characterized by the substantial post-leaf fall peak and the presence of cysts only at this time.

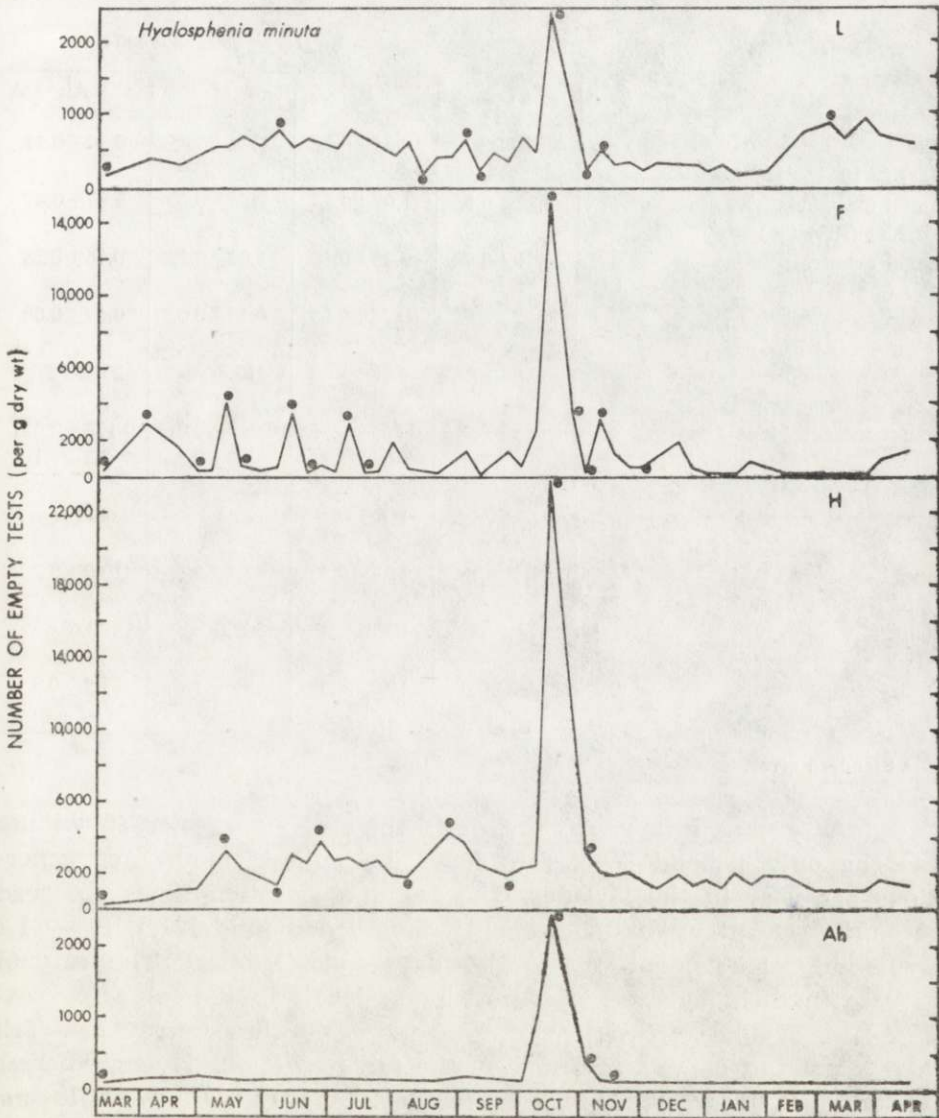


Fig. 1. The annual fluctuations of numbers of active, encysted and empty tests of *Hyalosphenia minuta* (— — — encysted tests; filled dot — indicates significant difference from previous filled dot — for active or empty tests; filled square — indicates significant difference from previous filled square — for encysted tests)

H. minuta had the lowest biomass of all 14 constant species in the L and F layers, the second lowest biomass in the H and Ah layers, and the lowest P_B in all layers (Table 2). However, in terms of r and P_B/B ,

Table 2

Summary of the population parameters calculated for *Hyalosphenia minuta*

	L	F	H	Ah
Annual mean density (D) ($\times 10^6 \text{ m}^{-2}$)	0.16 \pm 0.02*	0.55 \pm 0.22	1.74 \pm 0.45	0.34 \pm 0.11
Annual mean relative density (RD) (%)	1.23 \pm 0.15	1.07 \pm 0.29	1.55 \pm 0.20	2.87 \pm 0.47
Annual mean biomass (B) (mg m^{-2})	0.10 \pm 0.02	0.39 \pm 0.18	1.00 \pm 0.26	0.76 \pm 0.28
Annual mean relative biomass (RB) (%)	0.22 \pm 0.02	0.16 \pm 0.03	0.30 \pm 0.03	0.57 \pm 0.08
Production numbers (P_N) ($\times 10^6 \text{ m}^{-2} \text{ y}^{-1}$)	30.8	181.1	513.3	201.3
Annual mean r (day $^{-1}$)	0.182 \pm 0.012	0.245 \pm 0.028	0.257 \pm 0.011	0.133 \pm 0.011
Annual mean generation time (T) (days)	5.1 \pm 0.5	7.1 \pm 1.4	3.0 \pm 0.2	7.2 \pm 1.1
Number of generations (G) (y $^{-1}$)	86.5	116.5	122.5	68.8
Annual mean mortality rate (M) (% day)	11.1 \pm 0.1	11.3 \pm 0.3	12.3 \pm 0.1	10.0 \pm 0.2
Production biomass (P_B) (mg $\text{m}^{-2} \text{ y}^{-1}$)	18.5	108.6	308.0	120.6
Annual mean P_B/B (week $^{-1}$)	4.9 \pm 0.6	19.8 \pm 4.5	9.1 \pm 1.6	3.9 \pm 0.8

* Standard error.

the species ranked in the top third of all the constant species. *H. minuta* was the only nebelid species to have its highest number of generations per year in the H layer. The fastest generation times observed were: L — 1.4 days (mid-October), F — 1.0 day (mid-July), H — 1.2 days (mid-October), and Ah — 1.4 days (mid-October). The rate of increase in each layer achieved maximum levels in the following seasons: L — summer, fall, late winter, F and H — all seasons, Ah — fall only. Maximum P_B was recorded on the fall in all four layers, whereas maximum P_B/B occurred only in the fall in only H and Ah layers. Spring and fall were the seasons of maximum P_B/B in the L layer, while the F — layer *H. minuta* showed no seasonal trends. Only about 0.3% and 9% of the total annual production biomass of all 28 species and of the *Nebelidae* respectively were attributable to this species.

Hyalosphenia subflava

Despite being members of the same genus and having generally the same densities, there were some major differences between the populations of *H. subflava* and *H. minuta*. The cellular biomass of *H. minuta*

was ten times that of *H. minuta*, and living *H. subflava* were not recorded in the Ah layer. The patterns of population fluctuations in each layer were not quite so comparable in *H. subflava* as they were in *H. minuta* (Fig. 2). The general patterns of each species were, however,

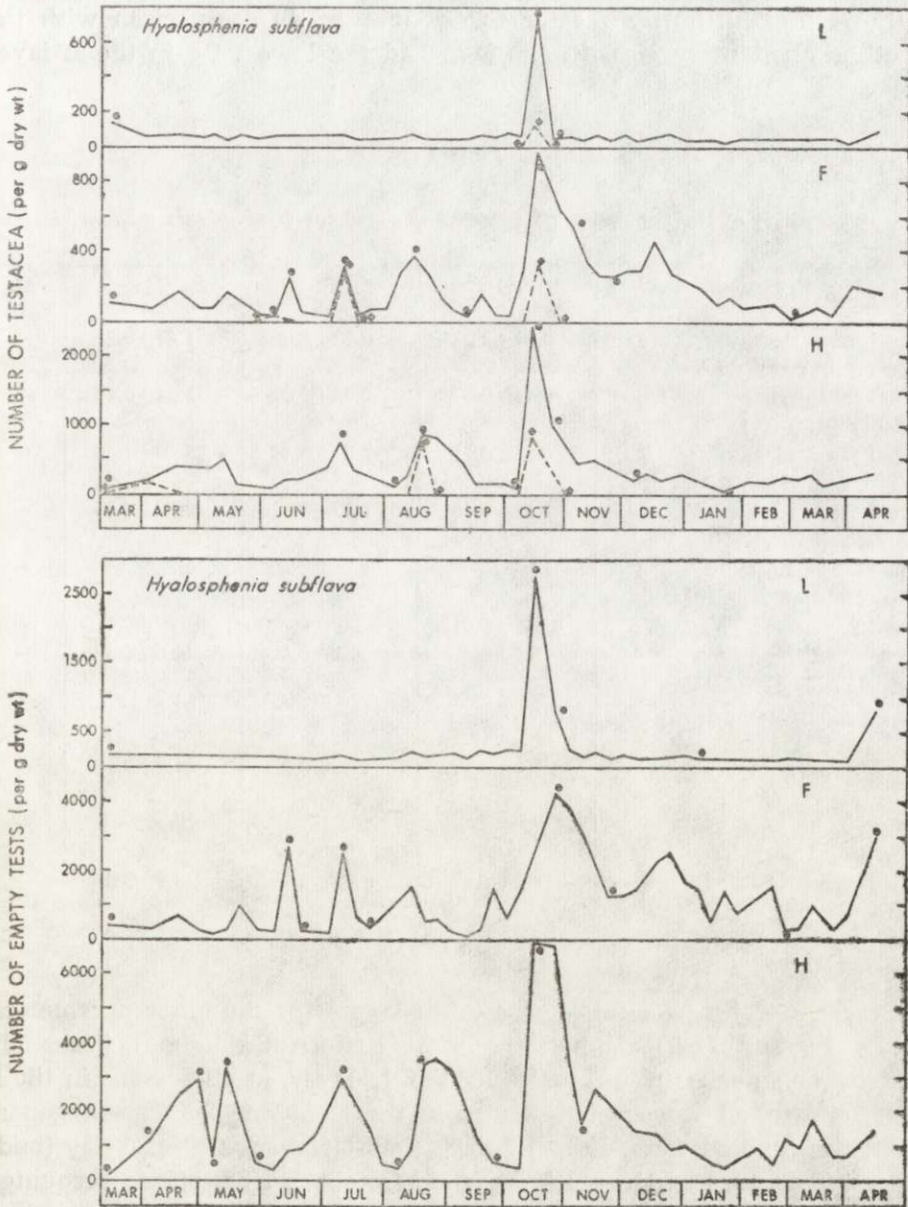


Fig. 2. The annual fluctuations of numbers of active, encysted and empty tests of *Hyalosphenia subflava* — (—) encysted tests; filled dot — indicates significant difference from previous filled dot — for active or empty tests; filled square — indicates significant difference from previous filled square — for encysted tests)

quite similar except that the population decreases after the post-leaf fall peak were more gradual for *H. subflava*. There were also more minor, significant fluctuations in the F- and H-layer populations of *H. subflava*.

While P_B was significantly greater for *H. subflava* (Table 3), P_N and P_B/B were significantly greater for *H. minuta*. In comparison with the 14 other constant species, *H. subflava* had the lowest P_N in the L layer

Table 3

Summary of the population parameters calculated for *Hyalosphenia subflava*

	L	F	H	Ah
Annual mean density (D) ($\times 10^6 \text{ m}^{-2}$)	0.09 \pm 0.02*	0.40 \pm 0.06	1.47 \pm 0.21	—
Annual mean relative density (RD) (%)	0.64 \pm 0.07	0.78 \pm 0.09	1.34 \pm 0.16	—
Annual mean biomass (B) (mg m^{-2})	0.53 \pm 0.11	2.64 \pm 0.43	8.60 \pm 1.30	—
Annual mean relative biomass (RB) (%)	1.12 \pm 0.08	1.25 \pm 0.13	2.62 \pm 0.24	—
Production numbers (P_N) ($\times 10^6 \text{ m}^{-2} \text{ y}^{-1}$)	14.4	129.9	330.4	—
Annual mean r (day^{-1})	0.131 \pm 0.011	0.249 \pm 0.020	0.197 \pm 0.016	—
Annual mean generation time (T) (days)	6.7 \pm 0.5	4.1 \pm 0.4	4.6 \pm 0.4	—
Number of generations (G) (y^{-1})	62.7	119.6	93.8	—
Annual mean mortality rate (M) ($\% \text{ day}^{-1}$)	9.9 \pm 0.1	11.8 \pm 0.2	11.4 \pm 0.1	—
Production biomass (P_B) ($\text{mg}^{-2} \text{ y}^{-1}$)	89.4	805.3	2048.5	—
Annual mean P_B/B (week^{-1})	4.2 \pm 1.1	13.5 \pm 3.8	8.9 \pm 3.0	—

but was generally near mid-range in all layers for the other parameters. The r , P_B and P_B/B all had the same pattern throughout the study periods, i.e., peaks in fall only in the L layer, in all seasons in the F layer, and in all seasons but winter in the H layer. The fastest generations times noted were: L — 1.2 days (mid-October), F — 1.0 day (mid-June, mid-July), and H — 1.0 day (mid-October). *H. subflava* accounted for about 1.4% of the total annual secondary production and almost 46% of the nebelid production for the year.

Heleopera petricola

The patterns of the active population fluctuations in each layer were similar in that there were two major peaks during the study period (Fig. 3), one in mid-summer and the other in mid-winter. *H. pe-*

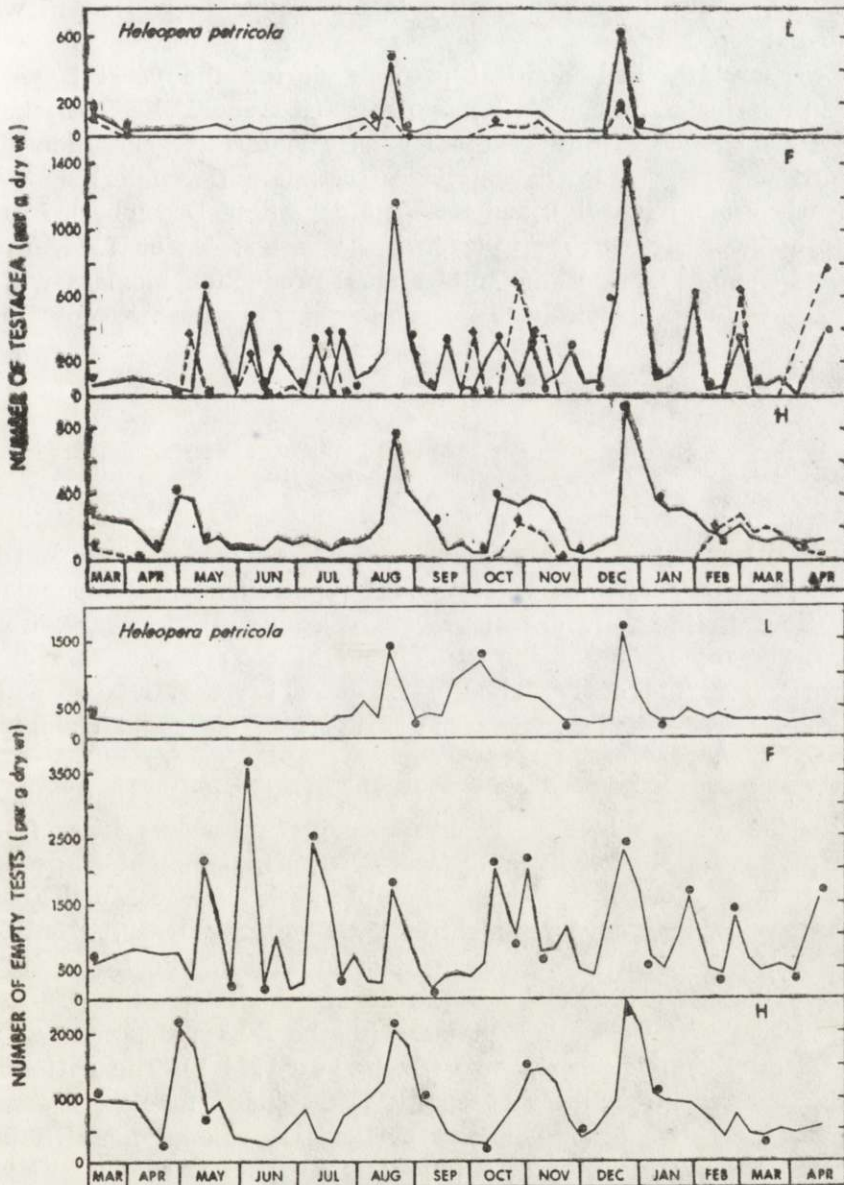


Fig. 3. The annual fluctuations of numbers of active, encysted and empty tests of *Heleopera petricola* (— — — encysted tests; filled dot — indicates significant difference from previous filled dot — for active or empty tests; filled square — indicates significant difference from previous filled square for — encysted tests)

tricola and *P. acropodia* (Lousier 1984 b) were only two species to peak in late December. This appeared to be unusual for the *H. petricola* because it was the largest pyriform species in the aspen woodland soil and, as such, should have required greater available moisture and space, commodities presumably less available in winter, in which to live and reproduce.

The r and P_B had identical patterns during the year: L — low values in spring only, F and H — no seasonal trends. The P_B/B showed no seasonal trends in any of the layers. The fastest generation times recorded were: L — 1.2 days (mid-August, late December), F — 1.0 day (early June, mid-July), and H — 1.5 days (late December). In relation to other species, *H. petricola* had the fastest T for L-layer species. Only about 1.4% of the total annual production biomass was accounted for by *H. petricola*. This species also represented just under 46% of the total annual *Nebelidae* production.

Discussion

Several general, summary comments can be made with regard to the information shown in Figs. 1–3. The patterns for the population fluctuations in the four organic layers were usually similar with all major peaks occurring at the same time in each layer. This differs somewhat from the euglyphid situation in which the L layer pattern was usually quite distinct (Lousier 1984 a). This could possibly be explained by the apparent preference for the deeper organic layers by the *Nebelidae* observed. Only the *Hyalosphenia* species had a major peak in the autumn in each layer either just after leaf litter fall or just after the onset of the winter freeze. The population of *H. petricola* in the H layer showed a prolonged autumn peak but the L and F layer populations did not have such a trend. *H. petricola* was the only nebelid species to reach its annual maxima in winter. The *Nebelidae* did not have the increases in abundance during the later winter — early spring period that were predominant with the *Euglyphidae* (Lousier 1984 a) and *Diffugiella oviformis* (Lousier 1984 b). The patterns of the fluctuations in numbers of empty tests tended to closely parallel those for the active tests. This was particularly evident for *H. minuta* indicating a rapid turnover of empty tests (Lousier and Parkinson 1981 b). The variations in cyst number, when the cysts were recorded, generally paralleled the fluctuations of active forms, i.e., coincident increases and decreases.

Tables 2-4 show that for all the constant species, annual mean weekly density and annual mean weekly biomass increased with profile depth to the H layer. The highest annual production totals for

Table 4

Summary of the population parameters calculated for *Heleopera petricola*

	L	F	H	Ah
Annual mean density (D) ($\times 10^6$ m ⁻²)	0.09 \pm 0.02*	0.54 \pm 0.08	0.82 \pm 0.10	—
Annual mean relative density (RD) (%)	0.69 \pm 0.09	1.23 \pm 0.19	1.18 \pm 0.26	—
Annual mean biomass (B) (mg m ⁻²)	10.1 \pm 0.17	5.71 \pm 0.84	8.12 \pm 1.03	—
Annual mean relative biomass (RB) (%)	2.37 \pm 0.41	3.46 \pm 0.57	3.09 \pm 0.43	—
Production numbers (P _N) ($\times 10^6$ m ⁻² y ⁻¹)	21.0	114.7	137.8	—
Annual mean r (day ⁻¹)	0.204 \pm 0.015	0.208 \pm 0.022	0.170 \pm 0.014	—
Annual mean generation time (T) (days)	4.6 \pm 0.5	7.1 \pm 1.2	6.1 \pm 0.7	—
Number of generations (G) (y ⁻¹)	97.6	98.5	81.2	—
Annual mean mortality rate (M) (% day ⁻¹)	11.5 \pm 0.1	10.8 \pm 0.3	10.2 \pm 0.3	—
Production biomass (P _B) (mg m ⁻² y ⁻¹)	224.3	1227.8	1474.6	—
Annual mean P _B /B (week ⁻¹)	6.3 \pm 1.2	14.8 \pm 4.6	6.3 \pm 2.0	—

* Standard error.

numbers and biomass were highest in the H and lowest in the L. The annual mean daily intrinsic rate of natural increase was highest in the F layer for *H. minuta* and *H. petricola* and in the H for *H. minuta*. While *H. minuta* had its highest number of generations in the H layer, the other two nebelid species had their highest number in the F layer. The annual weekly mean P_B/B was highest in the F layer for all of the species.

The three constant nebelid species represented 2% of the mean annual density and 4% of the mean annual biomass (Table 5). Laming-er (1978) found that the *Nebelidae* were only a minor component (<3%) of the total living *Testacea* in an alpine brown-earths-podsol. In terms of total production numbers and total annual production, the *Nebelidae* accounted for 2% and 3%, respectively.

Table 5

Comparisons between the 3 constant species of *Nebelidae*, the 14 constant species in the total community, and the 28 species comprising the total testacean community

		Totals for constant <i>Nebelidae</i>	Totals for the 14 constant species	Totals for all 28 species
Mean annual density (D) ($\times 10^6 \text{ m}^{-2}$)	L	0.3	17	18
	F	1.5	62	64
	H	4.0	156	158
	Ah	0.3	20	21
	Total	6.1	255	261
Mean annual biomass (B) (mg m^{-2})	L	1.6	36	46
	F	9.0	148	210
	H	18.0	276	346
	Ah	0.8	119	121
	Total	29.4	579	723
Production number (P _N) ($\times 10^6 \text{ m}^{-2} \text{ y}^{-1}$)	L	66	5034	5190
	F	426	19850	20677
	H	982	37052	38197
	Ah	201	25056	26862
	Total	1675	86992	90926
Production biomass (P _B) ($\text{mg m}^{-2} \text{ y}^{-1}$)	L	332	8295	14479
	F	2142	48158	59950
	H	3831	91428	100995
	Ah	121	30504	31107
	Total	6426	178385	206531

There are almost no data with which to compare the findings of this study. The annual total production for the *Nebelidae* (6.4 g m^{-2}) far exceeded the 0.003 g m^{-2} estimated for this family in a moss cushion under a German beechwood forest (Schönborn 1977) and the $0.5 \text{ g m}^{-2} \text{ y}^{-1}$ recorded by Schönborn 1978) for a beechwood soil.

Starting with the estimates of secondary production, it is possible to approximate the amount of food consumed by the testacean populations (Heal 1967, Laybourn 1976 (Table 6). The amount of bacteria consumed by the *Nebelidae*, $42.9 \text{ g m}^{-2} \text{ y}^{-1}$, comprised only 3% of the total annual mass of bacteria consumed (Lousier and Parkinson 1984). The ratio of secondary production to standing crop of *Nebelidae* (P_B/B) ranged from 150 to 310, averaging 230 for all species in all layers. The amount of carbon respired per year by the nebelid species was estimated as: 1.08 g m^{-2} (dry weight) $\times 47\%$ (carbon content of amoebae, Band 1959) = 0.51 g m^{-2} , representing 0.19% of the total an-

Table 6

Energy budgets for the species of *Nebelidae* in the aspen woodland soil (All estimates are wet weight of biomass, g m^{-2})

		<i>H. minuta</i>	<i>H. subflava</i>	<i>H. petricola</i>
Mean annual biomass, ($B \times 10^{-3}$)	L	0.1*	0.5	1.0
	F	0.4	2.6	5.7
	H	1.0	8.6	8.1
	Ah	0.8	—	—
	Total	2.3	11.7	14.8
Total annual production, P	L	0.02	0.09	0.22
	F	0.11	0.81	1.23
	H	0.31	2.05	1.47
	Ah	0.12	—	—
	Total	0.56	2.95	2.92
Total annual respiration losses, R	L	0.03(0.01)**	0.15(0.05)	0.38(0.11)
	F	0.18(0.06)	1.35(0.41)	2.05(0.62)
	H	0.53(0.16)	3.43(1.03)	2.45(0.74)
	Ah	0.20(0.06)	—	—
	Total	0.94(0.29)	4.93(1.49)	4.88(1.47)
Total annual egestion Losses, E	L	0.06(0.02)	0.36(0.09)	0.90(0.22)
	F	0.42(0.11)	3.24(0.81)	4.92(1.23)
	H	1.26(0.31)	8.22(2.05)	5.88(1.47)
	Ah	0.48(0.12)	—	—
	Total	2.22(0.56)	11.82(2.95)	11.70(2.92)
Total annual ingestion, I	L	0.1(0.05)	0.6(0.2)	1.5(0.6)
	F	0.7(0.3)	5.4(2.0)	8.2(3.1)
	H	2.1(0.18)	13.7(5.1)	9.8(3.7)
	Ah	0.8(0.3)	—	—
	Total	3.7(1.4)	19.7(7.3)	19.5(7.4)

* Values not in parentheses represent 10°C, where $P = 0.151$, $R = 0.251$ and $E = 0.601$ (Rogerson 1981; Lousier and Parkinson 1984)

Values in parentheses represent optimum temperatures (15–25°C), where $P = 0.41$, $R = 0.21$, and $E = 0.41$ (Heal 1967; Laybourn 1976)

nual carbon input to the soil and 3.1% of the total respiration losses of the entire testacean community, indicating a minor role for the *Nebelidae* in the aspen woodland soil.

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L'ingestion de Rhizopodes Thécamoebiens par *Thecamoeba terricola*

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Synopsis. Ce travail décrit le processus d'ingestion chez *Thecamoeba terricola* prédateur de Thécamoebiens des Mousses.

Dans une étude détaillée, Bovee (1960) a décrit le processus d'ingestion chez *Thecamoeba sphaeronucleolus*, nous avons étudié cette activité phagocytaire chez un autre Gymnamoebien de ce groupe: *Thecamoeba terricola* Greeff, 1866, qui s'avert être un grand prédateur de Thécamoebiens des Mousses; en effet les plus grands individus font preuve d'une grande voracité vis à vis de nombreuses espèces.

Matériel et méthode

De nombreux *T. terricola* ont été prélevés dans une culture de Mousses épigées maintenues en chambres humides.

Les coussinets de Mousses prélevés avec leur support sous-jacent de terre, ont été placés dans de grands cristallisoirs et régulièrement arrosés d'eau distillée, après quelques jours, l'eau aspirée à la pipette et examinée dans des boîtes de Petri au microscope inversé. Les espèces sont prélevées et placées en milieu nutritif (extrait de terre, infusion de blé à volume égal stérilisé sous U. V.)

Les études ont été faites entre lames et lamelles en fond clair et contraste de phase aux grossissements de $\times 100$, 400, 600 et 1000.

Observations

Ces Mousses contenaient outre l'espèce étudiée, de nombreux autres Protozoaires en activité: *Thecamoeba alba*, *Microchlamys patella*, *Euglypha rotunda*, *E. loevis*, *E. strigosa*, *Assulina muscorum*, *Trinema line-*

are, *T. penardi*, *T. complanatum*, *Heleopera sylvatica*, *H. petricola amethystea*, *Centropyxis aerophila*, *Diffugia lucida*, *Microcorycia flava*, *M. aculeata*, *M. penardi*, *Arcella arenaria*, *Corythion dubium*, des Ciliés et des Amibes de petite taille (Pl. I 1-8).

Parfait type de l'Amibe à pellicule, *Thecamoeba terricola* est entouré par une membrane souple, résistante et fortement plissée, toujours lâche et qui semble trop grande par rapport à l'endoplasme finement granuleux, massé vers le centre du corps autour d'un noyau ovale et souvent plusieurs vacuoles dont souvent une très volumineuse.

Ce Gymnamoebien extrêmement lent dans ses mouvements, peut montrer une grande activité cytoplasmique, qui se manifeste par des écoulements internes sans aucune progression de l'Amibe.

Ce prédateur semble attiré par la présence d'un Thécamoebien actif par une sorte de trophisme positif à une distance de quelques dizaines de microns; très lentement il y a progression vers la proie, on ne peut parler de pseudopode capteur, mais d'un prolongement ectoplasmique plus ou moins lamellaire enrobant la proie, structure temporaire qui se transforme en une poche phagocytaire. Lors de l'ingestion, la membrane cuticulaire semble seule participer à l'opération; la partie granuleuse de l'endoplasme reste massée au centre du corps.

Après l'avoir entièrement recouverte, l'enveloppe se soude complètement et il n'y a pas trace d'orifice d'entrée, la proie est dirigée vers l'intérieur par un phénomène d'invagination.

Le temps d'extention du pseudopode de contact (Pl. I 2) vers le Thécamoebien est d'environ une minute, le processus d'invagination demande de deux à quatre minutes suivant le cas. Il se forme alors progressivement une vacuole autour de la proie et le tout est lentement dirigé vers la partie granuleuse de l'endoplasme (Pl. I 3). L'ensemble des opérations dure de huit à quinze minutes.

La partie vivante du Thécamoebien est alors digérée et très souvent les thèques vides s'accumulent dans le cytoplasme du prédateur (Pl. I 4, 4 a) elles ne sont plus dès lors entourées d'une vacuole et sont déplacées au hasard des écoulements internes du cytoplasme.

Après ingestion complète, le temps de digestion de la partie vivante du Thécamoebien est difficile à contrôler; une seule observation sur ce sujet porte sur un individu de 290 μm dont le cytoplasme contenait déjà trois thèques vides de Thécamoebien: *Assulina muscorum*, *Euglypha rotunda* et *Corythion dubium*, ce prédateur venait d'absorber un *Trinema lineare*, l'ingestion complète était terminée à 11 h, à 11 h 30 le corps du Thécamoebien était concentré en une petite masse au centre de la thèque, après quatre heures, l'aspect n'avait pas changé ensuite progressivement, il s'est atténué, à 16 h seulement complètement digéré,

il n'était plus visible, il a donc fallu Cinq heures pour que la digestion soit complète dans ce cas-ci.

Certains *T. terricola* contiennent plusieurs thèques vides; nous avons compté jusqu'à sept *Euglypha rotunda* (Pl. I 4, 4 a).

Ce processus de capture ne se produit qu'envers un Thécamoebien vivant, émettant des pseudopodes, jamais nous n'avons observé la capture d'une thèque vide.

Il serait intéressant de connaître la raison de cette accumulation de thèques vides chez certains individus. En élevage pendant plusieurs jours, nous avons observé: trois, quatre et sept thèques vidées de leur contenu, circuler dans la masse mouvante au hasard des écoulements du cytoplasme, sans qu'aucune tentative d'expulsion ne se manifeste.

Tandis que chez d'autres, nous avons observé la défécation. Dans ce cas l'Amibe pour se débarrasser de la thèque se creuse pour former une sorte de poche entourant la proie, puis, très lentement, celle ci est poussée vers l'extérieur.

Le trou de passage dans la pellicule étant si rapidement ressoudé que nous n'en avons jamais observé trace, la réparation de la membrane a été observée et décrite par Penard (1938), cet auteur avait déjà fait remarquer l'importance de cette membrane dans son rôle de protection, en particulier aux agents nocifs; nous avons eu l'occasion de le vérifier au cours d'une expérience comparative de coloration: Dans une grosse goutte d'eau, nous avons isolé plusieurs spécimens de *T. terricola* en compagnie de divers Ciliés et nous les avons soumis à l'action d'une coloration directe, à l'aide d'une solution de Bleu de Toluidine glycerinée à 0,10%.

Les Ciliés témoins ont été immédiatement tués et leur noyau coloré en bleu intense, tandis que chez *T. terricola*, seule, la membrane s'est légèrement teintée de bleu, la partie centrale est restée vivante et active pendant trente minutes, puis progressivement toutes les vacuoles se sont fondues en une seule très grande, après une heure trente de nombreuses particules intracytoplasmiques polymorphes étaient colorées, deux heures ont donc été nécessaires pour que le colorant traverse la membrane, ceci démontre la grande efficacité qu'elle offre dans son rôle protecteur envers les conditions du milieu ambiant.

Signalons enfin qu'au cours de cette coloration, le Noyau ovoïde, bien visible en contraste de phase, ne s'est coloré faiblement qu'après rupture du protoplasme par compression avec le reste du cytoplasme.

Les parties non digérées du cytoplasme des Thécamoebiens ont pris le colorant, ce qui permet de les distinguer facilement des thèques déjà vidées de leur contenu.

SUMMARY

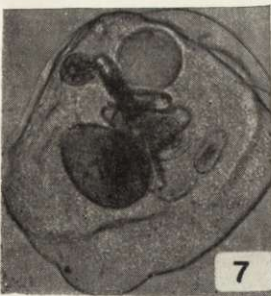
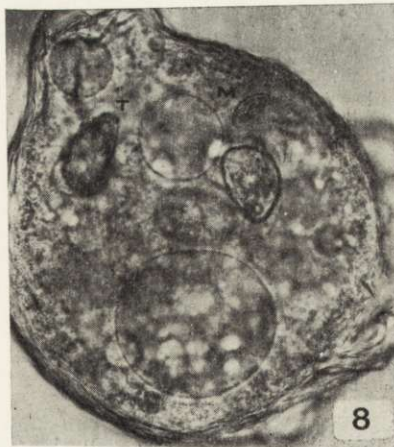
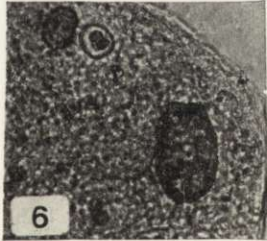
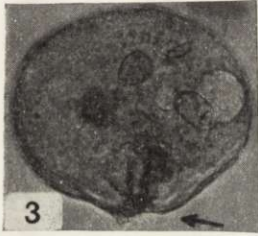
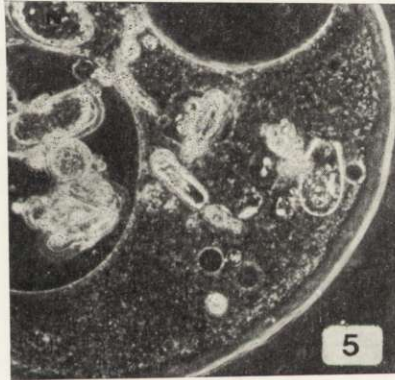
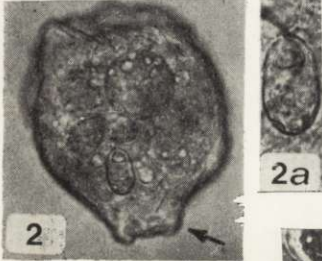
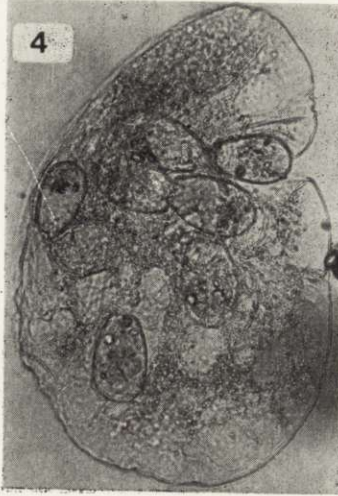
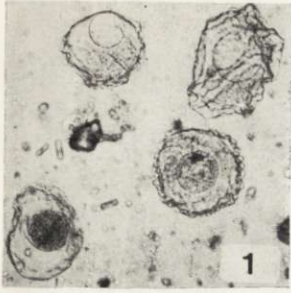
The results of investigations concerning the ingestion's process by *Thecamoeba terricola* predatory of Testate Amoebae from Moss.

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EXPLICATION DE PLANCHE I

- 1: *Thecamoeba terricola*, quatre individus $\times 100$
- 2: Absorption de *Trinema complanatum* $\times 200$ (la flèche indique le pseudopode de contacte)
- 2 a: *Trinema complanatum* dans le cytoplasme de l'Amibe $\times 400$
- 3: *Trinema complanatum* ingéré (la flèche indique le replis de passage dans la membrane)
- 4: *T. terricola* contenant 7 *Euglypha rotunda* $\times 600$
- 4 a: idem en contraste de phase
- 5: *Euglypha rotunda* dans le cytoplasme du prédateur N: noyau de *T. terricola* $\times 600$
- 5 a: *Euglypha rotunda* ingéré $\times 1000$
- 6: *Diffflugia lucida* ingéré $\times 400$
- 7: *Assulina muscorum* ingéré $\times 200$
- 8: *T. terricola* contenant 2 Thécamoebiens, *Trinema complanatum* T et *Microchlamys patella* M
- 8 a: *Trinema complanatum* ingéré $\times 1000$



D. Chardez

auctor phot.

Growth of a Ciliate Protozoan, *Tetrahymena pyriformis*
in the Presence of Different Isomers of Hexachloro-cyclohexane
(HCH)

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Synopsis. The effect of different HCH isomers, viz. α , β , γ and δ — on the cell population of a ciliate protozoan, *Tetrahymena pyriformis* was investigated. At a concentration of 5.0 ppm, γ -HCH reduced the cell number by 89% on the fifth day of treatment. The α -isomer reduced the cell number by 88% on the third day of treatment and β -HCH by 93% on the second day of treatment. The δ -isomer was lethal as all the cells died within 24 h of treatment. The order of toxicity of different HCH isomers in *T. pyriformis* was found to be $\delta > \beta > \alpha > \gamma$.

The consumption of technical grade pesticides in the areas of agriculture and public health in India, was estimated at 58,540 tons during the year 1976, out of which the consumption of HCH alone was 24,252 tons (Krishna-Murti et al. 1982). When these pesticides are released in the environment, in addition to having harmful effects on the pest, they may also adversely affect the non-target organisms. Among the non-target organisms in the aquatic environment, the ciliate protozoans are very important as they constitute a major group of microorganisms in aquatic ecosystem (Frenchel 1967, Butler 1977, Williams 1977) and are efficient nutrient regenerators (Johannes 1965, 1968). Protozoans occupy the first level of food chain and any adverse effect of insecticides on protozoans would ultimately lead to an imbalance at higher trophic level due to the disruption of the nutrient cycle in the ecosystem. Further, since protozoans feed on algae and bacteria, any effect of insecticides on protozoans will consequently affect both the algal and bacterial populations. Sessile ciliate protozoans are also reported to have been used to assess the level of water

pollution (Burbanck and Spoon 1967). The interaction of HCH isomers with ciliates is thus of vital importance. The present paper describes the effect of HCH isomers on the population and growth of *Tetrahymena pyriformis*.

Materials and Methods

Stock cultures of *Tetrahymena pyriformis* (Synngen-I) (obtained from Dr. J. G. Jones, Department of Biochemistry, University of Hull, Hull, U. K.) were maintained at $27 \pm 1^\circ\text{C}$ axenically, in 15 ml centrifuge tubes containing 2 ml of 1% proteoseptone (Difco) supplemented with 0.5% sodium chloride and 0.3% yeast extract.

Lindane (γ — 1, 2, 3, 4, 5, 6 hexachlorocyclohexane) was obtained from ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, Ohio. The other isomers of HCH viz., α , β , δ were obtained through the courtesy of Dr. Gunther Zweig, Environmental Protection Agency, USA.

The stock cultures of different isomers of HCH were prepared in acetone. The acetone concentration in the treated cultures was kept less than 0.5% as it was found that 0.5% acetone had no adverse effect.

The procedure adopted for conducting the experiments was essentially the same as described earlier by Rup Lal and Saxena (1979). A volume of 2 ml of 48 h old culture of *Tetrahymena* was transferred to 48 ml of sterile medium in 250 ml conical flasks and allowed to grow for 24 h. The number of cells per ml medium in each flask at the end of 24 h was determined by fixing 1 ml of the culture with an equal volume of 10% buffered neutral formalin and counting the cells with a haemocytometer. Different isomers of HCH were added separately from freshly prepared stock solutions to the 72 h old cultures. The concentrations of HCH-isomers used were 0.083, 0.5, 1.0, 2.5 and 5.0 ppm respectively. Simultaneously, two types of control were run, i.e., (1) normal culture without HCH and acetone and (2) cultures containing 0.5% acetone.

Cell number per ml medium was determined for 5 days at an interval of 24 h by taking out aseptically 1 ml of the culture medium from the controls as well as from the treated flasks. Five to six replicates were kept for every concentration of HCH used and out of these, three replicates were taken at one time and each of the replicates was counted thrice. Therefore, the cell counts used for calculations represent an average of nine counts each.

For recovery experiments, the ciliates were exposed to 2.5 and 5.0 ppm of lindane and α -HCH respectively and 1.0 and 2.5 ppm of β - and δ -HCH for three days. Subsequently, the animals were centrifuged, washed repeatedly with Chalkley's medium and transferred to 100 ml of HCH-free Chalkley's medium supplemented with 0.1% yeast extract. Cell counts were made from both treated and control cultures first at 30 min after transfer to the toxicant-free medium and later at 3, 6, 24 and 30 h.

Chalkley's medium used for recovery experiments contained (w/v, 0.01% sodium chloride, 0.0004% potassium chloride and 0.0006% calcium chloride (Randall and Jackson 1958).

Results

The data show that the growth cycle in *Tetrahymena pyriformis* follows a definite pattern. The active phase of division i.e., the log phase lasts 1-4 days. Subsequently, the cells enter the stationary phase and divide at a slower rate. Afterwards, unless the cultures are subcultured the growth rate further declines. There is no appreciable difference between the cell number in control and acetone control cultures (Table 1). The results were calculated as percentage effect as comp-

Table 1

Normal population growth of *T. pyriformis* (Syngen-1)

Number of days	Cell counts $\times 10^4$ /ml	
	Control	Acetone control
0	1.06	0.91
1	3.37	3.26
2	9.07	9.01
3	13.44	13.21
4	16.88	16.76
5	23.31	23.06

ared to acetone control. In populations of cells with a log normal distribution of generation times, cells with short generation times contribute more to the geometric increase in total cell number (Nachtwey and Cameron 1968). The net effect is that the culture doubling time is not the same as the mean cell generation time in mass cultures (Cameron 1973).

The different isomers of HCH used affected the growth of *T. pyriformis*, δ -HCH being the most toxic of all HCH isomers. In this case, a concentration of 0.083 ppm did not have any significant effect on the cell number. An increase in concentration to 0.5 ppm and 1.0 ppm reduced the cell number by 91% and 95% respectively on the fifth day of treatment. At a concentration of 5.0 ppm all the cells died on the first day of treatment itself. The data was statistically analyzed with F-test (Analysis of variance) and L.S.D. (least significant difference) at 0.05 was calculated for *a priori* comparisons of means among treatments (Sokal and Rohlf 1969). It was found that on the first day of treatment, a concentration of 2.5 ppm showed 5% significant effect with respect to control whereas on the second, fourth and fifth day of treatment, a concentration of 0.083 ppm showed $P = 0.05$. However, on the third day of treatment, 0.5 ppm showed 5% significant effect with respect to control (Fig. 1).

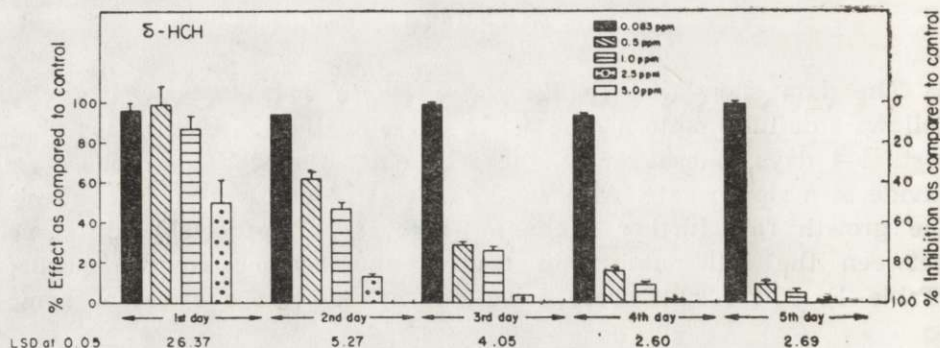


Fig. 1. Effect of δ -HCH on growth of *Tetrahymena pyriformis*

Next to δ -HCH in toxicity, was the β -isomer. In this case, lower concentrations of 0.083 ppm and 0.5 ppm did not produce any significant change in the cell counts. Treatment with 1.0 ppm reduced the cell number by 35% on the third day of treatment. Higher concentrations of 2.5 and 5.0 ppm were quite toxic and cell populations decreased by 85% and 93% respectively on the first day of treatment. Statistical analysis reveals that the concentration of 2.5 ppm showed 5% significant difference with respect to control on the first two days but on the last three days of treatment, a lower concentration of 0.5 ppm showed 5% significant difference (Fig. 2).

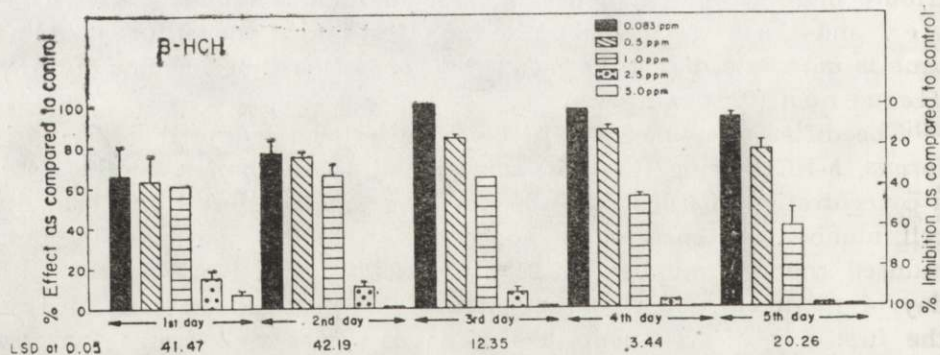


Fig. 2. Effect of β -HCH on growth of *T. pyriformis*

α -isomer was less toxic than β -HCH and a concentration of 0.083 ppm reduced the cell number by 55% on the fifth day of treatment. At 0.5 and 1.0 ppm the cell number reduced by 62% and 76% respectively on the fifth day of treatment. Higher concentrations of 2.5 and 5.0 ppm resulted in the reduction of cell number by 99% and almost 100% respectively on the fifth day of treatment. F-test indicates that on the first day of treatment, none of the concentrations used showed

5% significant difference with respect to control. However, on the remaining days of treatment a concentration of 0.083 ppm showed 5% significant difference with respect to control (Fig. 3).

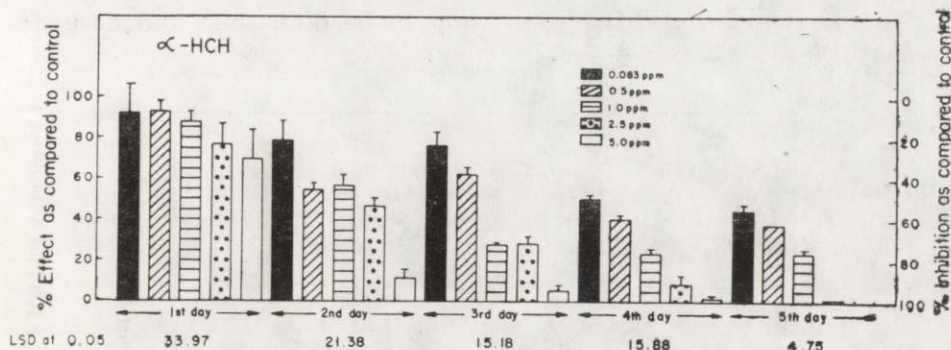


Fig. 3. Effect of α -HCH on growth of *T. pyriformis*

Among the isomers of HCH used, the γ -isomer was the least toxic. In this case, concentrations up to 1.0 ppm did not produce any significant effect on the cell number. On the fifth day of treatment with 5.0 ppm of γ -HCH, the cell number was reduced by 88%. Statistical analysis of results indicates that on the first day of treatment a concentration of 5.0 ppm showed 5% significant difference, whereas on the remaining days of treatment, 0.5 ppm was the concentration showing $P = 0.05$ with respect to control (Fig. 4).

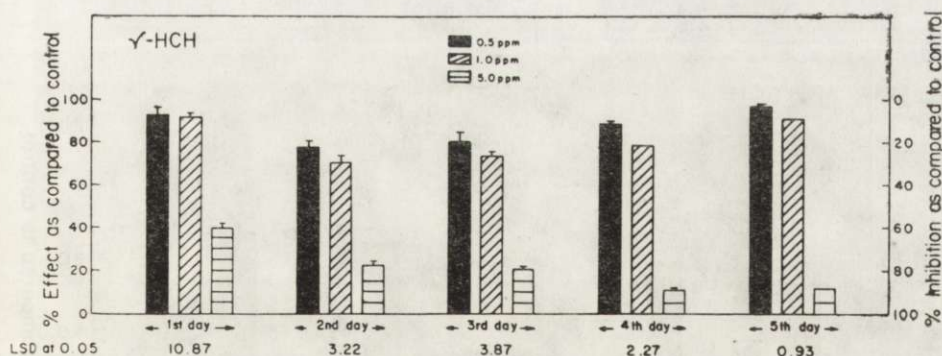


Fig. 4. Effect of γ -HCH on growth of *T. pyriformis*

The order of toxicity of different isomers of HCH in *Tetrahymena pyriformis* was, therefore $\delta > \beta > \alpha > \gamma$.

The retardation of cell growth of *Tetrahymena pyriformis* on treatment with HCH isomers was reversible as the cells started recovering

when the cells, treated with the sublethal concentrations of the insecticide for three days, were centrifuged, washed and transferred to a toxicant-free medium. The cell number in the treated cultures were comparable to that of the control. Furthermore, the inhibition of cell growth by various isomers, of HCH was found to be both time and dose dependent (Figs. 5-8).

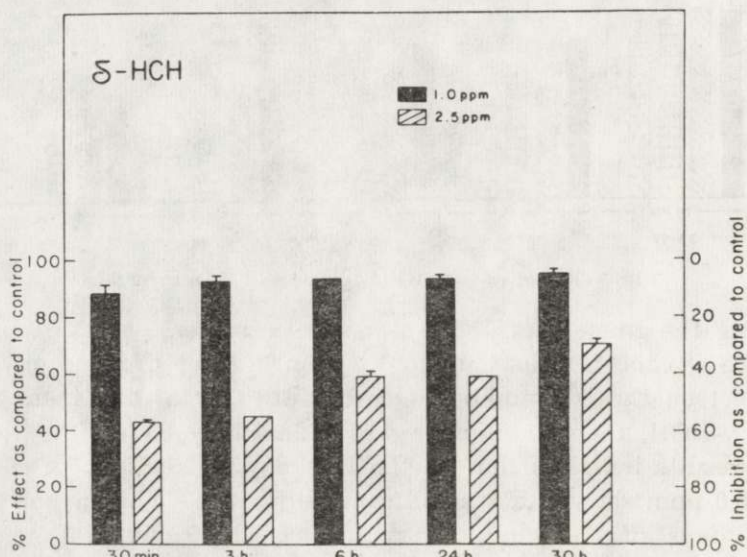


Fig. 5. Recovery of *T. pyriformis* after treatment with δ -HCH for 3 days

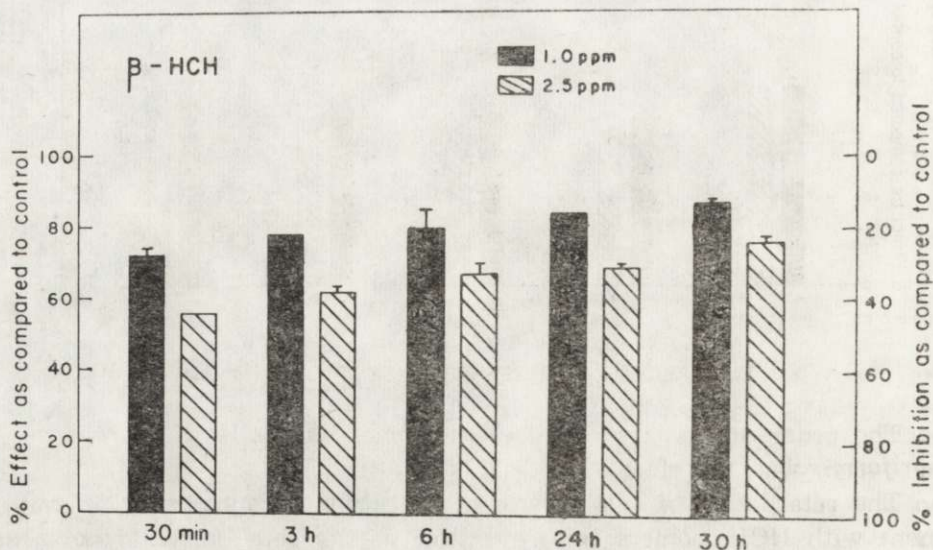


Fig. 6. Recovery of *T. pyriformis* after treatment with β -HCH for 3 days

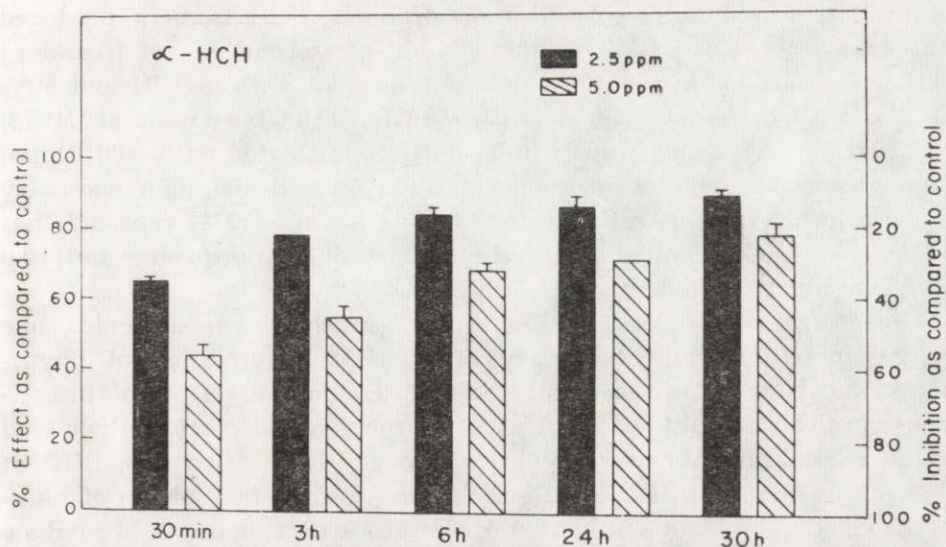


Fig. 7. Recovery of *T. pyriformis* after treatment with α -HCH for 3 days

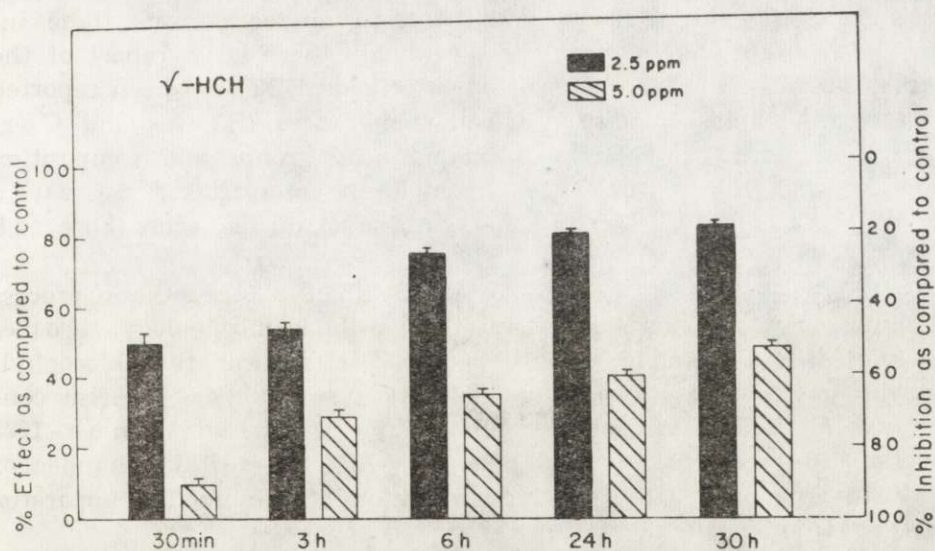


Fig. 8. Recovery of *T. pyriformis* after treatment with γ -HCH for 3 days

Discussion

Reports on the effects of HCH on protozoans indicate a lot of differences as regards the minimum effective concentration. The same is also evident from our results. Thus, while a concentration of 5.0 ppm of δ -HCH was lethal to *T. pyriformis* as all the cells died within 24 h

of treatment, the same concentration of other HCH isomers produced different degrees of inhibition viz. 93% on the second day of treatment with β -HCH, 88% on the third day of treatment with α -HCH and 89% on the fifth day of treatment with γ -HCH. Daubner et al. (1978) reported stimulation of growth in *T. pyriformis* treated with 0.0018 ppm lindane and higher concentrations between 50 and 100 ppm necessary to significantly inhibit cell division. Jeanne-Levain (1974) reported that 5.0 ppm γ -BHC inhibited the multiplication of *T. pyriformis* and 10.0 ppm completely inhibited the cell division.

Apart from the primary effects of organochlorine insecticides, one of the most important secondary effects is the formation of phytoplanktonic bloom. This is mainly due to the indirect effect of insecticides on algae because the more sensitive zooplankton species either feed on algae or compete for food with algae. The application of γ -BHC at the rate of 5 kg/ha in Philippine rice fields resulted in a bloom of blue-green algae due to the elimination of Ostracods (Raghun and MacRae 1967).

Quite contrary to higher organisms, where the site of action of the insecticides has been clearly identified, in microorganisms these insecticides affect one or more sites. Although there is no report of the target sites of HCH, another related insecticide DDT, has been reported to affect the lipid composition of cell membranes (Hicks and Corner 1973), ratio of polar phospholipid head groups and composition of fatty acid (Rosas et al. 1980), uptake of aminoacids (Czeczuga and Gierasimow 1977) and the synthesis of nucleic acids (French 1976).

HCH has, however, been reported to affect photosynthetic process of autotrophic microorganisms and their cellular morphology. Kopecek et al. (1976) found that 10 ppm γ -BHC reduced the chlorophyll content in *Ankistrodesmus braunii* and *Anacystis nidulans* whereas concentrations below 10 ppm stimulated photosynthesis. Jenne-Levain (1974) reported that 10 ppm of γ -BHC altered the number of cellular organelles and caused degeneration of the nuclear apparatus of *Dunaliella bioculata*.

Both microorganisms and higher organisms have been shown to recover from the toxic effects of HCH. It is clear from our results that *T. pyriformis* treated for 3 days with sublethal concentrations of different isomers of HCH, recovered from the toxic effect when cells were centrifuged and placed in a toxicant-free medium. *Acetabularia mediterranea* is also known to recover from the toxic effects of 50 and 100 ppm lindane (Borghi et al. 1973). Among higher organisms, male Swiss mice have been shown to recover two months after feeding of

500 ppm HCH was terminated (Babu et al. 1981). Although microorganisms degrade γ -HCH and its isomers, the extent of degradation is dependent on the spatial arrangement of chlorine atoms in the benzene ring. MacRae et al. (1967) found that the four isomers of HCH viz. α , β , γ and δ were decomposed in flooded rice soils at different rates (γ -HCH > α -HCH > β -HCH = δ -HCH). Despite a reasonable rate of anaerobic degradation, α -HCH is one of the most frequently observed environmental contaminants. This may be due to its release into the environment by the application of technical grade HCH which contains large amounts of α -HCH (Brooks 1974), and also to the conversion of γ -HCH to α -HCH by microorganisms (Benezet and Matsumura 1973). It is evident from our results that even low concentrations of stable isomers of HCH in the aquatic system are likely to produce adverse effects on the ciliate protozoan population such as *T. pyriformis*. However, these organisms are able to recover when the exposure to HCH is eliminated. Similar studies with other organisms at lower trophic levels of the food chains in an aquatic environment may prove to be of interest for the research on water pollution by insecticides.

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Mukundaella gulbargaensis, a New Actinocephalid Gregarine
from Odonate Insect, *Copera* sp.

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Synopsis: The morphology and life-history of a new species of cephaline gregarine, *Mukundaella gulbargaensis*, sp. n. from the midgut of an odonate, *Copera* sp., prevailing in Gulbarga (Karnataka, India) is described. The cephalonts are $175 \mu\text{m} \times 810 \mu\text{m}$ and the epimerite cup-like with 12-16 undulations on its wall. The cysts are spherical, $475 \mu\text{m}$ in diameter with an ectocyst. The spores are diamond-shaped, and in polar view hexagonal with spines.

Genus *Mukundaella* was created by Sarkar under the subfamily *Acanthosporinae* Léger emend. Grasse, (family *Actinocephalidae* Léger) to accommodate a gregarine species *M. undulatus* infecting odonate insects *Enallagma* sp., from Chinsurah and Naihati localities in West Bengal, India. An explorative study undertaken at our laboratory on gregarines infecting odonates of Gulbarga District revealed an actinocephalid gregarine species which for the reasons stated elsewhere is considered new and assigned to the genus *Mukundaella*.

Materials and Methods

Odonate hosts of the genus *Copera* sp. were collected around Gulbarga University Campus. Smear preparations of contents from various regions of the gut were made on a clean glass slide using insect ringer solution, fixed in Carnoy's fluid and stained with iron alum haematoxylin. Gametocysts collected from the mid and hind gut were subjected to moist chamber process to investigate their further development. Illustrations presented in this paper were drawn with the help of Camera lucida.

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Observations

Thirty per cent of the *Copera* sp. collected during November and December were found infected with a cephaline gregarine belonging to the genus *Mukundaella*.

Description of the Species

Cephalont: The early cephalont (Fig. 1 A, B and C) is a three segmented organism with fusiform deutomerite embodying an oval nucleus, dome-shaped protomerite and cup-like epimerite beset on a short neck. The exterior wall of the epimerite is marked with 12–16 undulations and the width of the cup is greater in the centre than at the apex. As the development progresses, the epimerite assumes a vase-shaped structure (Fig. 1 D) with upwardly directed finger-like folds. In the fully grown cephalont (Fig. 1 D, E) the neck of the epimerite shortens as it widens with the addition of numerous striations on its wall. The maximum length of the cephalont is 810 μm , the width being 175 μm .

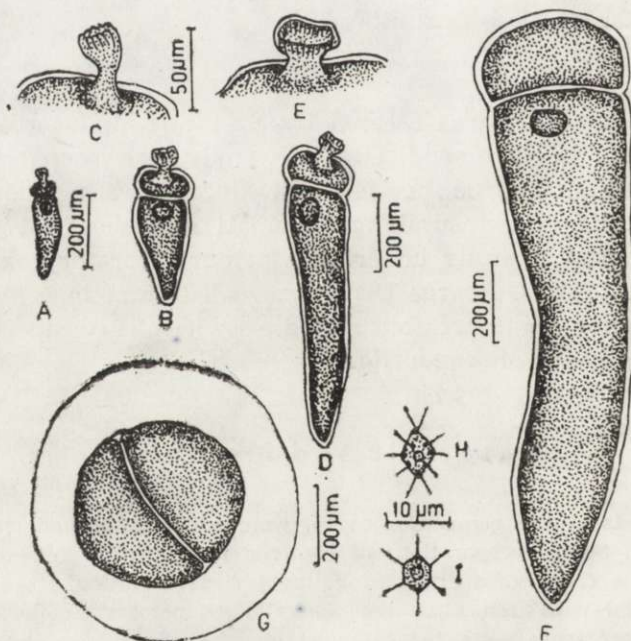


Fig. 1. A-I. *Mukundaella gulbargaensis* sp. n. A — Early cephalont, B — Later cephalont, C — Epimerite of the B enlarged, D — Matured cephalont, E — Enlarged epimerite of D, F — Adult sporont, G — Freshly-formed gametocyst, H — Diamond-shaped sporocyst, I — Polar view of the sporocyst

Sporont: Sporonts, on an average, measure 250 μm –1750 μm in length and 75 μm –310 μm in width. They have (Fig. 1 F) a rectangular protomerite (140 μm \times 186 μm) and a cylindroconical deutomerite (1070 μm \times 210 μm) enclosing an ovoidal nucleus (90 μm \times 65 μm) at the anterior region. The various ratios are:

PL:TL — 1 : 3.8–8.8

PW:DW — 1 : 0.57–1.3

Gametocyst: The gametocysts (Fig. 1 G) when freshly formed are spherical (710–775 μm in diameter) and milky white with thick (260–315 μm) ectocyst. They mature in about eight days and release by simple rupture the diamond-shaped sporocysts.

Sporocyst: The diamond-shaped (Fig. 1 H) sporocysts (7.5 μm \times 5.0 μm) are liberated from the matured gametocysts dehiscing by simple rupture. The sporocysts in plar view (Fig. 1 I) are hexagonal, with two spines at each pole and six meridional spines.

Systematic Position

The characteristics of the species such as solitary cylindroconical sporadins, cephalins with a cup-shaped epimerite having numerous striations on its wall and diamond-shaped sporocysts with polar and meridional spines assign it to the genus *Mukundaella*.

The only species, *M. undulatus* Sarkar described under this genus has small cephalins, sporonts and gametocysts. Moreover, the epimerite of the *M. undulatus*, in its early stage, has downwardly directed vertical folds, while in the presently described species they are upwardly directed. The cup of the epimerite is not widely open as in *M. undulatus*. The gametocyst of the present species bears a very thick (260 μm –915 μm) ectocyst unlike that observed in *M. undulatus*. In addition, sporocysts of the herein described species are small. Hence, this species is considered new to protozoological literature and the name *Mukundaella gulbargaensis* sp. n., is proposed. The specific name signifies the locality: Gulbarga, where odonate hosts, *Copera* sp. were found.

Parasite: *Mukundaella gulbargaensis*

Host: *Copera* sp.

Locality: Gulbarga University Campus, Gulbarga, Karnataka, India.

Site of infection: Midgut region.

Repository: The permanent slides of type specimens of this parasite are deposited in the Department of Studies and Research in Zoology, Gulbarga University, Gulbarga, Karnataka, India.

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Dorisiella graculae sp. n. from a Hill Myna *Gracula religiosa* Linn.
of Darjeeling Hills

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Synopsis. The communications includes the description of a new coccidium (*Eimeriidae*), *Dorisiella graculae* from the hill myna *Gracula religiosa* Linn. collected at Darjeeling (altitude 2100 m MSL). Disporocystid octozoic oocysts measure 16.66 μm (mean) in diameter and sporocysts measure 11.17 μm by 6.52 μm (mean).

There are many reports concerning the coccidian infection in birds and other vertebrates. Among the infected vertebrates birds occupy an important place as these parasites commonly occur in them. While investigating the protozoan parasites of the avifauna of Darjeeling area the authors encountered one coccidium of the genus *Dorisiella* in the faecal samples of *Gracula religiosa*.

Materials and Methods

Faecal samples of five hill myna *Gracula religiosa* collected at Darjeeling altitude 2100 m MSL were examined and one was found to contain the oocysts. The oocysts along with the faeces were kept in 2.5% potassium dichromate solution at room temperature. To facilitate the sporulation the hanging drop method (Mahrt 1968) was employed. Observations were made under oil-immersion lens of an Olympus binocular research microscope. Drawings and measurements were done by a Leitz camera lucida and an ocular micrometer respectively. Photomicrographs were taken by a Leica M4-2 camera with photomicrographic attachment. Twenty five specimens were taken for morphometric assesment.

Results

The oocysts are spherical in shape. The oocyst wall is double layered and about $1\ \mu\text{m}$ in thickness. The oocysts measure from 16.80 to $20.16\ \mu\text{m}$, being on an average $16.66\ \mu\text{m}$ in diameter. Neither a micropyle nor any refractile granule is detectable. The oocysts when voided along with the faeces were unsporulated and found to sporulate within 2 to 3 days at room temperature (11 to 16°C). The oocystic residuum is absent. The sporocysts are ellipsoidal without any Stieda body. The sporocysts measure 10.08 to $13.44\ \mu\text{m}$ (average $11.17\ \mu\text{m}$) by 5.04 to $6.72\ \mu\text{m}$ (average $6.52\ \mu\text{m}$). The sporocystic wall also reveals a bilayered structure. Each sporocyst contains eight elongated sporozoites. The sporozoites measure 6.72 to $10.08\ \mu\text{m}$ (average $8.56\ \mu\text{m}$) by 3.36 to $5.04\ \mu\text{m}$ (average $4.87\ \mu\text{m}$). A large amount of sporocyst residuum sometimes conceals the sporozoites. No significant difference in the morphology was found when the sporulated and unsporulated oocysts were compared (Fig. 1).



Fig. 1. Camera lucida drawing of a sporulated oocyst of *D. graculae* sp. n.

Type host: *Gracula religiosa* Linn.

Type locality: Darjeeling (alt. 2100 m MSL), West Bengal, India.

Localization: Gastro-intestinal tract.

Sporulation time: 2 to 3 days (at 11 to 16°C)

Diagnosis of *Dorisiella graculae* sp. n.

Disporocystid octozoic oocysts measure 16.80 to $20.16\ \mu\text{m}$, being on an average of $16.66\ \mu\text{m}$ in diameter. Micropyle and refractile granule absent. No oocystic residuum but a large amount of sporocystic residuum is present in each sporocyst. Ellipsoidal sporocysts with bilayered sporocystic wall and without any Stieda body. The sporocysts measure $11.17\ \mu\text{m}$ by $6.52\ \mu\text{m}$ on an average.

Discussion

The genus *Dorisiella* was instituted by Ray (1930) for a coccidium of marine polychaete. Pellérdy (1974) and Mandal (1976) recorded some species of *Dorisiella* from some birds but no mention of *G. religiosa* as a host of this coccidium was available. Levine (1980) has proposed a correction for the generic name as *Dorisa* (= *Dorisiella*) but in the present communication the former name has been adopted. From the available literatures it appears that this is the first report of *Dorisiella* from *G. religiosa* Linn. The protozoan under report shows some apparent similarities with *D. chakravartyi* Ray and Sarkar, 1967 in shape and in absence of oocystic residuum. But again it fails to differ sufficiently in morphometry not only from the latter but also from the other species of *Dorisiella* so far described from avian hosts, being smaller in size. Moreover, the parasite under report does not fit any other known species of the genus and is considered new. The species name has been proposed after the generic name of the host. The holotype and paratype will be deposited at the National Zoological Collection of Zoological Survey of India, Calcutta.

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A New Myxosporidan *Sphaeromyxa hareni* sp. n. (Myxozoa:
Myxidiidae) from an Indian Marine Teleost *Tachysurus*
platystomus (Day)

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Synopsis. This paper describes a new myxosporidan *Sphaeromyxa hareni* sp. n. (Myxozoa: Myxidiidae) from the gallbladder of an Indian marine teleost *Tachysurus platystomus* (Day) caught from the coastal water of the Bay of Bengal (Digha, West Bengal). It was compared with *Sphaeromyxa* spp. reported from India and other related spp. described from the other parts of the world.

The knowledge of the myxosporidan parasites of the estuarine and the marine fishes in India is scanty. Only a few reports have so far been made by Ganapati (1941), Narasimhamurti (1970), Narasimhamurti and Kalavati (1979 a, b) and Choudhury and Nandi (1973). Recently four more myxosporidan spp. have been recorded from the marine fishes by Sarkar and Mazumder (1982, 1983). This communication describes a new species of *Sphaeromyxa* Thelohan, 1895 from the gallbladder of *Tachysurus platystomus* (Day) caught in the West Bengal coastal water (Digha) of the Bay of Bengal during the winter of 1982-1983.

Material and Methods

All autopsies were made from the frozen fish collected from the West Bengal coast (Digha) of the Bay of Bengal. The parasites were studied from the wet smears of the gallbladder, treated with Lugol's iodine solution and also from the dry smears stained with Giemsa after fixation in Aceton-free absolute Methanol under the oil immersion lens of an Olympus research microscope. The extrusion of polar filaments was achieved with 2% KOH solution. The figures were drawn with the aid of a camera lucida. The measurements are given in micrometer (μm).

Observations

Cyst and vegetative forms: Not found.

Spore: The spores were found in bile of the gallbladder of the host. These were, in valvular view, nearly fusiform (Fig. 1 1), sometimes slightly curved (Fig. 1 2) with round ends and almost 'S'-shaped in sutural view (Fig. 1 3). Shell valves were smooth and thin-walled

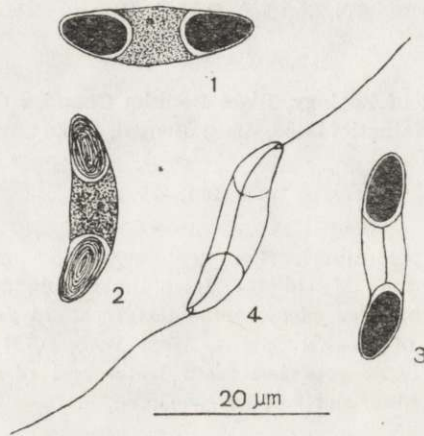


Fig. 1, 1—4. The spores of *Sphaeromyxa harenii* sp. n. 1 — Valvular view showing its fusiform shape, Giemsa stained; 2 — Valvular view appearing slightly curved, Lugol's iodine treatment; 3 — Sutural view appearing almost 'S'-shaped, Giemsa stained; 4 — The spore with extruded polar filaments, Giemsa stained after 2% KOH treatment

with 'S'-shaped suture. Polar capsules two, one on each end of the spore, were ovoid to ellipsoidal with coiled polar filament, the coiling being parallel to the long axis of the spore and equal. The extruded polar filament was filamentous and measured 34.5 μm in length, remaining perpendicular to the long axis of the spore (Fig. 1 4). The extracapsular space of the spore was filled with finely granular uninucleate sporoplasm. The capsular and the valvular nuclei were not distinct.

Dimensions (the mean value of 42 fresh spores and 84 polar capsules with range in parentheses is given below):

Length of the spore: 27.55 (23.35–28.95)

Breadth of the spore: 7.80 (5.60–8.87)

Thickness of the spore: 5.14 (4.67–5.60)

Length of the polar capsule: 9.34 (8.87–10.27)

Width of the polar capsule: 4.30 (3.00–5.10)

Length of the polar filament: 34.50 (32.69–37.36)

Infection locus: Gallbladder (bile)
Incidence: 1 infected out of 14 examined
Pathogenicity: Not apparent
Host: *Tachysurus platystomus* (Day)
Locality: West Bengal (Digha) coast of Bay of Bengal, India

Discussion

In India so far only three *Sphaeromyxa* Thelohan, 1895 spp. have been recorded of which *S. theraponi* Tripathi, 1951 and *S. pulti* Tripathi, 1951 have been described from the gallbladders of an estuarine fish *Therapon jarbua* Forsk. and a fresh water fish *Odontamblyopus rubicundus* (Ham.) respectively and *S. dighae* Sarkar and Mazunder, 1983 from the gallbladder of *Hilsa ilisha* (Ham.) caught from its marine habitat. The present species resembles *S. theraponi* Tripathi and also *S. reinhardti* Jameson, 1929 in having the spore smooth and slightly curved in valvular view and 'S'-shaped in sutural view. In the former character it also shows similarity with *S. gibbonsia* Noble, 1939 reported from the gallbladder of *Gibbonsia elegans elegans* (Cooper) and *G. metzi* Hubbs. However, the present myxosporidan, from *S. theraponi*, differs by the round ends (truncate ends in *S. theraponi*), oval to ellipsoidal polar capsule (pyriform in *S. theraponi*) and the dimensions of $27.55 \mu\text{m} \times 7.80 \mu\text{m}$ and $9.34 \mu\text{m} \times 4.30 \mu\text{m}$ of the spore and polar capsule respectively ($19.8 \mu\text{m} \times 5.4 \mu\text{m}$ and $7.2 \mu\text{m} \times 2.7 \mu\text{m}$ respectively in *S. theraponi*). It also differs from *S. reinhardti* by its round spore ends and its dimensions difference (truncate spore ends and $21.25 \mu\text{m}$ – $23.30 \mu\text{m} \times 3.75 \mu\text{m}$ – $5.00 \mu\text{m}$ spore dimensions in *S. reinhardti*) and from *S. gibbonsia* by 'S'-shaped suture (slightly angled to the main axis of the spore in *S. gibbonsia*). Moreover, the extruded polar filament is very long i.e., $34.50 \mu\text{m}$ ($20.0 \mu\text{m}$ long in *S. gibbonsia*). Taking all these features, including the different host of a different locality, into account, I consider the myxosporidan a new species and the name *Sphaeromyxa hareni* sp. n. is given to it after the late Prof. Dr. Harendra Nath Ray of the University of Calcutta, India.

Material: Syntypes specimens on slide No. MSH-12, deposited in the Dept. of Zoology, R.B.C. College and soon to be transferred to the National collection of the Zoological Survey of India, Calcutta.

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J. J. Nieto, P. Calvo, J. Martin and A. Torres: Divisional and Regenerative Morphogenesis in the Hypotrichous Ciliate, *Histiculus* sp. — E. E. Orlovskaja, L. N. Karvanen and L. N. Seravin: Susceptibility of Food Chemoreceptors in Carnivorous Protozoa — L. Szablewski: The Adaptation of *Tetrahymena pyriformis* GL to the Continuous Presence of Colistin in the Medium as Observed in Selected Physiological Functions — I. Wita: *Parastasia caudata* sp. n. (Euglenida) — a Parasite of Copepods — D. Chardez: Etude sur les Thécamoebiens du mésopsammon — T. K. Kundu and D. P. Haldar: *Lepismatophila cruzi* sp. n. New Cephaline Gregarine from Silver-fish *Acrotelsa collaris* (Fabricius) of West Bengal, India — N. K. Sarkar: *Pyxina reneae* sp. n. and *Gregarina chaetocnema* sp. n., New Cephaline Gregarines from the Coleopteran Insects of West Bengal, India.

Warunki prenumeraty

Cena prenumeraty krajowej: rocznie 400 zł, półrocznie 200 zł

Prenumeratę na kraj przyjmuje się:

- do 10 listopada na I półrocze roku następnego i na cały rok następny,
- do 1 czerwca na II półrocze roku bieżącego.

Instytucje i zakłady pracy zamawiają prenumeratę w miejscowych Oddziałach RSW „Prasa-Książka-Ruch”, w miejscowościach zaś, w których nie ma Oddziałów RSW — w urzędach pocztowych i u doręczycieli.

Czytelnicy indywidualni opłacają prenumeratę wyłącznie w urzędach pocztowych i u doręczycieli.

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Prenumerata ze zleceniem wysyłki za granicę pocztą zwykłą jest droższa od prenumeraty krajowej o 50% dla zleceniodawców indywidualnych i o 100% dla zlecających instytucji i zakładów pracy.

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