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Population dynamics of littoral rotifers (*Lecane inermis* and *Lepadella rhomboides*) (Rotifera) in relation to algal (*Chlorella vulgaris*) food density

TESIS PROFESIONAL

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B I O L O G A

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ÍNDICE

PÁGINA

ABSTRACT

We cultured two non-planktonic rotifers (*L. inermis* and *L. patella*) using four algal food densities ($0.1X10^6$, $0.2X10^6$, $0.4X10^6$ and $0.8X10^6$ cells ml^{-1} of *Chlorella vulgaris*). Under the tested food levels, *L. inermis* had long (about 15 days) lag phase, while *L. rhomboides* started to increase its population abundance from the 3rd day onwards. At low food density ($0.1X10^6$ cells ml^{-1}) *L. inermis* performed better, while at intermediate concentrations ($0.2X10^6$ and $0.4X10^6$ cells ml^{-1}), *L. rhomboides* grew to higher densities. The highest algal density resulted in reduced population growth of both the rotifer species. The rates of population increase (*r*) of *L. inermis* and *L. rhomboides* varied from 0.18 to 0.31 day^{-1}. For both the rotifer species the *r* was significantly affected by the algal concentration. When the daily rate of population increase was plotted as function of population density, a significant inverse relation was obtained for both the rotifer species. Results have been discussed to explain the differences in the relative abundances both the rotifer species in natural waterbodies with seasonally varying phytoplankton levels.

Keywords: *Lecane, Lepadella*, Rotifera, Food level, growth rate

INTRODUCTION

Rotifers are an important component of freshwater ecosystems. Compared to planktonic species, littoral rotifers are more diverse. Though members of the families Colurellidae and Lecanidae are frequently encountered in plankton collections, especially in shallow waterbodies, they are regarded as non-planktonic, and are also most common in both tropical and temperate regions (Koste 1978, Pontin 1978). They feed on detritus, bacteria, algae among other food items such as small flagellates. Littoral rotifers are mainly thought to participate in the detritus cycle of ponds and lakes. However, recent studies show even truly littoral-benthic rotifers such as *Lepadella* and *Lecane* are capable of feeding on planktonic algae and thus participate in the algal-herbivore trophic chain (Wallace et al. 2006). In addition, species of *Lecane* such as *L. bulla*, *L. closterocera* and *L. quadricornis* are used for feeding larval fish and as test organisms in ecotoxicological evaluations (Pérez-Legaspi and Rico-Martínez 2001).

Lepadella patella, *L. ovalis* and *L. rhomboides* generally occur in large numbers. *L. patella* may reach as high 1000 ind. ml^{-1} on a diet of *Chlorella vulgaris* at $1X10^6$ cells ml^{-1}. Such high density has been attributed to its smaller body size (<40 μm) (Nandini and Sarma 2001). The body size of *Lecane* species varies greatly (<50 - >400 μm) (Koste 1978). Though lecanid rotifers have been cultured on different algal species, their densities are generally lower than those of *Lepadella* of comparable body size (Pérez-Legaspi and Rico-Martínez 2001, Nandini and Sarma 2001). *Lecane inermis* is one of the smallest lecanid rotifer previously grown on alga, detritus and bacteria (Finesinger 1926). Due to its long toes and toe-tips, this species is often found attached to culture jars.

Both *Lepadella* and *Lecane* co-occur in many freshwater bodies (Nandini et al. 2006). Their relative abundances differ suggesting that the grow rates (*r*) vary even when the available food concentration is identical. However, quantitative data on growth rates are not available since the emphasis on the littoral rotifers has been from field-collections and only rarely studied under laboratory conditions (Pérez-Legaspi and Rico-Martínez 1998). Though it is possible to derive *r* for a particular rotifer species from the field-collections

(e.g., through egg ratio method, see Sarma et al. 2005), it is often to explain it because of confounding effects of several interacting factors. In addition, laboratory studies have largely focused on planktonic species, mainly of the genus *Brachionus* (Yúfera 2001). Growth patterns of *Brachionus* species may differ from those of *Lecane* and *Lepadella*, especially under different food levels (Pourriot 1982). It is therefore necessary to conduct experiments under controlled conditions for understanding the growth patterns of *Lecane* and *Lepadella*.

In this work, we aimed at studying population growth of two littoral rotifer species (*L. rhomboides* and *L. inermis*) under different algal food densities.

MATERIAL AND METHODS

We used two species of rotifers: *Lecane inermis* and *Lepadella rhomboides*, both of which were originally isolated from Lake Xochimilco (Mexico City) and separately mass-cultured using the single-celled green alga (*Chlorella vulgaris* at a density of about $0.5X10^6$ cells ml^{-1}) as the exclusive diet. We used re-constituted moderately hardwater (the EPA medium) as the medium for both maintaining zooplankton and for experiments. The EPA medium was prepared by dissolving 96 mg $NaHCO_3$, 60 mg $CaSO_4$, 60 mg $MgSO_4$ and 4 mg KCl in one liter of distilled water (Weber 1993). *Chlorella vulgaris* was batch-cultured in 2 L transparent bottles using Bold's medium (Borowitzka and Borowitzka 1988). The algal biomass was harvested from exponential phase by centrifuging at 4000 rpm for 5 min., rinsed and resuspended in distilled water. The density of alga was estimated using haematocytometer. From the stock alga, four algal concentrations ($0.1X10^6$, $0.2X10^6$, $0.4X10^6$ and $0.8X10^6$ cells ml^{-1}) were daily prepared using EPA medium.

Population growth experiments of both the rotifer species were conducted simultaneously. The experimental design for both rotifer species consisted of a total of 24 test jars (= 4 algal concentrations X 2 rotifer species X 3 replicates) of 50 ml transparent glass jars, each containing 20 ml medium of chosen algal density. The initial density of each rotifer species in the test jars was 1 ind. ml^{-1}, introduced individually using Pasteur Pipette under stereomicroscope (Nikon SMZ 645, Japan) at a magnification of 30X. The test conditions were: temperature 23±1° C, pH = 7.0 to 7.5, continuous photo period of diffused fluorescent illumination and the medium containing desired algal density daily replenished.

Following initiation of growth experiment, we daily counted all the live individuals of rotifers from each jar. When the rotifer density was higher than 5 ind. ml^{-1}, then we used 2 aliquot of 1 - 5 ml each from each jar. After the quantification of the rotifer density, we transferred the test rotifer populations to fresh jars containing appropriate algal concentration. The experiments were discontinued after 25 days by which time rotifers in most jars began to decline. Based on the data collected, we derived rate of population increase (*r*) using the following formula: The *r* was obtained from a mean of 3-5 values during the exponential phase of the population growth from

each replicate following Dumont et al. (1995). We used two-way analysis of variance (ANOVA) and post hoc test (Tukey) to quantify the differences among treatments for the rate of population increase following standard statistical procedure (Sokal and Rohlf 2000). Following Kerfoot et al. (1985), we correlated the daily rate of population increase of each rotifer species with its population density under the tested food levels.

RESULTS & DISCUSSION

Population growth curves of *L. inermis* and *L. patella* grown on different concentrations of *C. vulgaris* are presented in Figure 1. Regardless of food concentration, *L. inermis* had long (about 15 days) lag phase, while L. *rhomboides* started to increase its population abundance from the 3rd day onwards, especially at higher food levels. At lower algal density, *L. inermis* performed better, while at intermediate concentrations ($0.2X10^6$ and $0.4X10^6$ cells ml^{-1}), *L. rhomboides* grew to higher densities. Regardless of the rotifer species, the highest algal density used here ($0.8X10^6$ cells ml^{-1}) resulted in reduced population growth compared to the lower algal concentrations. The rates of population increase (*r*) of *L. inermis* and *L. rhomboides* cultured under different algal concentrations varied from 0.18 to 0.31 day^{-1} (Table 1). Statistically, for both the rotifer species the *r* was significantly affected by the algal concentration ($p<0.05$, F-test, Table 2). When the daily rate of population increase was plotted as function of population density, a significant inverse relation was obtained for both the rotifer species (Fig. 2).

Figure captions

Figure 1. Population growth curves of *Lecane inermis* and *Lepadella rhomboides* cultured under different algal (*Chlorella vulgaris*) concentrations. Shown are the mean±standard errors based on 3 replicates.

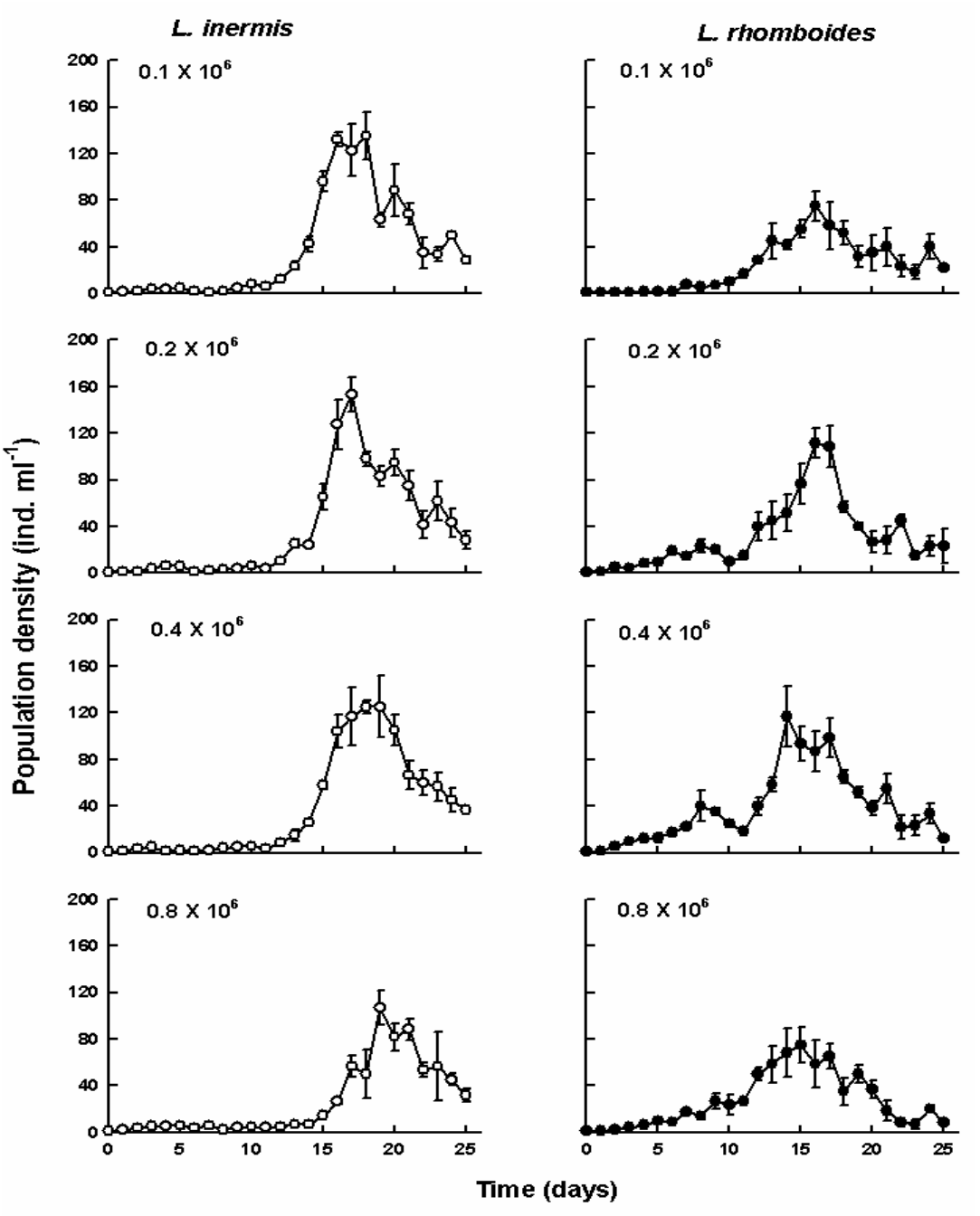


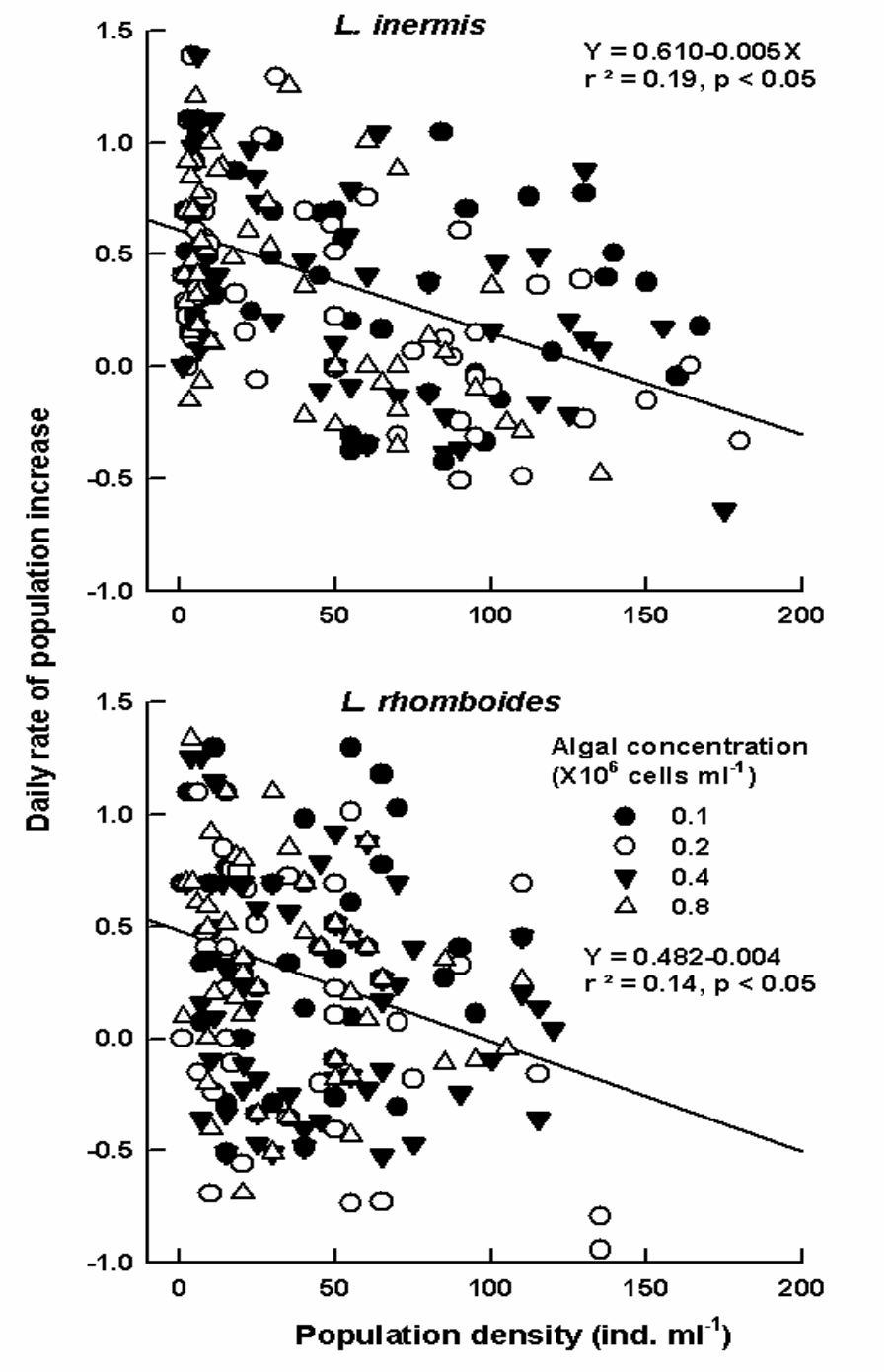
Table 1. Rate of population increase (d^{-1}) of *Lecane inermis* and *Lepadella rhomboides* grown under different concentrations of *Chlorella vulgaris*. For each species, data carrying similar alphabetic letter are not statistically significant ($p>0.05$, Tukey´s test).

Rotifer species	Algal food density ($X10^6$ cells ml^{-1})	Population growth rate (mean±standard error)
L. inermis	0.1	0.304 ± 0.005^{a}
	0.2	$0.277\pm0.011^{a,c}$
	0.4	0.243 ± 0.028^{a}
	0.8	0.176 ± 0.006^{b}
L. rhomboides	0.1	$0.263\pm0.012^{a,b}$
	0.2	$0.286\pm0.013^{a,b}$
	0.4	0.307 ± 0.005^{a}
	0.8	0.246 ± 0.010^{b}

Table 2. Results of the analysis of variance performed on the performed on the rate of population increase of *Lecane inermis* and *Lepadella rhomboides* grown under different concentrations of *Chlorella vulgaris*. DF: degrees of freedom; SS: sum of squares; MS: mean square; F: F-relation; ** = $p<0.01$; * = ($p<0.05$).

Source	df	SS	MS	F
		L. inermis		
Food level	3	0.027	0.009	12.07**
Error	8	0.006	0.001	
		L. rhomboides		
Food level	3	0.006	0.002	4.94*
Error	8	0.003	0.000	

Figure 2. Relation between the daily rate of population increase and the population density of *Lecane inermis* and *Lepadella rhomboides* cultured separately under different concentrations of algal food (*Chlorella vulgaris*). Plotted are the replicate data for each food level. Regression line was drawn using standard statistical procedure (Sokal and Rohlf 2000).



Of the two rotifer species used here, *Lecane inermis* was previously cultured axenically (Dougherty et al. 1961). However, a stable culture of *Lepadella rhomboides* has not been reported in literature, although some species of this such as *L. patella* have been successfully cultured on green algae (Nandini & Sarma, 2001). Food and feeding habits of many non-planktonic species is not well-known (Dumont 1977). It is generally believed that many *Lecane* and *Lepadell*a are not carnivorous and largely feed on algae, bacteria and detritus (Koste 1978). In the present work, both rotifer species utilized *Chlorella* and showed positive population growth. This implies that the non-planktonic herbivorous rotifers may also compete with planktonic ones due to the diet overlap (DeMott, 1989). We have not studied the gut content analysis of planktonic and non-planktonic rotifers from the field samples for evaluating diet overlap. However, there is enough evidence to support that non-planktonic rotifers such as *Lecane* and *Lepadella* are frequently encountered in planktonic samples and at times much higher abundances (> 1 ind. ml^{-1}) and the available food in water column is predominantly algae, it must be assumed that they feed on algal cells (Nandini et al. 2005).

The response of rotifers to increasing food levels is generally positive in that increased availability of food results in higher population abundances. This has been shown in great number of both planktonic and non-planktonic rotifers (Nandini et al. 2006). However, in some cases, certain rotifer species e.g., *B. variabilis* may not show increased population growth with increasing food levels (Sarma and Nandini 2001). Several factors such as species-specific incipient limiting levels, ability to filter in dense algal cultures, body size of the zooplankton, and adaptation to lower food density are responsible for this (Gliwicz 2003). In the present study, it was evident that both the tested rotifers did not show higher population abundances when cultured at $0.8X10^6$ cells ml. In addition, the rate of population increase of *L. inermis* decreased with increase in algal density of *Chlorella*, while for *L. rhomboides* the *r* under both the lowest and the highest algal concentrations was lower than that at intermediate levels. Our observations for *L. rhomboides* are similar to those on *L. patella* where higher algal concentrations actually reduced the population growth rates (Nandini and Sarma 2001). This suggests that the adaptation of the tested rotifer species to food levels may be different. In nature both the rotifer species are found in

eutrophic waters but with the relative abundances are different. Regardless of food concentration, the growth rates of both the rotifer species recorded in this study agree in general with those reported for other members of the same genera (*Lepadella*: 0.2-0.8 day^{-1}, Sarma and Nandini 2001, *Lecane*: 0.1-0.3 day^{-1}, Rico-Martinez et al. 2002). It is known that the daily growth rates of zooplankton decrease with increasing population density. This relation may be linear or curvilinear (Kerfoot et al. 1985). Increase in population density results in decreased availability of resources and hence decreased growth rates. This results in inverse relation between the population density and the daily growth rates, as also observed in this study.

In conclusion our study showed that the availability of food regulated the population abundances of both the rotifer species differently, i.e. at intermediate algal levels (0.2 $X10^6$ and 0.4$X10^6$ cells ml^{-1}), *L. rhomboides* showed higher growth rates, while *L. inermis* at 0.1$X10^6$ cells ml^{-1}. These may explain the differences in the relative abundances both the rotifer species in waterbodies with seasonally varying phytoplankton levels.

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