Peritrich epibiont protozoans in the zooplankton of a subtropical shallow aquatic ecosystem (Monjolinho Reservoir, São Carlos, Brazil)

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Peritrich epibiont protozoans and metazooplankton (rotifers, cladocerans and copepods) were investigated seasonally in a shallow eutrophic artificial reservoir in a subtropical region (Monjolinho Reservoir, Brazil). The ciliated peritrichs Rhabdostyla sp. and Scyphidia sp. were found to colonize individuals of different metazooplanktonic groups, especially copepods (47.20% of the total number of metazooplanktonic organisms found with epibionts and 57.14% of infection in the copepod population). Copepods also had the greatest epibiont load, followed by cladocerans and rotifers. Rotifers were the most important metazooplanktonic group (90.14% of the total) and the taxa observed with epibionts were Filinia longiseta, Polyarthra, Brachionus and Keratella, with highest preference for the first organism. F. longiseta was present at low density compared with the others and the preference as a substrate for epibionts may be related to its type of adhesion surface (soft) and/or feeding habits (particle feeding).

INTRODUCTION

Planktonic rotifers, copepods, cladocerans and protozoa may have small epibiont or ectobiont organisms such as bacteria, fungi, algae, protozoa or rotifers adhered to their body surface. Several species of protozoa belonging to the group of flagellates, suctorians, peritrichs etc can be found colonizing zooplankton organisms.

As a result of epibiosis, host and epibiont have benefits and disadvantages (Wahl, 1989). The negative effects of epibiosis on the host (basibiont) may involve a decrease in fecundity (Green, 1974; Henebry and Ridgeway, 1979; Threlkeld and Willey, 1993; Stirnadel and Ebert, 1997), decrease in survival ability (Willey et al., 1990; Xu and Burns, 1991; Allen et al., 1993), lesions and diseases (Sawyer and McLean, 1978; Turner et al., 1979; Nagasawa, 1987), perturbation of locomotion (Henebry and Ridgeway, 1979), competition for food (Kankaala and Eloranta, 1987), increase in susceptibility to predation (Green, 1974; Evans et al., 1979; Willey et al., 1990; Chiavelli et al., 1993; Threlkeld and Willey, 1993; Weisman et al., 1993; Willey et al., 1993; Wahl et al., 1997), increasing energy demands (Allen et al., 1993) and faster sinking rates (Herman and Mihursky, 1964; Allen et al., 1993). Henebry and Ridgeway (Henebry and Ridgeway, 1979) suggested, as a possible positive effect for basibionts, that copepods which eat peritrichs may benefit by carrying the easily available food supplement on their backs and Wahl et al. (Wahl et al., 1997) noted decreasing predation pressure when epibionts were less attractive than their host or when they were repellent.

Epibiosis is beneficial for epibiont protozoa, which can be transported to regions richer in food, competing (Kankaala and Eloranta, 1987) or not with their hosts (Evans et al., 1979), and to much more oxygenated sites (Smith, 1986). Also, by being fixed on moving substrates, they can avoid predation by zooplankton (Henebry and Ridgeway, 1979). Kudo (Kudo, 1966) mentioned that the dislocation of the host increases the chances of survival of epibionts due to the increased flow of water containing food items and to the fact that the epibionts are taken to sites with possibly more appropriate physical and chemical conditions. Wahl (Wahl, 1989) stated that the more intense water flow, in addition to ensuring a greater nutritional supply, facilitates the dispersal of excreta, while the mobility...
of the epibionts facilitates their own dispersion and improves the gene flow of their populations.

Although the first reports of epibionts were made long ago, there is not much known about the colonization of zooplankton by epibionts on tropical and subtropical systems. Lopez et al. (Lopez et al., 1998) worked in Tule Reservoir (Venezuela) where Epistylis sp. and Synedra sp. were examined on crustacean zooplankton during an annual cycle. They found cyclopid copepods as the preferred substrate for Epistylis sp. and the highest prevalence, for both epibionts, in the early dry season.

In the present study we examined the seasonal occurrence of bactivorous epibiotic peritrich ciliates (Rhabdostyla sp. and Scyphidia sp.) on metazooplankton in a subtropical shallow reservoir strongly dominated by rotifers. Samples were collected from the surface and the bottom and the possible relationships of epibionts with their hosts as well as with their prey (bacteria) were analyzed.

Study site

The Monjolinho Reservoir is a eutrophic environment located in the campus of the Federal University of São Carlos (São Paulo, Brazil) in a subtropical region (47°53′W and 22°01′S), with an area of 47,157 m² and a mean and maximum depth of 1.5 and 3 m, respectively. The region is characterized by two contrasting periods of the year, i.e. a cold dry and a hot rainy season. The dry season starts in May and ends in September and the rainy season lasts from November to February. The retention time of the reservoir is 22.9 days during the dry period and 2.1 days during the rainy period (Nogueira and Matsumura-Tundisi, 1994). The reservoir is highly unstable and turbulent because of its small dimensions, shallow depth, short retention time, precipitation and wind, which affect not only the phytoplankton and zooplankton communities (Nogueira and Matsumura-Tundisi, 1996), but also the protozooplankton and bacterioplankton communities. This high instability promotes a frequent resuspension of the sediment, which increases the nutrients in the water column and favors the occurrence of phytoplankton blooms at the end of the dry season.

METHOD

Surface and bottom (0.5 m above the sediment) water samples were collected biweekly from January to December 1990 at a central station in the reservoir. Samples (100 mL) for zooplankton and protozooplankton were collected in triplicate, fixed with mercuric chloride and stained with bromophenol blue (Pace and Orcutt, 1981). Unfixed protozoan samples, concentrated with 10 and 25 µm mesh plankton nets, were analyzed under the microscope for taxonomic features observation.

The zooplankton and protozooplankton of each flask were concentrated by discarding the supernatant liquid after sedimentation and counted in three Sedgwick–Rafter chambers at 100× magnification, under an optical microscope with phase contrast. The organisms were identified, based on morphologic characteristics according to the literature descriptions (Kahl, 1930–35; Pennak, 1953; Edmondson, 1959; Kudo, 1966; Foissner et al., 1992).

Water samples (100 mL) for live bacterial counts were kept under refrigeration and counted within 4 h from collection. From each sample, a sub-sample of 0.5–2 mL, stained with acridine orange (0.01 g L⁻¹ final concentration), was filtered onto 0.45 µm pore size black cellulose membrane (Sartorius), and counted by epifluorescence microscopy under blue excitation with an Olympus BH-2 microscope. For each membrane, a total of 300 cells were counted in random fields.

The physical and chemical variables were measured at the surface and bottom. The temperature (°C) and water transparency were measured in the field with a mercury bulb and a Secchi disc, respectively. The pH was measured in the laboratory with a MicroNal potentiometer B271 and the dissolved oxygen concentration (DO, mg L⁻¹) was determined by the Winkler method (Golterman et al., 1978).

Student’s t-test, Pearson’s correlation and Sliding covariance analysis were calculated with ‘Statistica Program’ (Copyright © 1984–1999 by StatSoft, Inc.) using both biological and limnological parameters.

RESULTS

Analysis of the physical and chemical variables by the Student’s t-test showed no significant differences between surface and bottom samples, and the mean values and ranges are shown in Table I. Comparing the dry and wet periods, the changes were significant for temperature and transparency.

The proportion and composition of the metazooplanktonic organisms found in this study are shown in Table II. Rotifers dominated the plankton (90.14% of the total metazooplanktonic population), followed by copepods (mainly naupliar forms) and cladocerans. The copepods were represented by only one cyclopid species (Thermocyclops decipiens). The most representative cladocerans present in the plankton were Bosminopsis deitersi and Moina minuta. The main genera of rotifers observed were Keratella, Polyarthra and Brachionus (Table III). The species of rotifers that occurred in the samples were Keratella americana, K. cochlearis, K. cruciformis, Polyarthra longissima, P. vulgaris, Brachionus angularis, B. calyciflorus, B. caudatus, B. falcatus, B. plicatilis, Lecane spp., Trichocerca agnatha, Trichocerca sp., Filinia longiseta, Platys quadricornis, Conochilus unicornis,
Synchaeta stylata, S. pectinata, Philodina sp., Asplanchna sieboldi, Asplanchna sp., Collotheca spp., Hexarthra mira, Hexarthra sp., Lepadella triptera and Lepadella sp.

The epibionts observed on the rotifers, cladocerans and copepods throughout the sampling period were the peritrich ciliates *Rhabdostyla* sp. and *Scyphidia* sp., except for four collection dates during the dry season when they were absent from both surface and bottom collected zooplankton. *Rhabdostyla* sp. and *Scyphidia* sp. were identified in the live samples but they couldn’t be distinguished in the fixed ones due to distortion and shrinkage after preservation with fixative. Consequently, the epibiont–host specificity was not determined. Both peritrichs were found colonizing all vital stages (eggs, nauplii, copepodids and adults), as well as all body parts of their hosts, except for the appendages.

With respect to epibiont burden (number of epibionts on a substrate organism), copepods presented, on average, the largest number (24.5 ± 2.05 epibionts ind.⁻¹), followed by cladocerans (22 ± 1.48 epibionts ind.⁻¹) and rotifers (5 ± 4.21 epibionts ind.⁻¹).

The annual averages of the surface and bottom densities of each group of organisms studied through a Student’s *t*-test showed no significant differences. Therefore, the mean values (from surface and bottom data) are presented for each group of organisms studied (Figure 1). With the peritrich peak there was an abrupt decrease in metazooplankton. At the beginning of November, when peritrich densities (free and attached) were very low, the rotifer and crustacean populations reached a peak.

Free-swimming forms of epibionts (peritrich telotrochs) were recorded in similar numbers, on average, to those of adhered forms, i.e. 1.4 ind. mL⁻¹ and 1.7 ind. mL⁻¹, respectively. A predominance of free-swimming forms...
were recorded 15 days before the peak of adhered epibionts at the end of October (Figure 1).

The values found for prevalence (percentage of substrate organisms with epibionts) through the year at the surface and bottom samples were variable and not always associated with high densities of zooplankton (Figure 2). The highest prevalence of epibionts on metazooplankton was 33.36% on bottom samples on October 29, and the mean prevalence for the year was 4.86%.

Although the differences found with depth were not statistically significant, the mean yearly densities of the total zooplankton were higher in surface samples (7.0 ind. mL$^{-1}$) than in the bottom ones (3.9 ind. mL$^{-1}$) and the mean yearly metazooplanktonic prevalence was higher in the bottom (5.4%) than in the surface (3.4%).

Table II shows that rotifers were the numerically dominant metazooplankton group (90.14% of the total) and despite the fact that they are smaller than the copepods, they presented almost four times more surface area than that of nauplii, copepodids and adults summed. Even considering these facts, the copepods were the organisms most frequently observed with peritrichs (adults, copepodids and nauplii summed 47.2% of the total infested organisms). Comparing the different copepod life stages, nauplii were proportionally the most frequently found with epibionts, but considering the percentage of individuals

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**Fig. 1.** Mean numerical density (surface and bottom) of peritrichs (attached and free), bacteria and metazooplanktonic organisms in the water column of the Monjolinho Reservoir during 1990.

**Fig. 2.** Metazooplankton prevalence values in surface and bottom samples of the Monjolinho Reservoir during 1990.
infected in each group, only 11.89% of the total nauplii population was colonized against 59.34% of the copepodids and adult copepods.

Among the rotifers found to carry peritrichs (*Filinia longiseta*, *Polyarthra*, *Keratella* and *Brachionus*), *F. longiseta* was the most frequently infested one, followed by *Polyarthra*, *Brachionus* and *Keratella*. However, in terms of numerical dominance in the reservoir, *F. longiseta* was the sixth in the rotifer group, *Polyarthra* the second, *Brachionus* the third and *Keratella* the first (Table III).

**DISCUSSION**

The studied reservoir is quite unstable and homogeneous due to the shallow depth of the environment, with a consequent strong action of precipitation and wind on the circulation and mixture of the water column. Transparency, in the wet period, is decreased by precipitation, the shorter retention time of the reservoir and the algal blooms, which started at the end of the dry period.

The proportion and composition of the metazooplanktonic organisms found in this study (Table II) was similar to that detected by Nogueira and Matsumura-Tundisi (Nogueira and Matsumura-Tundisi, 1996) at the same reservoir, who inferred that its short retention time permitted a predominance of zooplanktonic groups with a shorter life cycle, such as the rotifers. Similarly, according to the same authors, the environment is selective for most copepod species since the group was represented by only one cyclopoid species (*Thermocyclops decipiens*). The dominance of nauplar forms of copepods instead of copepodids and adults is also explained by the short retention time of the reservoir.

The peritrich ciliates observed in this work (*Rhodostyla* sp. and *Scyphidia* sp.), which are frequently observed in other environments colonizing the surfaces of different hosts (Green, 1974; Henebry and Ridgeway, 1979; Smith, 1986; Weissman et al., 1993), were seen attached to all their body parts except to their appendages. The presence of peritrichs on different body parts has been mentioned by Henebry and Ridgeway (Henebry and Ridgeway, 1979) and Weissman et al. (Weissman et al., 1993). Henebry and Ridgeway (Henebry and Ridgeway, 1979) proposed that, since peritrichs primarily consume bacteria, their location on the body of the host is indifferent. However, adhesion to appendages is probably inadequate because epibionts may be lost with the attrition caused by movements and, according to Green (Green, 1974), the frequent perturbation by the antenna of the host interrupts the feeding of peritrichs, causing zooid contraction. *Colacium*, *Synedra*, *Amoebidium* and *Lyngbya* were observed on the appendages, but since they are finer and smaller photosynthesizing organisms than peritrichs, they are probably not affected by movements (Green, 1974; Chiavelli et al., 1993).

In the present work, the greater epibiont burden found for the copepods, followed by the cladocerans and the rotifers, seems to indicate that infestation was associated with the available adhesion surface offered by each group (host size and available area), and also with the duration of its instars. Henebry and Ridgeway (Henebry and Ridgeway, 1979) observed that the number of vorticelloids per cladoceran changed according to length class, with more individuals adhering to larger than to smaller hosts. According to Threlkeld et al. (Threlkeld et al., 1993), there is a positive relationship between epibiont burden and body size of the plankton organism because larger organisms (i) are easier targets for epibionts passively and randomly dispersed, (ii) swim more rapidly and find more epibionts, (iii) can filter water and attract more epibionts, and (iv) can create greater hydrodynamic perturbations that attract mobile epibionts capable of detecting them.

The occurrence of *Synura* sp. and *Mallomonas* sp. blooms at the end of the dry season with the increased food supply for different components of the food chain altered the balance of the system, generating a succession of density peaks of bacteria, protozoa and zooplankton (Figure 1). Enhanced bacterial densities in the blooming period can be seen and, although epibiont populations changed with the changes in their food (i.e. bacteria), a Pearson's correlation analysis between these two populations throughout the year was not significant ($r^2 = 0.2689$). Probably other factors (e.g. predation on bacteria, epibionts or metazooplankton) are contributing to mask the relationship.

The highest epibiont densities were observed on October 29 (30.7 ind. mL$^{-1}$ on the surface and 29.4 ind. mL$^{-1}$ on the bottom) and were associated with the high zooplankton densities, possibly due to the increase in the adhesion surface (Figure 1). With the epibiont peak there was an abrupt decrease in metazooplankton, possibly as a consequence of the large amounts of epibiont protozoa. At the beginning of November, when epibiont densities were very low, the rotifer and crustacean populations reached a peak. Sliding covariance analysis (with a 2 month window for the first and last periods, and a 4 month window for the other periods) for zooplankton and epibiont populations along the year showed high positive values during the increase in zooplankton population up to October 29 (Figure 3). After this date, high negative covariances were detected possibly indicating that epibiosis permits the normal growth of host populations when present at certain rates. However, a high incidence of infestation can be pointed out as a direct and/or indirect controlling agent influencing the seasonal
succession of these populations. The controlling action of the epibiont populations on their hosts has been suggested by various investigators [e.g. (Herman and Mihursky, 1964; Green, 1974; Evans et al., 1979; Turner et al., 1979; Kankaala and Eloranta, 1987; Willey et al., 1990; Xu and Burns, 1991; Weissman et al., 1993)].

The population peak of free peritrich epibionts observed before the peak of adhered epibionts (Figure 1) can be explained by the normal life cycle of peritrichs, with telotrochs (swarmers or ciliated free-swimming larvae) in the early stages, followed by the sessile mature phase attached to a new substrate. Thus, the peritrich epibiont populations were probably reproducing intensely in response to the population increase of their hosts.

The values found for metazooplanktonic prevalence through the year at the surface and bottom samples were variable and not always associated with high densities of zooplankton (Figure 2). This explains the absence of correlation found between metazooplanktonic prevalence and metazooplanktonic densities ($r^2 = 0.012$). The maximum metazooplanktonic prevalence (33.36%) found could be considered low since these values vary widely in the literature, reaching 100% at certain sites and in certain situations. However, these literature high values are associated with cases of specific infestation, a fact that was not observed in the present study. According to Threlkeld et al. (Threlkeld et al., 1993), obligatory epibions probably show stronger relations with zooplankton abundance.

Despite the fact that the differences found with depth were not statistically significant in this work, the results showing proportionally more infected zooplankters in the bottom are in accordance with laboratory experiments conducted by Allen et al. (Allen et al., 1993) that showed higher sinking rates of colonized individuals than the non-colonized ones.

The results shown in Table II lead us to consider that bigger organisms are easier targets for the epibionts than smaller ones, even if these are more abundant. The higher percentage of copepod infestation compared with other zooplanktonic groups may be related to epibiont preference for these organisms or to the presence of a comparatively larger adhesion surface offered by each individual (Table II). The higher densities of the nauplii compared with the adult copepods can perhaps explain the higher percentage of nauplii found with peritrich epibionts. However, comparing the percentage of individuals infected in the nauplii populations (11.89%) with the copepodids and adult populations (59.34%) we can see that epibiosis is proportionally more important in the adult populations. Willey et al. (Willey et al., 1990), in a study on Lake Michigan, reported a higher susceptibility of nauplii to adhesion by Calacium, in relation to the total zooplankton on one occasion. By another point of view, the dominance of naupliar forms of copepods instead of older forms can be explained by the short retention time of the reservoir (as explained above, in the beginning of the Discussion) and also by the fact that the older forms are more infected and consequently die more rapidly due to the epibiont load.

In the literature, there are no studies dealing with epibions on rotifer populations and the high percentage of rotifer infestation observed at Monjolinho Reservoir was probably associated with the high rotifer densities found in the plankton of this environment, that increases the available adhesion surface for the epibions. The lack of reports of infestations in rotifer populations may be due to the low rotifer density in the plankton where epibiosis was most studied in the past, i.e. marine environments.

The preference for particle feeding rotifers was evident in our work (with the exception of Polyarthra that have different feeding habits) and this interaction is interesting vis a vis the epibions since they can be carried to regions with higher bacterial concentrations.

The body surface of the prey may play a primary role in its interactions with consumers (Wahl et al., 1997), and, therefore, we can expect the same for epibiont-host interactions. Our work verified epibiont preference for soft-bodied rotifers (F. longiseta and Polyarthra—Table III) in comparison with the loricated ones. Keratella was the first in population dominance among the rotifers and its
rates of epibiosis are proportionally the lowest. This can be related to the type of carapace it has (loricated), often with spines that can impair adhesion, the escape strategy of this rotifer to avoid predation (Gilbert and Kirk, 1988), which can also be important to avoid epibiosis and for the possible mechanism of chemical antifouling that occurs in several marine invertebrates (Davies et al., 1989).

The preference for *F. longiseta* was probably associated with the type of adhesion surface (soft bodied) and to the feeding habits (particle feeding-bacterivory) of the basibiont. On the other hand, the low density of *F. longiseta* itself may be related to the negative effect of epibiosis on the population, since it may lead to competition for food, increase the susceptibility to predation and cause infectious diseases. Turner et al. (Turner et al., 1979) suggested that the loss of body fluids through the lesions close to the sites of adhesion of *Epistylis* in *Acartia tonsa* may contribute to the seasonal decrease of that population. Ooms-Wilms et al. (Ooms-Wilms et al., 1995) measured bacterivory of several rotifers with the microscopes technique and found that *F. longiseta* and *Amphiariopsis fissa* exhibited the highest uptake rates. Ooms-Wilms (Ooms-Wilms, 1997), in an experiment involving the growth of different rotifers with bacteria, detected higher mortality rates and difficulties of cultivation for *F. longiseta*. Considering these literature data, we can suppose that, in the Monjolinho Reservoir, the possible competition for food between *F. longiseta* and its epibiont may be more harmful for this rotifer population than to other infested rotifer populations.

Although predation impact on the zooplanktonic community was not evaluated in the Monjolinho Reservoir, this work suggests that epibiosis plays an important role in the control and dynamics of the planktonic populations.

**ACKNOWLEDGEMENTS**

We thank Dr. Paulo Seleighim Jr. for help and advice during the study, Darci D. Javarotti for technical assistance and CNPq (National Council for Scientific and Technological Development) for a scholarship to M.H.R.S.

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Received on October 9, 2000; accepted on February 10, 2004; published online on March 9, 2004