

SHORT COMMUNICATION

A preliminary study of freshwater protozoa in tank bromeliads

JEAN-FRANÇOIS CARRIAS*¹, MARIE-EVE CUSSAC* and BRUNO CORBARA†

**Laboratoire de Biologie des Protistes, UMR CNRS 6023, Université Blaise Pascal, Clermont-Ferrand II, 63177 Aubière Cedex, France*

†*LAPSCO, UMR CNRS 6024, Université Blaise Pascal, Clermont-Ferrand II, 34 avenue Carnot, F - 63037 Clermont-Ferrand Cedex, France*

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Bromeliads are common as epiphytes in warm neotropical forests (Benzing 1990). Species native to relatively wet forests impound water in a central cup and/or in separate leaf axils. These tanks receive enough leaf litter and rain-water to support aquatic life (Maguire 1971). In these complex microcosms many groups of freshwater organisms ranging from algae, fungi, bacteria and protozoa through insects to frogs are represented and constitute considerable animal populations (Frank 1983, Laessle 1961, Maguire 1971, Picado 1913, Richardson 1999). Nutrients originating from the decomposition of litter and animal waste are absorbed by specialized trichomes on the bases of the leaves which form the tanks (Benzing 1980, 1990).

Most investigations on aquatic organisms inhabiting these plant-held waters have focused on particular groups and have usually been restricted to aquatic insects, especially mosquito larvae (Fish 1983, Frank 1983). Inventories of the total aquatic fauna have been made by Picado (1913) and Laessle (1961) but no accurate quantitative data on the microbiota were obtained by these authors. The value of studying phytotelmata biota for answering questions of theoretical importance in ecology was emphasized by Maguire (1971) and a

¹ Corresponding author. Email: J-Francois.CARRIAS@lbp.univ-bpclermont.fr

few recent studies have focused on these topics. Cotgreave *et al.* (1993) examined the relationship between the abundances and body size of morphospecies from epiphytic and terrestrial bromeliad tanks in Brazil. More recently, the fauna of bromeliad phytotelmata was used to measure diversity in three forest types in Puerto Rico (Richardson 1999). However, neither of these studies took into account the smallest aquatic organisms such as metazoan zooplankton and protozoa. A preliminary account of the food webs in phytotelmata was presented by Kitching (1987) but protozoa were not considered. It is, therefore, necessary to evaluate the importance of protozoa in this kind of microecosystem. Their role is indeed essential in the functioning of many aquatic ecosystems (Caron 1991, Sanders & Wickham 1993, Sherr & Sherr 1988). Marine and freshwater protozoans (e.g. flagellates and ciliates) are considered important predators of bacteria and small phytoplankton, as prey for zooplankton, and as mediators for recycling nutrients essential for phytoplankton and microbial growth (Sanders & Wickham 1993, Sherr & Sherr 1988). Thus, protozoa may be key organisms in bromeliad microcosms when large amounts of organic matter are present.

The aim of this study was therefore to evaluate the numerical importance of flagellates and ciliates in tank bromeliads. The densities of the major metazoans were also estimated. To our knowledge, no quantitative data on protozoa in plant-held waters are available, although some early studies (Laessle 1961, Maguire 1971) indicated their presence in phytotelmata.

Water samples were collected on 7 and 8 July 1998 from the tanks of epiphytic bromeliads growing between 1 and 5 m off the ground, in small valleys (elevation: 35–40 m) of the lowland tropical rain forest along the road leading to the Petit Saut Dam (approx. 5°05'N, 53°00'W), Sinnamary, French Guiana. Ten specimens belonging to three species (*Aechmea mertensii* (Meyer) Schultes, *Vriesea splendens* (Brongniart) Lemaire and *Vriesea pleiosticha* (Grisebach) Gouda) were sampled. All of these specimens were sterile plants, 20–60 cm tall, with leaves forming a crateriform rosette (*A. mertensii*), a funnelliform rosette (*V. splendens*) or an open rosette (*V. pleiosticha*) (Mori *et al.* 1997). A 50-ml sample of water from each plant was collected at the water-detritus interface in each central tank. Therefore, the densities of aquatic organisms estimated during this study were not representative data of the tank contents as a whole. They must be considered as estimates of a particular habitat unit within the tanks. Sampling was done using a 60-ml syringe, equipped with a tip of 2 mm in diameter. Samples were transferred to 60-ml glass bottles, fixed with 1% (final concentration) glutaraldehyde, and then stored at 4 °C in the dark before analysis. The upper inner diameter and the height of water content in the sampled tank were measured. The volume of water was therefore roughly estimated assuming a cone shape for the sampled chamber. In the laboratory, samples were gently mixed by inversion and processed for microorganism counts. Water used to count microorganisms was taken from the surface of

each sample after the visible organisms had sedimented out. This simple process prevented size-fractionation and the consequent loss of organisms in very concentrated samples.

Three 2-ml subsamples were analysed for flagellates after primulin (Sigma-Aldrich chimie, France) staining (Caron 1983) and filtration onto black-stained 0.8- μm -pore-size Nuclepore filters (Whatman, England) using epifluorescence microscopy (Olympus, Japan). Two sets of filters were used: UG-1, DM 400, L 435 (UV light) for heterotrophic flagellates and BP 490, DM 500, O 515 (blue light) for pigmented flagellates and non-flagellated algae. A total of 100–200 flagellated cells from each slide were enumerated ($\times 1250$ magnification) by 3–5 transects ($\text{SD} < 10\%$, Carrias *et al.* 1996). Pigmented cells were absent in all samples. The abundances of ciliates and rotifers were determined from settled samples (Utermöhl 1958) using an inverted microscope (Wild, Switzerland). Triplicate 5-ml subsamples were concentrated by settling and examined at $\times 400$ magnification by scanning the whole chamber. The remaining water in each sample was transferred to Dolffus chambers (Leune, France) and large zooplankton and macroinvertebrates were counted under a binocular microscope (Leica, Germany). Dipteran larvae, nematodes, oligochaetes and copepods were the most numerous organisms. Some rare forms (unknown larvae, nymphs, mites) were not included in the counts. Flagellate and ciliate abundances ranged from 5×10^2 to $5 \times 10^4 \text{ ml}^{-1}$ (mean: $1.5 \times 10^4 \text{ flagellates ml}^{-1}$) and from 0 to 200 ml^{-1} (mean: $50 \text{ ciliates ml}^{-1}$), respectively (Figure 1a, 1b). Colourless euglenids (5–35 μm in length) and hymenostomes (15–120 μm) largely dominated the protozoan communities, whereas pigmented protists were always absent. Rotifers were the most numerous aquatic metazoa (range: 0 to 55 individuals ml^{-1} , mean: 17 individuals ml^{-1}) and were present in all but one plant (Figure 1c). A small unidentified taxon (length: 60 μm , width: 40 μm) appeared to be most common and occurred in nine plants. Copepods (adults and nauplii) were only present in three plants and their density was highest (three individuals ml^{-1}) in the smallest bromeliad (No. 7: *Vriesea splendens*) (Figure 1c). The densities of macroinvertebrates ranged from 0.2 to 1.6 individuals ml^{-1} (mean: 0.8 individual ml^{-1}). Diptera larvae were present in all but one plant (Figure 1d). Nematodes and oligochaetes were only recorded in some of our samples. On average, culicidae larvae outnumbered the other macroinvertebrates (32%) followed by nematodes (29%), oligochaetes (23%) and chironomid larvae (16%).

Protozoan abundance was positively correlated with the densities of rotifers ($n = 10$, $r^2 = 0.59$, $P = 0.01$) and macroinvertebrates ($n = 10$, $r^2 = 0.59$, $P = 0.005$). In addition, the number of taxa occurring in each chamber tend to increase with the volume of water impounded ($n = 10$, $r^2 = 0.38$, $P = 0.05$). For example, the smallest plant sampled (No. 7: *Vriesea splendens*) which had the smallest volume of water only contained flagellates, copepods and chironomidae. In contrast, the highest number of taxonomic groups was recorded in the

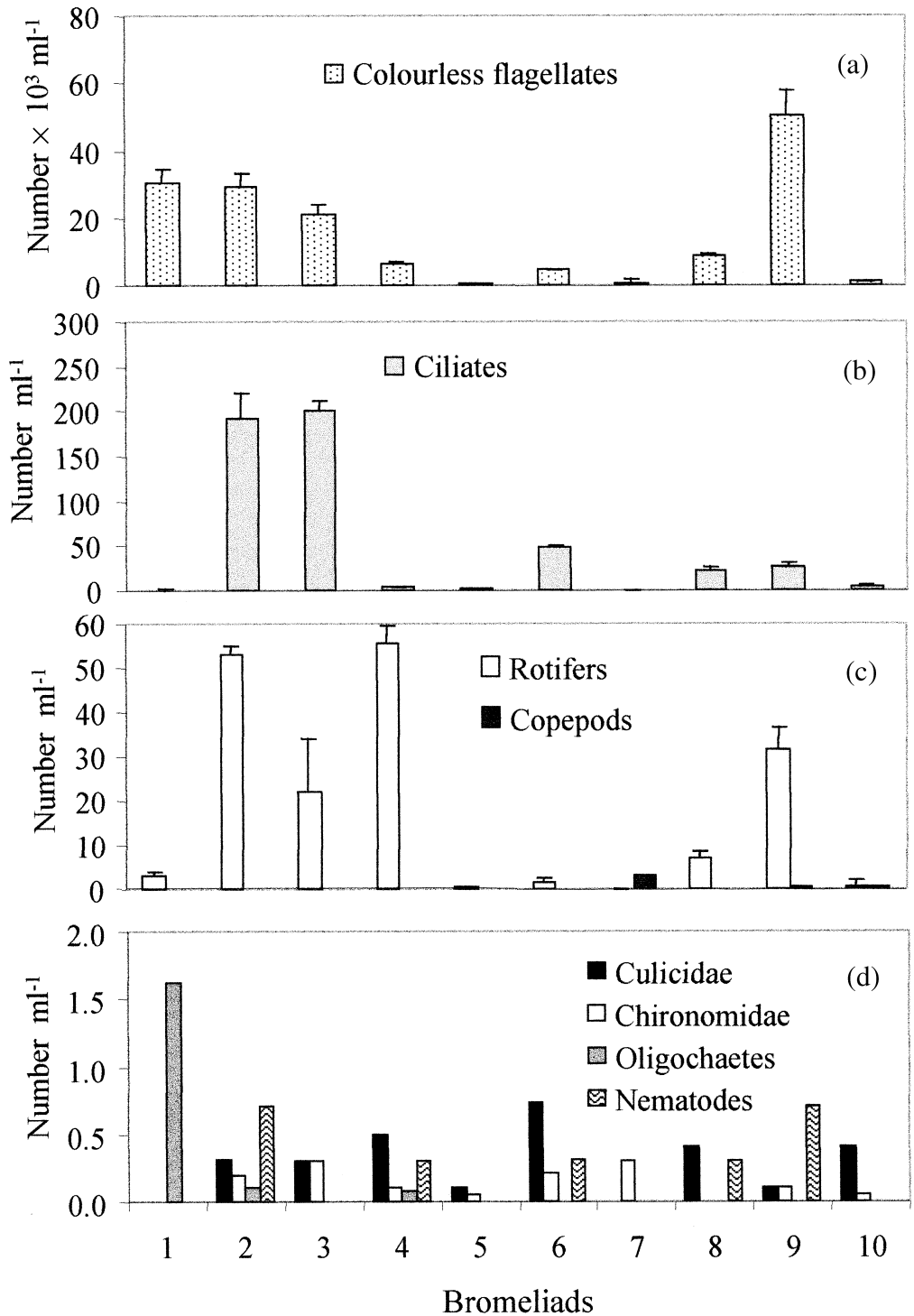


Figure 1. Numerical abundance of protozoa (flagellates (a), ciliates (b)), small metazoa (c), and major macro-invertebrates taxa (d) in 10 tank bromeliads from French Guiana. 1–6: *Aechmea mertensii*, 7–8: *Vriesea splendens*, 9–10: *Vriesea pleiosticha*.

largest bromeliads that contained large volumes of water (No. 2: *Aechmea mertensii*, No. 9: *Vriesea pleiosticha*).

Based on our results, protozoa appear clearly to be of major importance within the aquatic community of tank bromeliads. Both flagellates and ciliates were present in all samples with similar densities to those reported for freshwater ponds and productive lakes (Berninger *et al.* 1991, Finlay *et al.* 1988). Colourless euglenids are typically abundant in aquatic habitats enriched with organic debris where they feed both on bacteria and organic solutes (Sanders 1991). Hymenostomes are important filter-feeding bacterivorous ciliates in many aquatic habitats (Foissner & Berger 1996). Thus, heterotrophic protozoa may act as an essential pathway for dissolved organic matter and as important consumers of bacteria in tank bromeliads. Due to their high, weight-specific metabolism, aquatic protozoa may contribute more significantly to nutrient remineralization than metazoa (Caron 1991). It suggests that their role in the release of nutrients from particulates may be essential for epiphytic bromeliad growth. Photosynthetic protists were probably absent because all the sampled plants had been grown in deep shade. They also contained many dead leaves, detrital particles and bacteria (*pers. obs.*). As noted by Laessle (1961) and Frank (1983), algae are relatively uncommon in shaded bromeliads, probably because the light is not sufficiently intense. The food chain in these bromeliads should be based mainly on detritus. In contrast, plants exposed to sunlight may contain algae and support a more complex food chain (Frank 1983, Laessle 1961).

Although no accurate taxonomic determination of metazoa was performed during this study, it is likely that metazoan abundance in phytotelmata is largely dominated by detritivorous taxa (Kitching 1987). Thus, the positive relationship between protozoan and metazoan abundances probably indicates a similar response to the same environmental or biological factor such as an increase in a major food resource rather than predator–prey interactions. Our results support the idea that amensalistic and mutualistic interrelationships between protozoa and metazoa may be more common than competition or predation (Alongi & Hanson 1985), particularly in systems with high detritus content such as tank bromeliads. Protozoan densities may be enhanced by detrital particle breakdown by detritivorous metazoa resulting in an increased surface area for bacterial and protozoan colonization. Our results also indicate that the number of taxonomic group increased with the volume of water in the shoot. Based on a large number of plants, Richardson (1999) found that total organic matter, animal abundance, animal species richness and, to a less extent, water volume were all related to the plant size. This suggests that the food web in bromeliads is probably more complex in large plants.

Studies of the metazoan food webs in phytotelmata are scarce (Frank 1983, Kitching 1987, Naeem 1988), and the trophic coupling between protozoans and metazoans has never to our knowledge been studied. A common handicap of these types of study is the difficulty in identifying the potential predators of

protozoa accurately. Both Culicidae and Chironomidae contain taxa which prey on other macro-invertebrates. However, the feeding activity of these taxa does not necessarily avoid small-size prey such as protozoa. Maguire *et al.* (1968) recorded a strong negative interaction between mosquito larvae and *Paramecium* in the field. In the laboratory, natural densities of *Paramecium* were reduced to zero within a week by *Culex americanus* (Neueu–Lemaire). However, the ciliate genus *Paramecium* generally comprises large-size species (> 100 µm) that might not be representative of the whole ciliate diversity in freshwater ecosystems. Further investigations on feeding behaviour and ecology of bromeliad-inhabiting metazoa are therefore needed to evaluate their ability to consume protozoa. Nevertheless, the extent to which rotifers, copepods and other metazoa consume protozoa can be estimated from planktonic studies. The sparse data available suggest that rotifers, copepods and mosquito larvae may be important predators of protozoa. Rotifers seem to be significant feeders on the smaller organisms (bacteria, flagellates and ciliates) of the planktonic microbial web (Arndt 1993). Copepods are also able to capture planktonic protozoa, especially ciliates (Sanders & Wickham 1993). In riverine systems, filter-feeding insect larvae are the likely consumers of protozoa (Carlough & Meyer 1989). Oligochaetes can also ingest planktonic ciliates (Archbold & Berger 1985). These limited data suggest that protozoa may be an important food resource for the numerous aquatic metazoa that inhabit the tanks of bromeliads. However, results from limnetic and lotic studies do not necessarily apply to bromeliad microcosms. Moreover, many organisms are bromeliad specialists not found in other habitats (Benzing 1990). It is clear that data are needed on the role of aquatic organisms in detrital decomposition, trophic interaction and in nutrient regeneration in plant-held waters.

Considering their abundance and their potential role, protozoa are undoubtedly active participants within phytotelmata food webs. Surprisingly, these communities and the microbial food webs in this ecosystem have not yet been considered.

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