Population dynamics and food preferences of the testate amoeba Nebela tincta major-bohemica-collaris complex (Protozoa) in a Sphagnum peatland

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Acta Protozool. (2003) 42: 99 - 104

ACTA
PROTOZOOLOGICA

Population Dynamics and Food Preferences of the Testate Amoeba *Nebela tincta major-bohemica-collaris* Complex (Protozoa) in a *Sphagnum* Peatland

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Summary. Population dynamics and food preferences of the testate amoeba species complex *Nebela tincta major-bohemica-collaris ("Nebela collaris sensu lato")* were described from a *Sphagnum* peatland over one growing season. The average abundance of *Nebela collaris sensu lato* was 29582 ind. l^{-1} active, and 2263 ind. l^{-1} encysted forms. On average, 17.4% of *Nebela collaris sensu lato* specimens were observed associated with prey, 71% of which could not be identified because of their poor preservation state. Among the identified prey, those most frequently ingested were micro-algae (45% of the total predator-prey associations, especially diatoms: 33%), and spores and mycelia of fungi (36%). Large ciliates, rotifers and small testate amoebae were also ingested, but mainly in summer. The seasonal variations in the proportions of prey categories in the ecosystem and the percentage of identifiable prey lead us to hypothesise that (1) *Nebela collaris sensu lato* ingest mainly immobile, senescent or dead organisms, and (2) that the more mobile micro-organisms such as ciliates and micro-Metazoa become more accessible, in relatively dry conditions, when the water film is thin.

Key words: microbial food web, Nebelidae, population dynamics, Rhizopoda, soil micro-organisms, Wetland.

INTRODUCTION

In aquatic environments, knowledge on the trophic interactions within microbial food webs has benefited from the use of artificial or fluorescent-labelled prey. This approach is used to study the bacterivorous activity of micro-organisms in marine and lacustrine pelagic ecosystems (Borsheim 1984, Pace and Bailiff 1987, Sanders *et al.* 1989, Simek *et al.* 1990, Carrias *et al.* 1996). More recently the same principle has been applied using labelled eukaryotes (Premke and Arndt 2000). By contrast in environments where an interface between the solid and liquid exists (including the benthic zone of fresh- and salt-water bodies in aquatic ecosystems, the upper horizons of soils in terrestrial ecosystems, bryophytes, and lichens) these techniques are difficult to use mainly because many predators feed on

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fixed and/or mobile prey and that the potential range of these prey is impossible to reconstruct artificially.

As a result, although the functional importance of the microbial loop has been established in several interface environments such as soils (Clarholm 1994, Coleman 1994, Bonkowski and Brandt 2002), and peatlands (Gilbert *et al*. 1998a, b), little data is available on the feeding habits of protozoa in these environments. Information on the feeding habit of testate amoebae especially is very scarce (Coûteaux and Pussard 1983, Coûteaux 1984, Chardez 1985, Yeates and Foissner 1995, Gilbert *et al*. 2000), although this group represents an important part of the protozoan biomass in these interfaces (Gilbert *et al*. 1998a, b; Mitchell *et al.* 2003). This study therefore aimed at establishing the feeding habits of one of the most abundant testate amoeba group in *Sphagnum* peatland, the *Nebela tincta major-bohemica-collaris* complex (hereafter "*Nebela collaris sensu lato*") (Deflandre 1936, Heal 1964, Warner 1987), in natural conditions, in relation to the seasonal dynamics of their potential prey.

MATERIALS AND METHODS

The study was carried out in the Pradeaux peatland (Puy de Dôme, France, 3°55 E, 45°32 N, altitude 1350 m. a. s. l.), a drained *Carex rostrata/Sphagnum fallax* fen covering a surface of 10 ha (Francez 1992).

Three PVC tubes 25 cm in diameter and 30 cm in length were permanently inserted vertically in the peat in macroscopically identical locations in order to get triplicate measurements. Once a month, from April to November 1995, 50 ml water samples were collected in each tube by pressing the moss surface with a sieve (mesh size 1.5 mm) and sucking the water up with a syringe. During each site visit pH and conductivity were measured *in situ* with a Chekmate M90 multiparametric probe (pH: ± 0.01 unit; conductivity: ± 0.01 μScm^{-2}). Water levels were measured with 30 cm piezometers inserted in the peat. Because of the high water holding capacity of *Sphagnum* and the relatively high water table levels it was possible to sample water every month except in August. This method, which was already used by Grolière (1977) for ciliates and by Francez (1988) for rotifera, allows the recovery of most microorganisms. However, this method probably underestimates the densities of at least some microbial groups because they may be attached to the *Sphagnum* mosses, or because they may be living inside the large, empty cells of *Sphagnum* called hyalocysts. Conversely, an advantage of this method is that as the *Sphagnum* carpet is very little affected by the sampling, repeated sampling at the exact same place is possible. This is important because fine-scale heterogeneity in testate amoeba distributions may occur even in a macroscopically homogeneous *Sphagnum* moss carpet (Mitchell *et al.* 2000). The water samples were fixed with glutaraldehyde (2% final concentration) and stored at 4°C in the dark. Cyanobacteria, fungi, micro-algae, protozoa, rotifers, and nematodes were identified using a sedimentation (plankton) chamber and an inverted microscope (Utermöhl 1958). Biovolumes of morphotypes within each community were estimated by assuming geometric shapes and converted to biomass using conversion factor according to Gilbert *et al.* (1998a).

For each sample, we observed a minimum of 20 specimens of *Nebela collaris sensu lato* (total for this study: 684 active or encysted specimens). Among the active specimens, we distinguished those with an attached prey. We defined as "prey" any organic matter particle, even if precise identification was not possible because of poor preservation (e.g. decomposed organic matter or partly decomposed micro-organisms). The glutaraldehyde solution fixes efficiently all micro-organisms and we were able to verify that this method does not separate the prey from the predator, even after homogenizing the sample through the vortex.

RESULTS AND DISCUSSION

Physico-chemical environment

The water table level fluctuates during the year according to the relative importance of precipitations and evapotranspiration and the drainage of the site. These fluctuations are especially important in the case of drained peatlands such as the site we studied. At our site, the water table level remained high in spring (11 cm below the surface of the moss carpet). It then became gradually lower, until the beginning of summer when it reached 19.3 cm. During August, the water table fell below 30 cm, and it was impossible to sample surface water. The autumn rainfall did not fully compensate for this water deficit and the water level had only risen to 14 cm by November (Table 1). The water temperature at the surface of the peatland fluctuated between 0.7 and 24.4°C during the course of the study. The mean values for water pH and conductivity were 4.7 and 43.1 μScm^{-2} respectively. The concentration of dissolved oxygen fluctuated between 2.0 mg l^{-1} in June and 9.8 mg l^{-1} in November (Table 1). The dissolved oxygen concentration was logically negatively correlated to water temperature ($n = 12$, $r = -0.99$, $P < 0.001$). The conductivity was also negatively correlated to water temperature ($n = 18$, $r = -0.457$, $P = 0.05$), probably because the activity of *Sphagnum*, and therefore the nutrient uptake, increases with the temperature.

Population dynamics

Nebela collaris sensu lato was regularly present in *Sphagnum* mosses (averages: 29582 ± 9650 ind l^{-1}, for the active forms, and 2263 ± 1620 ind l^{-1}, for the encysted forms) (Fig. 1). The highest abundance of active

Table 1. Temporal variations in physico-chemical variables (mean ± SE, ND not determined).

Months	Water table depth (cm)	Temperature (°C)	pH	Dissolved oxygen (mg l^{-1})	Conductivity (µS cm^{-2})
April	11.0 ± 2.6	5.4 ± 0.6	4.5 ± 0.2	ND	56.4 ± 4.7
May	14.0 ± 2.0	13.2 ± 0.5	4.7 ± 0.1	5.8 ± 0.6	33.7 ± 8.6
June	16.3 ± 4.7	20.7 ± 0.1	4.5 ± 0.0	2.1 ± 0.2	26.7 ± 3.9
July	19.3 ± 2.1	19.8 ± 0.6	4.4 ± 0.2	ND	48.5 ± 7.0
August	> 30.0 ± 0.0	ND	ND	ND	ND
September	22.3 ± 2.5	ND	ND	ND	ND
October	22.3 ± 3.5	11.9 ± 0.4	4.3 ± 0.1	6.3 ± 0.2	47.2 ± 11.5
November	14.3 ± 2.1	6.7 ± 0.6	5.4 ± 0.2	9.5 ± 0.1	43.6 ± 10.7

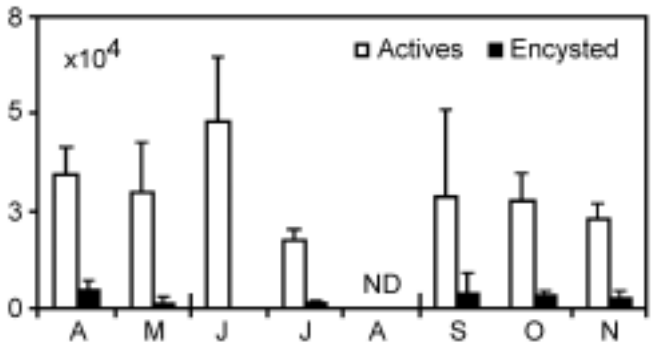


Fig. 1. Temporal variations of the abundance (ind. l^{-1}) of active and encysted *Nebela tincta major-bohemica-collaris* complex (ND - not determined).

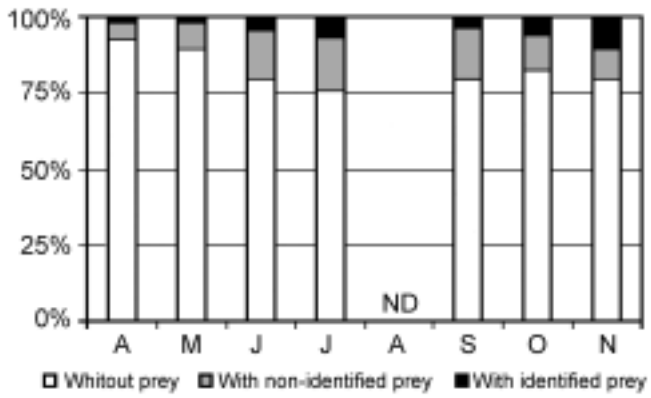


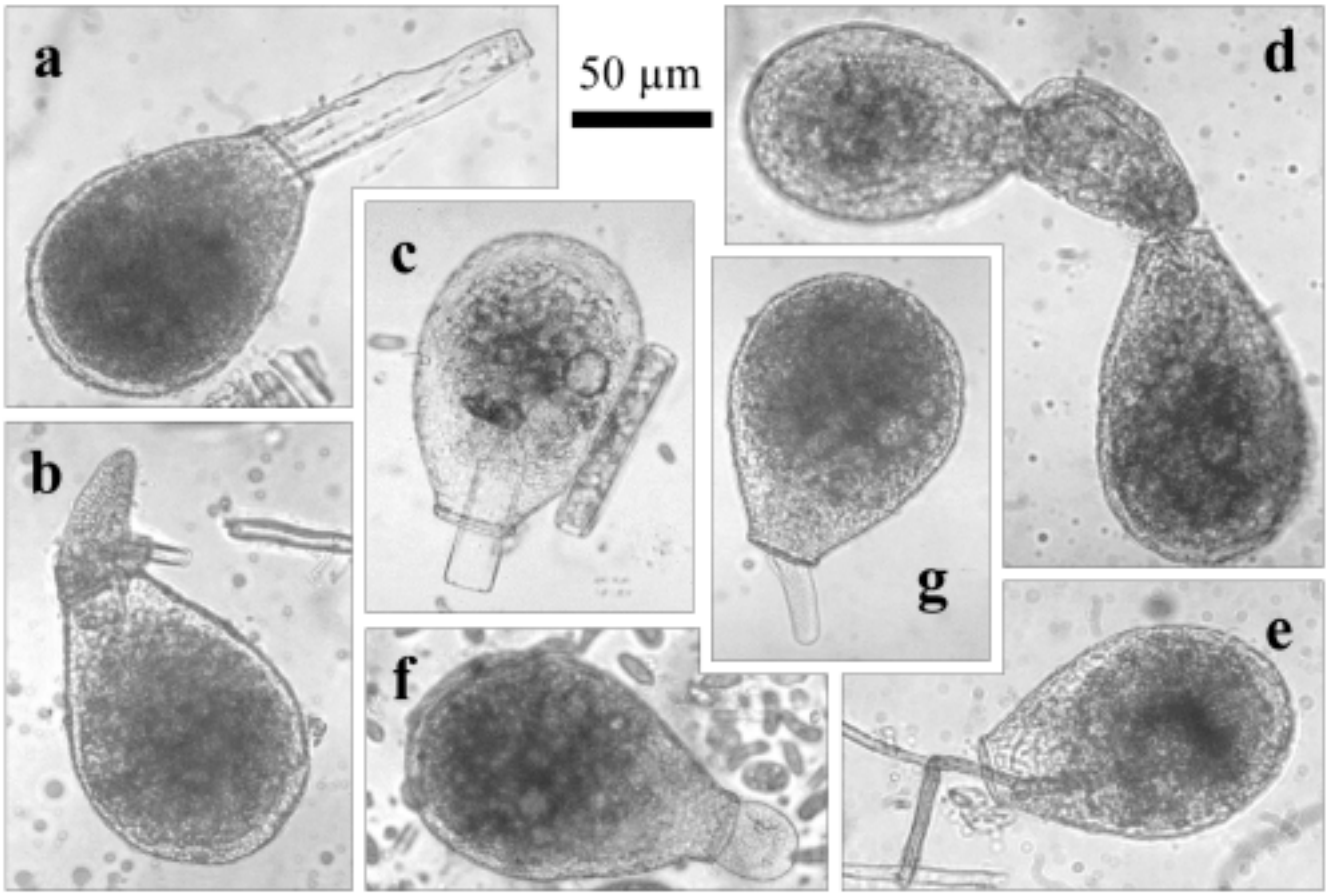
Fig. 2. Temporal variations of the relative proportion (%) of *Nebela tincta major-bohemica-collaris* complex individuals associated with a prey (ND - not determined).

forms was observed at the end of June (up to 37947 ± 9843 ind. l^{-1}) and the lowest in July (13172 ± 3137 ind. l^{-1}) (Fig. 1). This pattern seems to be characteristic for drained peatland, with winters being too cold and summers being too dry for microbial development Similar temporal patterns were described before in *Sphagnum* for microalgae (Schoenberg and Oliver 1988) and ciliates (Grolière 1977). In the same way, the encysted forms were most abundant at the beginning of spring (4634 ± 2347 ind. l^{-1} in April) when the water temperatures were still low, and after the dry period (mean for September, October and November: 2934 ± 2845 ind. l^{-1}) (Fig. 1). Cyst abundance was negatively correlated with temperature (r = -0.86, P = 0.03) and positively correlated with conductivity (r = 0.86, P = 0.03).

Seasonal feeding activity and general feeding habits

The frequencies of *Nebela collaris sensu lato* specimens observed associated with a prey (M=17.4 ± 6.0%) were low in spring (8.0% in April), rising until July (24.1%), and then decreasing in autumn (Fig. 2). However, these variations were not significant, and the number of specimens observed in association with a prey was positively correlated with the number of active individuals (n = 21, r = 0.53, P = 0.01). Thus our data suggest that the fraction of *Nebela collaris sensu lato* actively feeding is relatively constant throughout the year.

Among the identified prey, those most frequently ingested were micro-algae (45% of the total identified predator-prey associations, especially diatoms 33%), and spores and mycelia of fungi (36%). Predation on large ciliates (12%), rotifers (3%) and small testate amoebae (4%, among which *Trinema* spp. and *Euglypha* spp.) appeared to be more marginal (Figs 3, 4). However, on average, 71 ± 27% of the prey could not be identified because of their poor preservation state. These unidentified prey were most likely *Sphagnum* leaf fragments, other plant cells, or prey without rigid skeleton, such as green algae, ciliates, and most bdelloïd



Figs 3 a-g. *Nebela tincta major-bohemica-collaris* complex associated with; **a** - plant cell; **b** - ciliate; **c** - diatom; **d** - rotifer; **e** -fungi mycelium; **f** - testate amoeba; **g** - individual without prey.

rotifers. Fungi spores and mycelia as well as diatoms and testate amoebae were easily identifiable, even dead, because of their internal or external skeleton, or rigid cell walls. Thus the ingestion frequencies obtained for fungi, diatoms, and testate amoebae are probably quite accurate, whereas those obtained for rotifers and ciliates are most likely underestimated. However, glutaraldehyde being a very efficient fixative, we do not believe that the poor preservation stage of the majority of the prey is due to their degradation between the time of sampling and the time of analysis. We rather infer that the unrecognisable prey might have been dead before the amoeba started to feed on them. If true this would suggest that *Nebela collaris sensu lato* is mainly detritivorous. Nevertheless, *in vivo* observations of carnivorous Nebelidae populations (*Nebela* spp., *Hyalosphenia papilio*) showed that they are also capable of attacking living or senescent ciliates (Gilbert *et al*. 2000).

Seasonal feeding habits

The dominant kind of ingested prey varied with time. Because of the relatively low numbers of predators-prey associations observed in each sample, we grouped the sampling periods into three categories: spring (April and May), summer (June and July), and autumn (September, October and November). In spring and autumn, identified prey were dominated by fungi and autotrophic microorganisms, while during the summer period the main prey were autotrophic microorganisms, protozoa, and micro-metazoa (Fig. 5a). The biomass of different categories of selected prey was estimated in order to compare the nature and variations of their feeding habits to the seasonal dynamics of their prey. Figure 5b shows the biomass variations of the same microbial groups: fungi (mycelia and spores), micro-algae, protozoa and micro-metazoa. A significant relationship was found between the biomass of fungi in the environment and the

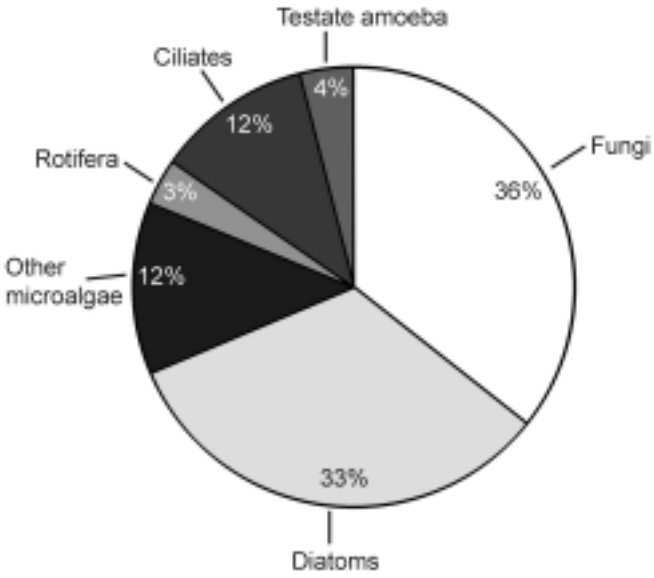
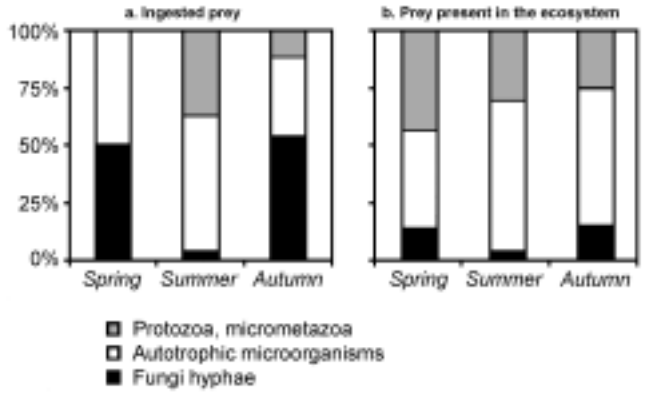


Fig. 4. Relative proportions (%) for the different identified prey categories (ND - not determined).



Figs 5 a, b. Temporal relative proportion of variations (**a**) of identified ingested prey and (**b**) of the relative proportion of the biomass of the same categories of prey in *Sphagnum* (ND - not determined).

frequency of their ingestion by *Nebela collaris sensu lato* ($r = 0.99$, $P = 0.04$), but no such relationship was found for the other groups. Because the size of our data set is limited, it is difficult to draw strong conclusions regarding the ability of *Nebela collaris sensu lato* to select their prey. Direct observations of microcosms with a range of prey types could clarify this question. Furthermore we described the predator-prey relationships with a low taxonomic resolution for the prey, but *Nebela collaris sensu lato* may select their prey at the species level. Thus, it remains possible that *Nebela collaris sensu lato* selects specific species of ciliates and rotifers that are more abundant or more accessible in summer when the lower water content of mosses makes these prey types more accessible. Indeed, the water content of *Sphagnum* mosses may play an important role in determining the ability of predators to catch their prey. *In vivo* observations showed that micro-organisms were located primarily between the leaves and on the stems of *Sphagnum*. In dry periods the water film in which micro-organisms live becomes thinner and their concentration therefore increases. In such conditions slow moving organisms like testate amoebae may be able to catch mobile predatory micro-organisms, such as ciliates or rotifers, otherwise too fast for them, whereas immobile, senescent or dead organisms, are accessible all year round.

The methodology we used neither allowed us to quantify the consumption of bacteria, because they are invisible, nor of naked amoebae, because they are difficult to observe, even alive. Furthermore, although glutaraldehyde is a fixing agent widely used in predation experiments of micro-organisms (Borsheim 1984, Pace and Bailiff 1987), the interpretations of results is difficult because many prey were not identifiable when they were fixed. Nevertheless, our study establishes that (1) *Nebela collaris sensu lato* feed on a wide range of living, senescent, or dead micro-organisms (fungi, microalgae, ciliates, other testate amoebae, rotifers) and organic remains, and (2) the proportion of predators among the identified prey is higher during the summer. Some prey seems to be selected, but further studies are needed to determine the importance of this phenomenon.

Acknowledgements. This study was conducted as part of a European Community environmental programme (3rd Framework, Contract No. EV5V-CT92-0099) and was financed by the CEREMCA Association. We also thank two anonymous reviewers for valuable comments on an earlier version of this paper.

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Received on 18th November 2002; revised version on 11th February, 2003; accepted on 25th February 2003