

YOLANDA ZALOCAR DE DOMITROVIC¹, MARINA E. FORASTIER¹, SYLVINA L. CASCO¹ and VISITACIÓN CONFORTI^{2*}

- ¹ Centro de Ecología Aplicada del Litoral (CONICET). Departamento de Biología, Facultad de Ciencias Exactas y Naturales y Agrimensura (UNNE), Corrientes, Argentina
- ² CONICET, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales (UBA), Buenos Aires, Argentina

With 8 figures and 1 table

Abstract: Studies on taxonomy and seasonal variations of density and biomass were carried out over a year on epibiont algae on planktic microcrustaceans from a subtropical shallow lake (Corrientes, Argentina). Four taxa, 3 Chlorophyta and 1 Euglenophyta, were documented. Among them, *Chloromonas anuraeae* (KORSHIKOV) GERLOFF et ETTL and *Chlorangiopsis systilis* KORSHIKOV were documented for the first time for South America, and *Korshikoviella setosa* (FILARSZ.) SILVA for the first time in Argentina. *Colacium vesiculosum* EHRENB. was the most frequent taxon observed. The infestation percentage was high in adult microcrustaceans of calanoid copepods. The absence of a significant correlation between the density of epibionts and microcrustaceans or between both populations and the environmental variables suggests that epibiont algae might not play a key role in the regulation of planktic microcrustaceans.

Key words: biomass, Chlorophyta, epibiont algae, Euglenophyta, seasonality

Introduction

Microcrustaceans from continental water plankton usually have epibionts (or ectosymbionts) bound to the surface of their exoskeletons (protozoan, rotifers, algae and/or bacteria). Some studies demonstrate that epibionts affect zooplankton sedimentation rate, hindering locomotion, and interfering with food acquisition and the ability to escape from predators (WILLEY et

DOI: 10.1127/1864-1318/2008/0127-0029

1864-1318/08/0127-029 \$ 2.50

© 2008 E. Schweizerbart'sche Verlagsbuchhandlung, D-70176 Stuttgart





^{*} corresponding author



al. 1990, Allen et al. 1993, Chiavelli et al. 1993, Threlkeld & Willey 1993). Most data come from research carried out in lakes of Europe and USA, since the subject has been hardly explored in tropical and subtropical areas, where it was limited to the identification of a few epibiont taxa (Seckt 1931, Margalef 1961).

The aim of this study was to identify epibiont algae, to determine the seasonal variations of density and biomass in each group of planktic microcrustaceans, and to relate population changes (of algae and microcrustaceans) to the environmental variables that might favour the interaction of both organisms.

Materials and methods

Studies were carried out in Paiva pond, located near Corrientes city (27° 29'S; 58° 45'W), Argentina. The surface of the pond has 70 ha, but between 50% and 70% of it is occupied by aquatic and marsh vegetation.

Between May 2002 and May 2003, 17 samplings were carried out in the vegetation-free area of the pond. Their frequency varied according to the infestation percentage (weekly, bimonthly or monthly). Integrated zooplankton samples were taken with a PVC collection tube, 1 m long and 10 cm diameter. The collected water (50 liters) was filtered through a plankton net with a mesh size of 53 µm and fixed with 4 % formaldehyde.

Microcrustacean counting was performed in Bogorov chambers using a stereoscopic microscope, and counting 50 % of the samples. From this fraction, organisms with epibionts (infested) were separated and observed with the light microscope to carry out the taxonomic determination of algae, calculate density (expressed as cells microcrustacean⁻¹), and measure cells to determine biomass as biovolume (µm³ microcrustacean⁻¹).

During samplings, *in situ* determinations of some environmental variables such as water transparency (using a Secchi disk), depth, temperature, pH, conductivity, and dissolved oxygen were carried out. Samples were collected to perform nutrient analyses in the laboratory following techniques by APHA (1981). Biotic and abiotic variables were correlated using the Spearman correlation coefficient (STEEL & TORRIE 1988). This study does not include microcrustacean predator organisms.

Results and discussion

Four species of epibiont algae from Chlorophyta and Euglenophyta were identified. The highest number of taxa was observed in Chlorophyta: Volvocales (*Chloromonas anuraeae*), Tetrasporales (*Chlorangiopsis systilis*) and Chlorococcales (*Korshikoviella setosa*). Only one taxon of Euglenophyta was observed: *Colacium vesiculosum*.







a - Description of epibiont algae

CHLOROPHYTA, Chlorophyceae

Volvocales

Chloromonas Gobi emend. WILLE 1903

Ch. anuraeae (Koršhikov) Gerloff et Ettl, Nova Hedwigia 34, p. 83, Lam. 18, fig. 1. 1970. (Fig. 1 a, b)

Basionym: Chlamydomonas anuraeae Koršhikov 1938

Ovoid cells (5.5–10 µm long and 3–7 µm wide) fixed to the substrate by two short flagella. They have an asymmetrical parietal chloroplast without pyrenoid.

This species was originally documented in Russia as epizoic on planktic rotifers of the genus Anuraea EHRENB. (= Keratella BORY DE ST. VIN-CENT). It was documented on Cyclops in Romania (see ETTL 1970), as well as on other organisms in Slovakia (HINDÁK 1998) and Slovenia (REKAR & HINDÁK 2002). In Paiva pond, it was found on cyclopoid and calanoid copepods, with a sporadic presence during the year. A high density of this taxon was observed on calanoids in a single sampling (see Fig. 7). It was documented in South America for the first time.

Tetrasporales, Chlorangiellaceae

Chlorangiopsis Koršhikov 1932

Ch. systilis Koršhikov 1932

(Figs 2 a, b)

Ovoid cells similar to *Chlamydomonas*. They are bound to the exoskeleton of microcrustaceans by two short jelly-like styles located in the anterior part. They present a parietal chloroplast with one pyrenoid. Vegetative cells 7.5–10 µm long and 6.5–7.5 µm wide. Zoosporangia: 17.5–25 x 12–18 µm, zoospores: 7.5–8 x 3.5–5 µm. Most of these organisms were represented by zoosporangia containing 2, 4 and 8 zoospores.

Species documented in Russia, usually found on Systilis flava (see FOTT 1972). It was documented fo the fist time for South America.

Chlorococcales, Chlorococcaceae

Korshikoviella Silva 1959

K. setosa (Filarsz.) Silva 1959

(Figs 3 a, b)

Synonym: Characium setosum Filarsz. 1914, Lambertia setosa (Filarsz.) Koršhikov 1953

Cylindrical cells attenuated at the poles in long setae. They have a basal

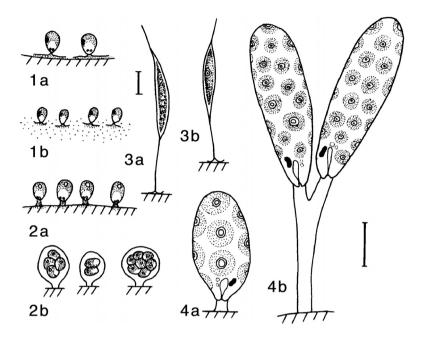
style of bifid fixation (23–28 µm long) and longer than the apical seta (11– 15 μ m long). Cells are 30–63.5 μ m long, 4.5 – 6 μ m wide. They exhibit a parietal chloroplast with one pyrenoid.

Most of the observed forms presented zoosporulation with transversal divisions in the protoplast.





32 Y. ZALOCAR DE DOMITROVIC et. al.



Figs 1–4: 1 – Chloromonas anuraeae, 2 – Chlorangiopsis systilis, 3 – Korshikoviella setosa, 4 – Colacium vesiculosum. [Scale bars = $10 \mu m$].

In South America, this species was documented in Venezuela as *Lambertia setosa* (MARGALEF 1961). This is the first time it is documented in Argentina.

EUGLENOPHYTA

Euglenophyceae, Colaciales, Colaciaceae

Colacium Ehrenberg 1833

C. vesiculosum Ehrenberg

(Figs 4 a, b)

Long cells fixed by a jelly-like peduncle that can be simple or branched when they form colonies. They have parietal chloroplasts with pyrenoids. Vegetative cells 20 to 35 μ m long and 10 to 13 μ m wide.

This is a cosmopolitan species.

b – Temporal variations of density and biomass of epibiont algae in relation to microcrustaceans

Planktic microcrustaceans showed moderate density (32 to 460 ind.L $^{-1}$), with copepods predominating over cladocerans. The infestation percentage was between 1 % and 62 %, showing maximum values in spring and autumn and minimum values in winter (Figs 5, 6).







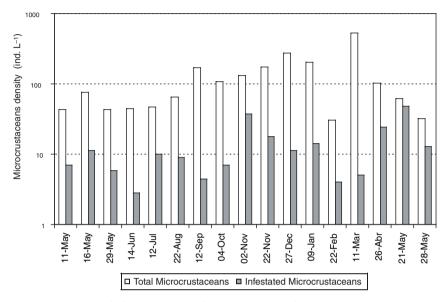


Fig. 5: Annual variation of the density of total planktic microcrustaceans in relation to infested microcrustaceans (ind. L-1).



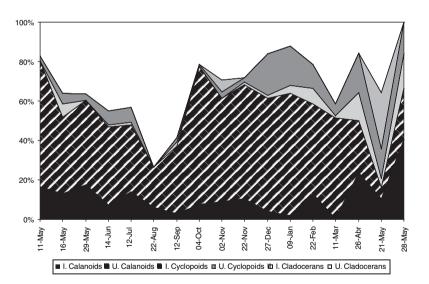


Fig. 6: Relative abundance of calanoids (Calan.), cyclopoids (Cyclop.) and cladocerans (Clad.) infested (I) and uninfested (U).







There was a positive correlation between the infestation percentage and the abundance of microcrustaceans ($r_s = 0.679$, p < 0.05). Among them, calanoid copepods exhibited higher infestation than cyclopoids, *Notodiaptomus spiniger* and *Thermocyclops minutus* being the dominant populations, respectively. However, infestation was occasional in cladocerans, affecting *Diaphanosoma birgei*, *Bosminopsis* sp. and *Daphnia laevis*.

The infestation percentage was higher in adult microcrustaceans than in larvae and juveniles (values below 5 %), probably due to the lower biomass and the frequent moltings experimented by the latter. The high infestation observed in calanoid copepods could be related to their high biomass and to their renovation rate, which is slower than that of other microcrustaceans.

Among epibiont algae, the most abundant was *Colacium vesiculosum* (Euglenophyta). It is usually the dominant species in lakes of the north hemisphere (WILLEY et al. 1990, AL-DHAHERI & WILLEY 1996). Its abundance in Paiva pond was directly related to the density of calanoid copepods and cladocerans. Medium values of density oscillated between 168 and 6049 cells microcrustacean⁻¹ and biomass, between 0.4.106 and 10.8.106 µm³ microcrustacean⁻¹ (Figs 7, 8).

Chlorophyta species showed low density and biomass. *Chlorangiopsis systilis* exhibited higher infestation in cyclopoid copepods (r_s = 0.489, p< 0.05), *Chloromonas anuraeae* was observed sporadically in both copepod groups, and *Korshikoviella setosa* was detected in cladocerans and only occasionally in cyclopoid copepods.

Epibiont algae did not show a uniform distribution over microcrustacean exoskeletons. Both unicellular and colonial forms (between 2 and 200 cells) were observed in *C. vesiculosum*. Adult microcrustaceans of calanoid copepods presented colonies with more cells. Cyclopoid copepods and cladocerans, on the contrary, tended to have more unicellular forms, and colonies with fewer cells (between 2 and 45).

The other epibionts (*Chloromonas anuraeae*, *Chlorangiopsis systilis* and *Korshikoviella setosa*) exhibited groups of 3 to 13 cells.

The microcrustacean population (infested + non-infested organisms) showed a positive correlation with water conductivity (r_s = 0.577, p < 0.02), and a negative correlation with the depth of the pond (r_s = -0.597, p < 0.01) and the water transparency (r_s = -0.566, p < 0.02). No statistically significant correlation was observed between the populations of epibiont algae and microcrustaceans, or between both populations and the environmental variables described in Table 1.

According to the bibliography, some epibionts affect the population dynamics of their substrate organisms reducing the energy used for food acquisition and increasing the predation hazard (WILLEY et al. 1990, ALLEN et al. 1993). In the present study, algae did not seem to affect microcrustacean populations, since these did not suffer significant decreases during most of the year. This may be due to the low infestation percentage ob-







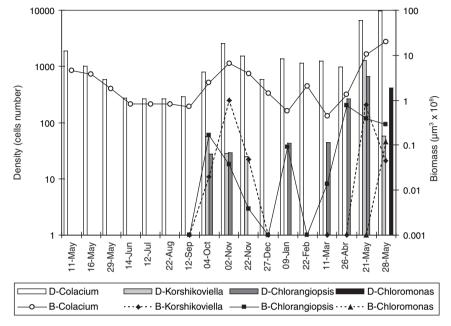


Fig. 7: Density (D) and biomass (B) of epibiont algae observed on microcrustaceans.

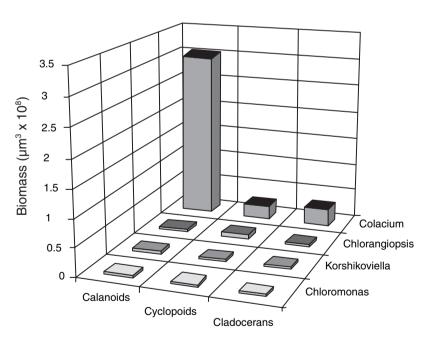


Fig. 8: Biomass (μ m³) of epibiont algae (*Chloromonas anuraeae*, *Chlorangiopsis systilis*, *Korshikoviella setosa* and *Colacium vesiculosum*) on calanoids, cyclopoids and cladocerans.





36 Y. ZALOCAR DE DOMITROVIC et. al.

Table 1. Average value (± SD) of environmental variables in which each epibiont was observed.

Parameter	Colacium (n= 17)	Chlorangiopsis (n= 8)	Korshikoviella (n= 4)	Chloromonas (n=1)
Depth (cm)	100.6 (25.7)	96.5 (25.9)	111.2 (25.9)	100
Secchi disk (cm)	91.3 (25)	85.2 (22.5)	101.5 (13.17)	100
Conductivity (µS cm ⁻¹)	48.4 (9)	49.1 (11.3)	44.0 (10.42)	35
Temperature (°C)	21.4 (3.9)	22.17 (3.79)	20.15 (3.57)	18
Dissolved oxygen (% saturation)	79.5 (16)	70.5 (10.4)	70.7 (4.57)	70
pH	6.66 (0.74)	6.59 (0.77)	7.1 (0.43)	6.96
Nitrites+Nitrates (µg L ⁻¹)	24.1 (37.3)	30 (56)	2.50 (3.53)	38.2
Ammonium (µg L ⁻¹)	15.5 (17.5)	19.20 (13.8)	30.5 (14.85)	22.3
Ortophosphates (µg L ⁻¹)	191.7 (96.6)	269 (97.6)	223.5 (19.09)	110

served (Fig. 5), or to the fact that food acquisition would not involve a high demand of energy considering the availability of phytoplankton observed during the year (represented by small green algae and Cryptomonas) (Zalocar De Domitrovic et al. 1998), as indicated by the positive correlation between Colacium density and phytoplankton ($r_s = 0.609, \, p < 0.01$). In the autumn of 2003 there was an important decrease in microcrustaceans with respect to the summer of 2002, in which the highest infestation percentage was registered. It is not easy to determine if this reduction was a consequence of the high infestation of epibiont algae, the dilution of the community due to water level increases caused by the heavy rains that occurred during this time of the year, or the interaction of both factors.

The ecological significance of epibiont algae is still unclear. Apparently, they benefit from their host, i.e. nutrient supply, mobility, and optimum light conditions for photosynthesis. In order to further study the interaction between epibionts and microcrustaceans, samplings were completed with laboratory bioassays (ZALOCAR DE DOMITROVIC et al., in prep.).







Conclusion

Four species of epibiont algae were identified: 3 Chlorophyta (two documented for the first time in South America and the third, in Argentina), and 1 Euglenophyta. The latter, represented by *Colacium vesiculosum*, was the alga with the highest density and biomass.

The infestation percentage was higher in adult microcrustaceans with respect to juveniles, probably because these have a lower biomass and suffer frequent moltings during their growth. Calanoid copepods were the most infested group due to their high abundance and biomass, and their renovation rate, which is slower than that of other microcrustaceans.

The absence of a significant correlation between the density of epibionts and microcrustaceans and the environmental variables would suggest that epibiont algae might not play a key role in the regulation of planktic microcrustacean populations in Paiva pond.

Resumen

Se presenta un estudio de la taxonomía, densidad y biomasa de las algas epibiontes en microcrustáceos planctónicos de la laguna Paiva (Corrientes, Argentina) realizados durante un año de estudio. Se registraron 4 taxa: 3 Chlorophyta y 1 Euglenophyta. *Chloromonas anuraeae* (Koršhikov) Gerloff et Ettl y *Chlorangiopsis systilis* Koršhikov se registran por primera vez para Sud América y *Korshikoviella setosa* (Filarsz.) Silva se menciona por primera vez para Argentina. *Colacium vesiculosum* Ehrenb. fue la especie más frecuente y abundante. El porcentaje de infestación fue elevado en microcrustáceos adultos de copépodos calanoideos en relación a su mayor abundancia, biomasa y tasa de crecimiento más lenta que la de los demás microcrustáceos.

Palabras clave: Algas epibiontes, taxonomía, densidad, biomasa, microcrustáceos, plancton, laguna subtropical, Argentina.

References

- AL-DHAHERI, R. S. & WILLEY, R. L. (1996): COLONIZATION AND REPRODUCTION OF THE EPIBLOTIC HAGELIATE *Colacium vesiculosum* (Euglenophyceae) on *Daphnia pulex*. J. Phycol. **32**: 770–774.
- Allen, Y.C., De Stasio, B.T. & Ramcharan, C.W. (1993): Individual and population level consequences of an algal epibiont on *Daphnia*. Limnol. Oceanogr. **38**: 592–601.
- American Public Health Association (1981): Standard Methods for the Examination of Water and Wastewater, 15th edn. APHA, AWWA & WPCF, Washington: 1134 pp.
- Chiavelli, D. A., Mills, E. L. & Thr elkeld, S. T. (1993): Host preference, seasonality, and interactions of zooplankton epibionts. Limnol. Oceanogr. **38**: 574–583.









ETTL, H. (1970): DIE GATIUNG *Chloromonas* Gobi emend. Wille. – Nova Hedwigia **34:** 1–283.

Fott, B. (1972): Chlorophyceae (Grünalgen) Ordnung: Tetrasporales. Das Phytoplankton des Süsswassers: Systematik and Biologie **6:** 1–211. Stuttgart. E. Schweizerbart.

Hindák, F. (1998): Morphology and taxonomy of some unicellular chlorophytes attached to planktic organisms. – Biologia, Bratislava, **53**: 381–401.

Mar gal ef, R. (1961): La vida en los charcos de agua dulce de Nueva Esparta (Venezuela). – Memoria de la Sociedad de Ciencias Naturales La Salle 21: 75–110.

Rekar, Š. & Hindák, F. (2002): *APHANIZOM ENON SLOVENICUM*, sp. nov. – morphological and ecological characters of a new cyanophyte/cyanobacterial species from Lake Bled, Slovenia. – Annls Limnol. **38:** 271–285.

Seckt, H. (1931): Fenómenos de epifitismo en algas de agua dulce. – Rev. Univ. Córdoba 8: 86–133.

Steel, G. D. & Torrie, J. H. (1988): Bioestadística: Principios y Procedimientos. – McGraw-Hill, México, 622 pp.

Thr el kel d, S. T. & Willey, R. L. (1993): Colonization, interaction, and organization of cladoceran epibiont communities. – Limnol. Oceanogr. **38**: 584–591.

Willey, R. L., Cantrell, P. A. & Threlkeld, S. T. (1990): Epibiotic euglenoid flagellates increase the susceptibility of some zooplankton to fish predation. – Limnol. Oceanogr. **35**: 952–959.

Zalocar de Domitrovic, Y., Casco, S. L. & Assel born, V. M. (1998): Estudio de la biomasa y diversidad del fitoplancton de la laguna Paiva, Corrientes, Argentina. – Physis, Buenos Aires, Serie B 55: 1–13.

Manuscript received May 25, 2007, accepted April 23, 2008.

The authors' addresses:

YOLANDA ZALOCAR DE DOMITROVIC MARINA E. FORASTIER SYLVINA L. CASCO Centro de Ecología Aplicada del Litoral (CONICET) C. C. 291, 3400-Corrientes, Argentina e-mail: yzalocar@arnet.com.ar, marinaforastier@hotmail.com, sylvina@arnet.com.ar

VISITACIÓN CONFORTI Facultad de Ciencias Exactas y Naturales (UBA) – CONICET Departamento de Biodiversidad y Biología Experimental Ciudad Universitaria, Pab. II 1428-Buenos Aires, Argentina e-mail: conforti@bg.fcen.uba.ar



