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Research Paper

# Population Growth of Zooplankton (Rotifers and Cladocerans) Fed *Chlorella vulgaris* and *Scenedesmus acutus* in Different Proportions

In the present work we tested the effect of *Chlorella vulgaris* and *Scenedesmus acutus* in different proportions on the population growth of *Brachionus calyciflorus*, *Brachionus patulus*, *Ceriodaphnia dubia,* and *Moina macrocopa*. In general, both rotifer species grew well on either type of algae. Regardless of algal mixture, *B. calyciflorus* had a shorter initial phase, while *B. patulus* needed more than a week to begin the exponential phase of growth. Both the rotifer species showed consistently better population growth with *Chlorella* than on *Scenedesmus*, or the mixture. At any given algal combination, *B. patulus* had higher peak values than *B. calyciflorus*. The rate of population increase (*r*) for both the rotifers varied from 0.18 to 0.48 $d^{-1}$ depending on the algal type and combination. Regardless of algal type and combination, *B. calyciflorus* had a much higher value of *r* than *B. patulus*. Both *C. dubia* and *M. macrocopa* grew on the algal types, whether offered separately or in mixture. Regardless of the treatment type, *C. dubia* needed a longer period (about 2 weeks) than *M. macrocopa* to reach peak abundances. Thus, our study did not support the view that *Scenedesmus* is consistently superior to *Chlorella* as a basic diet to the tested species of zooplankton.

**Populationswachstum von Zooplanktern (Rotatorien und Cladoceren) bei Ernährung mit *Chlorella vulgaris* und *Scenedesmus acutus* in unterschiedlichen Anteilen**

Untersucht wird der Einfluss von *Chlorella vulgaris* und *Scenedesmus acutus* in unterschiedlichen Anteilen in der Nahrung auf das Populationswachstum von *Brachionus calyciflorus*, *Brachionus patulus*, *Ceriodaphnia dubia* und *Moina macrocopa*. Generell wachsen beide Rotatorien-Arten gut mit jedem Algentyp, wobei *B. calyciflorus* eine kürzere Initialphase hat, während *B. patulus* länger als eine Woche bis zum Beginn der exponentiellen Wachstumsphase benötigt. Beide Rotatorien-Arten zeigen besseres Populationswachstum bei Ernährung mit *Chlorella* als mit *Scenedesmus* oder Algenmischungen. In jedem Fall erreicht *B. patulus* höhere Populationsdichten als *B. calyciflorus*. Die Wachstumsraten *r* liegen zwischen 0.18 $d^{-1}$ und 0.48 $d^{-1}$, wobei die Wachstumsrate für *B. calyciflorus* stets größer ist als jene von *B. patulus*. *C. dubia* benötigte in allen Versuchsreihen stets eine längere Zeit bis zum Erreichen der maximalen Populationsdichte als *M. macrocopa*. Die Untersuchungsergebnisse bestätigen somit nicht die Auffassung, dass *Scenedesmus* eine stets bessere Basisdiät für die untersuchten Zooplankton-Arten sei als *Chlorella*.

**Keywords:** Alga, Population Dynamics, *Brachionus*, *Moina*, *Ceriodaphnia*

**Schlagwörter:** Nahrung, Algenmischung, *Brachionus*, *Moina*, *Ceriodaphnia*

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## 1 Introduction

Freshwater zooplankton are mainly composed of protozoans, rotifers, and crustaceans (cladocerans and copepods) [1, 2]. In terms of numerical abundance, biomass, production, and regeneration of nutrients, rotifers and crustaceans dominate in freshwater bodies [3]. The density and diversity of rotifers, cladocerans, and copepods are influenced by a great variety of factors both abiotic and biotic. Among the biotic factors, phytoplankton density and diversity strongly affect zooplankton composition and abundance [4]. Just as strong seasonal changes occur in the zooplankton community, phytoplankton also show variations in the composition and abundance throughout the year [3]. Rotifers in general prefer algae in the size range of 0.2...5.0 μm while cladocerans feed on algae in the range of 2...25 μm. The size structure and dominance of certain algae therefore controls, to a large extent, the zooplankton community density and diversity.

While studies related to food selectivity by zooplankton through short term feeding experiments give information on the type of algae preferred by a given rotifer or cladoceran, this does not necessarily reflect a positive population growth on an exclusive diet of that algae [5, 6]. Similarly, data obtained from zooplankton population growth studies conducted separately using one or two food types could be different when mixed food types are used. For example, some studies have shown that when a mixture of edible algae and toxic cyanobacteria was offered as food for the rotifer *Brachionus calyciflorus*, the population growth was higher than when fed either type of algae separately [7].

For zooplankton feeding and population growth studies, two genera of green algae are widely used, *Chlorella* and *Scenedesmus* [8–10]; a few others such as *Chlamydomonas* [11], *Selenastrum* [12], and *Ankistrodesmus* [13] have also been used. There has not been much agreement among different workers on the nutritional adequacy of *Chlorella* or *Scenedesmus* for zooplankton studies. For example, most species of rotifers of the genus *Brachionus* could be grown successfully using *Chlorella*, while *Scenedesmus* apparently is not as good as the former, especially when population growth rates are considered [14, 15]. However, for cladocerans, *Scenedesmus* seems appropriate, although *Chlorella* also promotes positive growth in several genera [16].

It is doubtful if any species of zooplankton would feed exclusively on a single algal species consistently [5]. Population growth of zooplankton could also be different on a single alga or a mixture. A comparative study on the population growth of zooplankton employing *Chlorella* and *Scenedesmus* is rare, although results in most studies are discussed from the point of view to the nutritional adequacy of algal species [11]. The purpose of the present work was therefore to test the effect of two species of algae (*Chlorella vulgaris* and *Scenedesmus acutus*) in different proportions on the population growth of four zooplankton species (two rotifers: *Brachionus calyciflorus* and *Brachionus patulus* and two cladocerans: *Ceriodaphnia dubia* and *Moina macrocopa*).

## 2 Materials and methods

In our study we used the rotifers *Brachionus calyciflorus* and *Brachionus patulus,* and the cladocerans *Ceriodaphnia dubia* and *Moina macrocopa*. These were originally isolated from Lake Xochimilco (Mexico city), Santa Elena Dam (State of Mexico) and the waterbody Manuel Avila Camacho (State of Puebla), respectively. All species were mass-cultured separately using *Chlorella vulgaris*, *Scenedesmus acutus,* or their mixture. The culture conditions in the laboratory were: temperature (24 ± 2) °C, pH 7.5, continuous fluorescent illumination and change of culture media every other day. Certified strains of *C. vulgaris* (CL-V-3, CICESE, Ensenada, Baja California, Mexico) and *Scenedesmus acutus* (*Scenedesmus acutus* f. *alternans* Hortobagyi (No. 72) from the University of Texas) were separately cultured using 2 L transparent bottles using defined medium (Bold's Basal medium [17]). Algae harvested during the log-phase were centrifuged and resuspended in distilled water. The density of the stock algal concentrate of each species was estimated using a haemocytometer. For zooplankton mass cultures and for experiments, we used reconstituted moderately hard water (EPA medium) [18]. The EPA medium was prepared by dissolving 96 mg $NaHCO_3$, 60 mg $CaSO_4$, 60 mg $MgSO_4$ and 4 mg KCl per litre of distilled water.

In order to provide an equal biomass of both algae, we determined the dry weights of *C. vulgaris* and *S. acutus* using a Cahn electrobalance following standard procedures [19]. On the basis of dry weight, $1 \cdot 10^6$ cells of *C. vulgaris* were equal to $0.546 \cdot 10^6$ cells $mL^{-1}$ of *S. acutus*. For population growth experiments we used 75 mL capacity transparent jars, each with 50 mL of medium with the specified algal type, proportion, and zooplankton species. We used one food level (*C. vulgaris* $1 \cdot 10^6$ cells $mL^{-1}$ of *Chlorella* or its equivalent ($0.546 \cdot 10^6$ cells $mL^{-1}$) for *S. acutus* but at five different algal mixtures: 100% *C. vulgaris*, 75% *C. vulgaris* + 25% *S. acutus*, 50% *C. vulgaris* + 50% *S. acutus*, 25% *C. vulgaris* + 75% *S. acutus,* and 100% *S. acutus*. The inoculation density of each of the four zooplankton species was one ind. $mL^{-1}$. For each species of zooplankton we kept four replicates. Thus, for each zooplankton species, we used a total of 20 jars. All experiments were conducted at 25 °C under continuous but diffused fluorescent illumination. Following initiation of the experiments, every day we estimated the density of zooplankton using either total count (cladocerans) or 3 aliquots of 1 to 5 mL (rotifers). Following estimation of their density, every day the zooplankton was transferred to fresh jars containing the appropriate algal combination. Depending on the zooplankton species, the experiments were continued for 15 (for *B. calyciflorus* and *M. macrocopa*) to 21 days (for *B. patulus* and *C. dubia*).

Based on the data collected, we derived the rate of population growth per day ($r$) using the exponential equation [20]: $r = (\ln N_t - \ln N_0)/t$, where $N_0$ and $N_t$ are the initial density and that at time $t$ in days. For each treatment we obtained 4 to 5 values and their mean was expressed as the growth rate per replicate.

We used one-way analysis of variance (ANOVA) [21] for statistically evaluating the differences among the food types and combinations on the rate of population increase and peak population abundances of the tested zooplankton species.

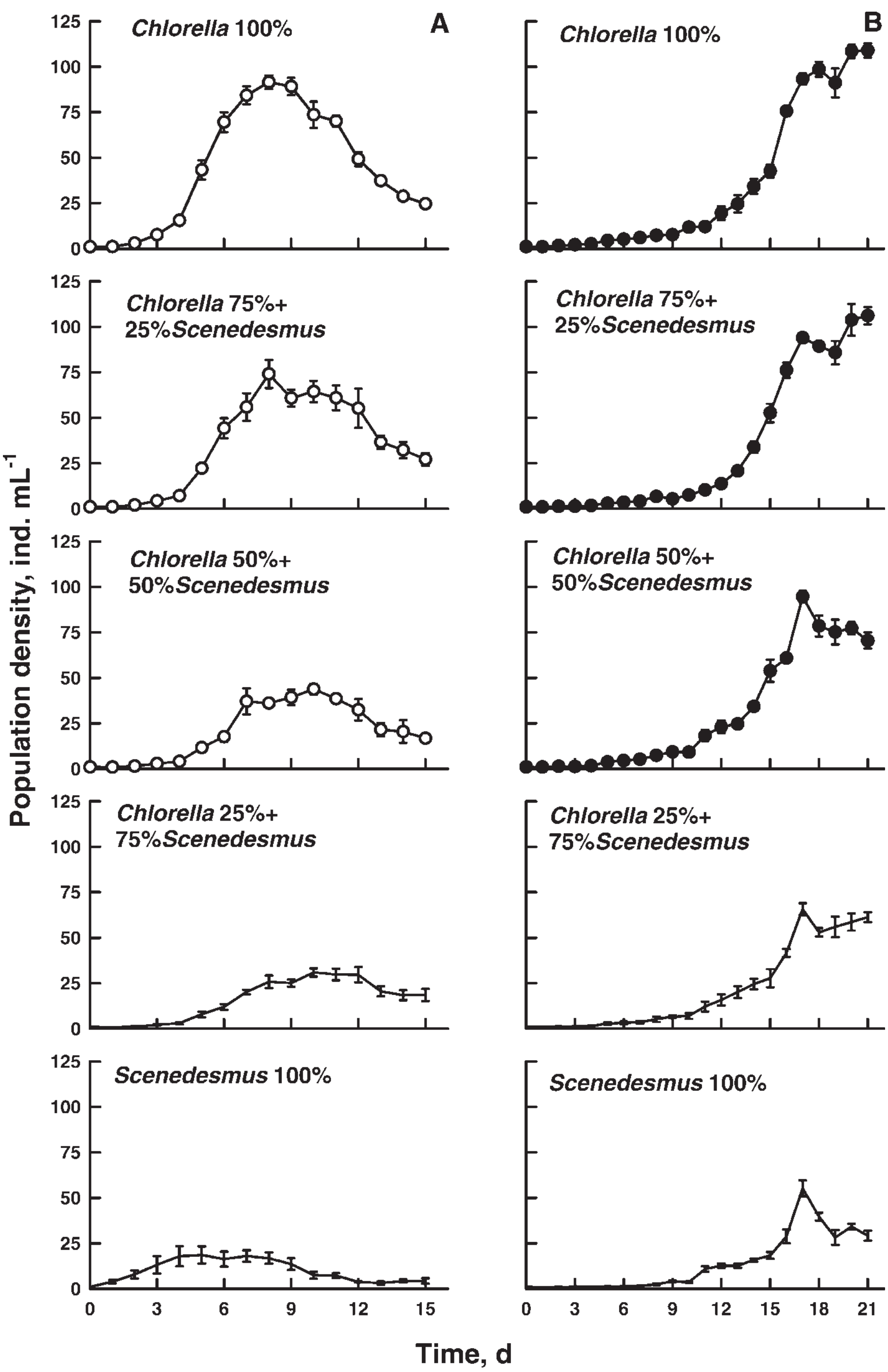


**Fig. 1:** Population growth curves of the rotifers *Brachionus calyciflorus* (A) and *B. patulus* (B) grown on *Chlorella*, *Scenedesmus* separately or their mixture in different proportions. For each treatment, shown are the daily mean population densities ± standard errors based on four replicates.

Kurven des Populationswachstums für die Rotatorien *Brachionus calyciflorus* (A) und *B. patulus* (B) bei Ernährung mit *Chlorella*, *Scenedesmus* einzeln oder in unterschiedlichen Anteilen. Dargestellt sind mittlere Populationsdichte ± Standardabweichung aus vier Wiederholungen.

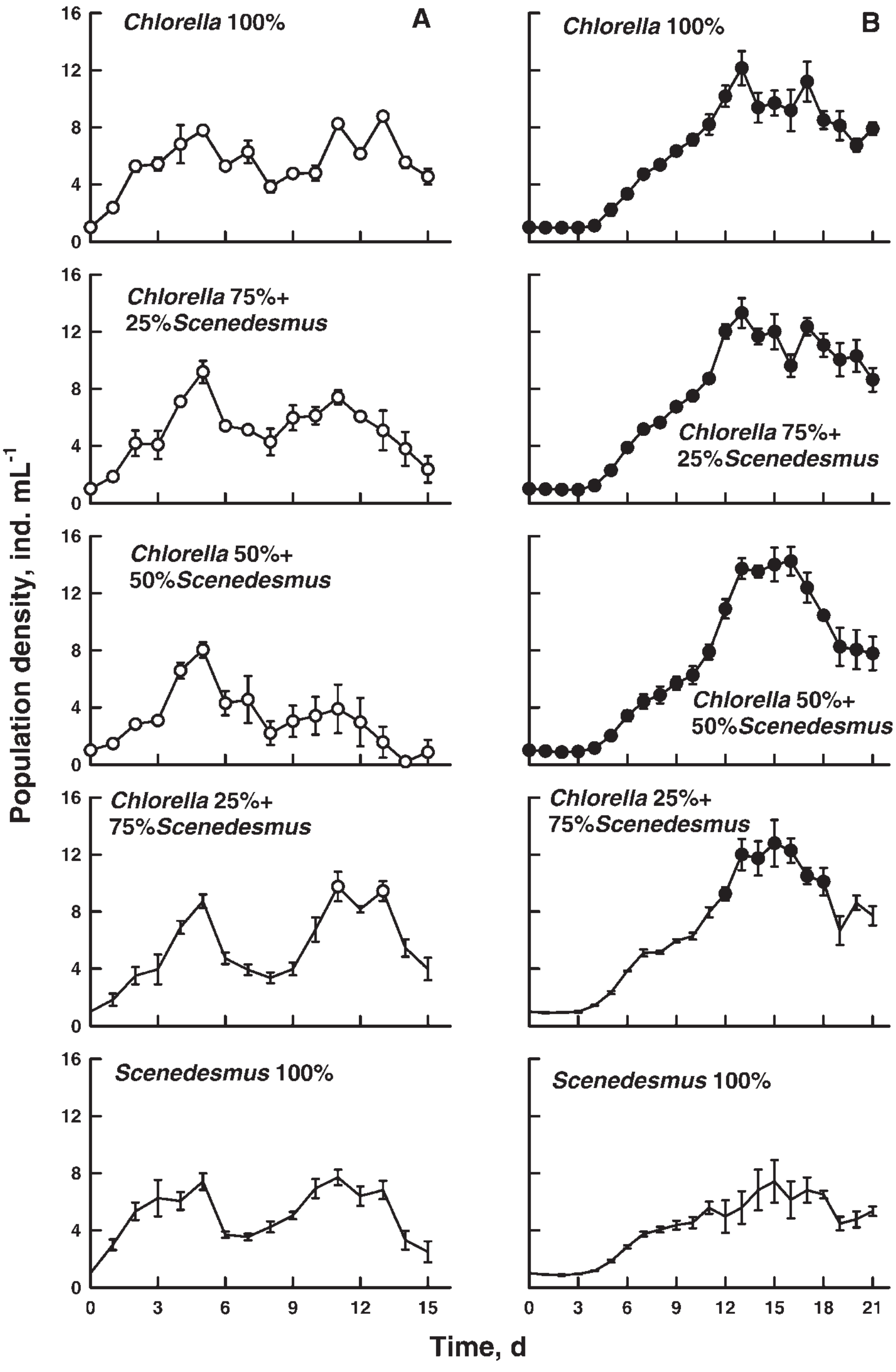


**Fig. 2:** Population growth curves of the cladocerans *Moina macrocopa* (A) and *Ceriodaphnia dubia* (B) grown on *Chlorella*, *Scenedesmus* separately or their mixture in different proportions. For each treatment, shown are the daily mean population densities ± standard errors based on four replicates.

Kurven des Populationswachstums für die Cladoceren *Moina macrocopa* (A) und *Ceriodaphnia dubia* (B) bei Ernährung mit *Chlorella*, *Scenedesmus* einzeln oder in unterschiedlichen Anteilen. Dargestellt sind mittlere Populationsdichte ± Standardabweichung aus vier Wiederholungen.

## 3 Results

Population growth curves in relation to the algal species *Chlorella* and *Scenedesmus*, separately and mixed, are presented in Figure 1 for the rotifers *B. calyciflorus* and *B. patulus* and in Figure 2 for the cladocerans *M. macrocopa* and *C. dubia*. In general, both rotifer species grew well on either type of algae. Regardless of algal mixture, *B. calyciflorus* had a shorter initial phase while, *B. patulus* needed more than a week to begin the exponential phase of growth. Both the rotifer species showed consistently better population growth with *Chlorella* than on *Scenedesmus*, or the mixture. An increase in the

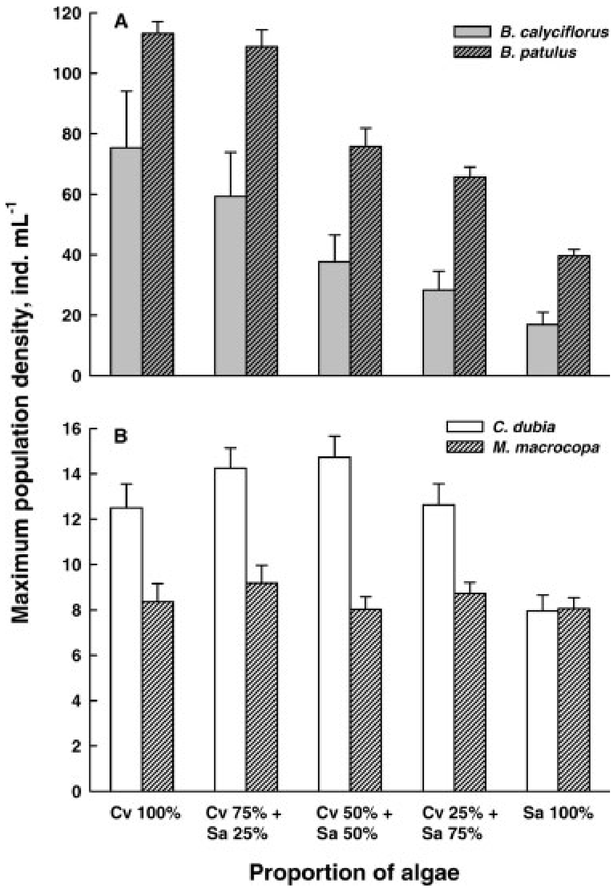


**Fig. 3:** Peak population densities of the zooplankton (rotifers: A, cladocerans: B) grown on *Chlorella* (Cv), *Scenedesmus* (Sa) separately or their mixture in different proportions. Shown are the mean densities ± standard errors based on four replicates.

Maximale Populationsdichten des Zooplanktons (Rotatorien: A, Cladoceren: B) bei Ernährung mit *Chlorella* (Cv), *Scenedesmus* (Sa) einzeln oder in unterschiedlichen Anteilen. Dargestellt sind mittlere Populationsdichte ± Standardabweichung aus vier Wiederholungen.

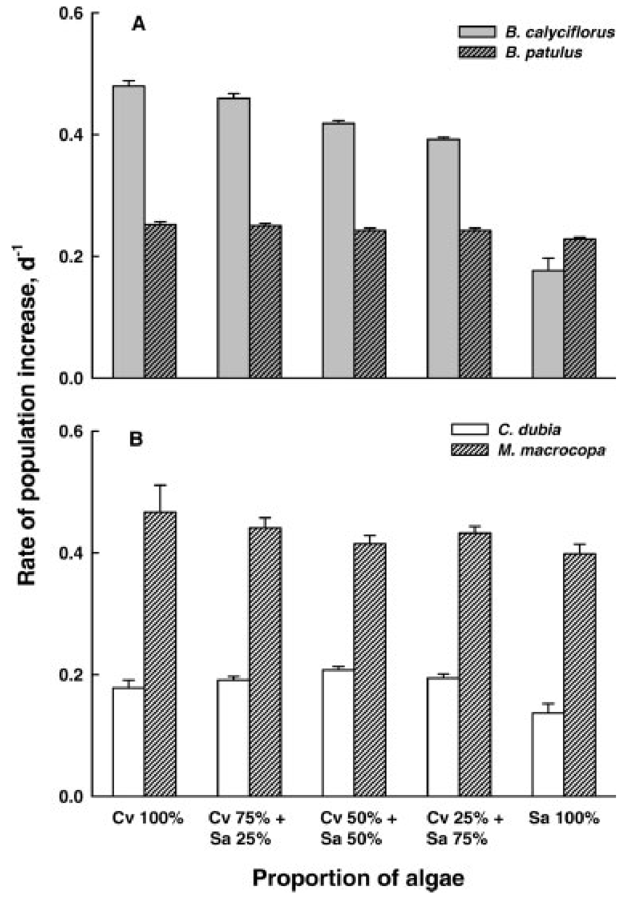


**Fig. 4:** Rate of population increase per day of the zooplankton (rotifers: A, cladocerans: B) grown on *Chlorella* (Cv), *Scenedesmus* (Sa) separately or their mixture in different proportions. Shown are the mean ± standard errors based on four replicates.

Raten des täglichen Populationswachstums des Zooplanktons (Rotatorien: A, Cladoceren: B) bei Ernährung mit *Chlorella* (Cv), *Scenedesmus* (Sa) einzeln oder in unterschiedlichen Anteilen. Dargestellt sind mittlere Wachstumsrate ± Standardabweichung aus vier Wiederholungen.

proportion of *Scenedesmus* with *Chlorella*, resulted in reduced peak population abundances of *B. calyciflorus* but this was less pronounced for *B. patulus* (Fig. 1A, B). However at any given algal combination, *B. patulus* had higher peak values than *B. calyciflorus*. Thus the highest peak population density of *B. patulus* observed in this study was 115 ind. $mL^{-1}$ (Fig. 3). The rate of population increase (*r*) for both the rotifers varied from 0.18 $d^{-1}$ to 0.48 $d^{-1}$ depending on the algal type and combination. Regardless of algal type and combination, *B. calyciflorus* had much higher *r*-values than *B. patulus* (Fig. 4A). For the cladocerans, both *Ceriodaphnia dubia* and *Moina macrocopa* grew on the algal types, whether offered separately or in mixture (Fig. 4B).

Regardless of the treatment type, *C. dubia* needed a longer period (about 2 weeks) to reach peak abundances while *M. macrocopa* showed the first peak in less than one week. Independent of algal type and combination, *Ceriodaphnia* had

**Table 1:** Results of analysis of variance conducted on the peak population density and the rate of population increase $r$ (in $d^{-1}$) of the tested zooplankton species in relation to algal food combination (*Chlorella* and *Scenedesmus* separately and in mixed forms). *DF:* degrees of freedom, *SS:* sum of squares, *MS:* mean square, *F:* *F*-ratio; $^{***}p < 0.001$, $^{**}p < 0.01$, ns: $p > 0.05$.

Ergebnisse der Varianzanalyse, durchgeführt für die maximale Populationsdichte und die Wachstumsrate $r$ (in $d^{-1}$) der untersuchten Zooplankter in Bezug auf die Nahrungszusammensetzung der Algen (*Chlorella* und *Scenedesmus* allein und in unterschiedlichen Kombinationen).

| Source of variation | *DF* | *SS* | *MS* | *F* |
|---|---|---|---|---|
| Peak population density | | | | |
| *B. calyciflorus* | | | | |
| Algal food type | 4 | 14 600 | 3650.19 | 61.19*** |
| Error | 15 | 894 | 59.66 | |
| *B. patulus* | | | | |
| Algal food type | 4 | 10 365 | 2591.42 | 40.00*** |
| Error | 15 | 971 | 64.79 | |
| *C. dubia* | | | | |
| Algal food type | 4 | 120.403 | 30.10 | 8.60*** |
| Error | 15 | 52.474 | 3.50 | |
| *M. macrocopa* | | | | |
| Algal food type | 4 | 18.947 | 4.74 | $2.73^{ns}$ |
| Error | 15 | 25.994 | 1.73 | |
| Rate of population increase per day | | | | |
| *B. calyciflorus* | | | | |
| Algal food type | 4 | 0.236 | 0.06 | 128.69*** |
| Error | 15 | 0.007 | 0.00 | |
| *B. patulus* | | | | |
| Algal food type | 4 | 0.002 | 0.00 | 7.54*** |
| Error | 15 | 0.001 | 0.00 | |
| *C. dubia* | | | | |
| Algal food type | 4 | 0.012 | 0.00 | 7.23** |
| Error | 15 | 0.006 | 0.00 | |
| *M. macrocopa* | | | | |
| Algal food type | 4 | 0.011 | 0.00 | $1.23^{ns}$ |
| Error | 15 | 0.033 | 0.00 | |

higher peak population densities than *M. macrocopa*. However, *M. macrocopa* had higher population growth rates than *C. dubia* (Fig. 3B).

Statistically, both the peak population abundance and the rate of population increase (Table1) of *B. calyciflorus*, *B. patulus,* and *C. dubia* were significantly affected by the algal type and combination ($p < 0.001$, *F*-test). However, these variables were not significantly affected by algal type or combination for *M. macrocopa* ($p > 0.05$).

## 4 Discussion

Among the different groups of phytoplankton, green algal species especially *Chlorella* and *Scenedesmus* are widely used for growing zooplankton both under laboratory and field conditions. While there is little evidence that *Chlorella* could develop anti-grazer morphological changes, some species of *Scenedesmus* such as *S. quadricauda* could develop structures to deter grazing by zooplankton [22]. However, in our experiments we did not observe such grazer-induced morphological changes in *Scenedesmus,* because a) the medium in which experiments were conducted lacks essential nutrients for phytoplankton to develop, b) fresh algae were provided everyday, c) *S. acutus* is not known to develop strong anti-grazer defenses [23], d) the zooplankton continuously feed on the algal cells allowing little growth, if any at all. Thus, the low growth rates of zooplankton, especially rotifers on *Scenedesmus* or the mixture containing higher proportions of it, could be due to factors other than those interfering with induced defense mechanisms by the algae [9].

Algal nutritional quality and digestibility of the cells by the rotifers and cladocerans could be conceived as the possible factors influencing the growth patterns of the tested zooplankton taxa [15, 24]. Since both the algal species were cultured using identical nutrients under similar conditions, there may not be strong differences in the nutritional quality as a result of the culture conditions. However, if there are differences inherent in the nutritional quality including protein or fatty acid content of the algal species, they could be attributed to the differences on both the peak population density and the rate of population increase of the tested zooplankton. Although we did not measure the nutritional quality of algal species used here, published data do not support that great differences, especially in terms of the major elemental composition, exists between them [25]. The possibility of lower filtration rates by both cladocerans and rotifers on the two algal species could be one of the reasons why the latter showed lower growth rates. This, coupled with low digestibility and/or assimilation of the alga *S. acutus* by the rotifers, could have caused lower growth rates of *B. calyciflorus* and *B. patulus* compared to cladocerans. In the present study though we did not measure the assimilation efficiency of the zooplankton, there is some indication that at least for rotifers fed *Chlorella*, it could be as high as 80% [26, 27].

The response of zooplankton to mixed diet is of some interest not only from aquatic ecologists [28] but also from aquaculturists [29]. For example, since the first use of baker's yeast as food, several attempts have been made to combine it with green algae for both freshwater and saline rotifer species [30, 31]. Numerous studies have been also made using two more types of marine algal species on the population growth of marine or saline water rotifer species such as *Brachionus plicatilis* and *Synchaeta cecilia* [32, 33]. Arévalo-Stevenson et al. [34] have also shown that *B. calyciflorus* shows higher growth rates on wastes from the tortilla industry with *Chlorella* than on the latter alone. In most of these studies the usual result is that mixed food types yield higher rotifer growths than when offered separately. In the present study, however, in most treatments, mixed *Chlorella* + *Scenedesmus* yielded lower growth rates and peak abundances. This is especially the case for *B. calyciflorus*. The lack of statistically significant influence (for both peak population density and *r*) of the mixed algal types for *M. macrocopa* suggest that this could be grown using either type of algae.

Food and feeding habits of most rotifers and cladocerans have focused on the importance of green algal components in their diet especially in live form [25, 35]. Studies conducted so far, for both short-term growth or mass cultures, have shown that peak abundances and growth rates as some of the important variables often considered to understand the role of a given algal diet on the life of zooplankton. When grown on an edible algal type, generally smaller-bodied zooplankton reach higher abundances at a given food level. Thus among the four zooplankton species used here, *B. patulus* is the smallest while *M. macrocopa* is the largest. These differences were thus reflected in their peak abundances. Regardless of algal combination, the peak abundances of rotifers and cladocerans are within the range (50...200 ind. $mL^{-1}$ for *Brachionus* [28], and 5...50 ind. $mL^{-1}$ for cladocerans [16] observed in previous studies.

At population level, many life history traits such as age at first reproduction, clutch size and the lifespan sum up so as to maximize the rate of population increase of a zooplankton species under given environmental conditions [36]. While both cladocerans and copepods have large clutch size to achieve higher *r*, rotifers in spite of their low clutch size, have much reduced age at first reproduction and inter-clutch period so that their growth rates are comparable or even higher than most crustaceans [37]. Except for a few species of *Daphnia* and *Moina*, most cladocerans have population growth rates lower than 0.5 $d^{-1}$, while most genera of rotifers have growth rates nearly twice this under comparable conditions. In the present study, the *r* for brachionid rotifers varied from 0.18 $d^{-1}$ to 0.48 $d^{-1}$ depending on the food type and combination, while those for cladocerans varied from 0.14 $d^{-1}$ to 0.47 $d^{-1}$, a range observed for most species of zooplankton [10, 28]. When mixed algal types were used for both rotifers and cladocerans the values of *r* were significantly influenced suggesting that this is a sensitive variable. There are several different types of studies, e.g., competition [38], predation [39], and ecotoxicology [40] which have also indicated that the population growth rate *r* is a sensitive variable affected by both biotic and abiotic factors.

In conclusion our study does not support the view that *Scenedesmus* is consistently superior to *Chlorella* as a basic diet for all cladocerans. For the tested rotifers, *Chlorella* promoted better peak abundances and population growth rates. In the case of cladocerans *C. dubia* showed trends similar to those of rotifers, while *M. macrocopa* did not differ significantly of its growth rates or peak abundances when grown on either algal type separately or in different combinations. The fact that *Scenedesmus* was extensively used for tests on the genus *Daphnia* [41], which is often twice the size of *Moina* or *Ceriodaphnia* led to this hypothesis that *Scenedesmus* is the ideal algae for all cladocerans. This may not be true in tropical water bodies where the size structure of the cladoceran community is significantly smaller than in temperate lakes and daphniids are conspicuous by their absence [42].

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