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Research Article

Effect of *Brachionus rubens* on the growth characteristics of various species of microalgae

Reda A.I. Abou-Shanab [1], Manjinder Singh *, Anangelica Rivera-Cruz, Grace Power, Thomas Bagby-Moon, Keshav Das

Biorefining and Carbon Cycling Program, College of Engineering, The University of Georgia, Athens, GA 30602, USA

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ABSTRACT

Background: Cultivation of algae for conversion to biofuels has gained global interest. Outdoor raceway cultivation is preferred because of its lower capital and operating costs. A major disadvantage of outdoor cultivation is susceptibility of algal crops to attack by predatory rotifers. In order to quantify the impact of rotifer attack on different species of algae, we evaluated the growth of eleven microalgal species over a 21-d period after being infected by the predatory rotifer *Brachionus rubens*.
Results: Of the eleven species, *Chlorella sorokiniana* was the most susceptible with rapid decline in algal growth concomitant with increase in rotifer population growth (3.82/d). In contrast, *Synechococcus elongatus* and *Scenedesmus dimorphus* were both resistant to the rotifer and suppressed rotifer growth (-0.06/d). An index of algal species susceptibility to be consumed by the rotifer was generated with *C. sorokiniana* as the baseline (index = 1.000) indicating most susceptible among species tested. Other species' susceptibilities are indicated in parenthesis as follows: *Monoraphidium* spp. (0.997), *Chlamydomonas globosa* (0.827), *Botryococcus braunii* (0.740), *Chlorella minutissima* (0.570), *Chlamydomonas augustae* (0.530), *Chlamydomonas yellowstonensis* (0.500), *Scenedesmus bijuga* (0.420), and *Haematococcus pluvialis* (0.360). Two species, namely, *S. dimorphus* and *S. elongatus* were unique in that they exhibited an ability to suppress the growth of the rotifer as indicated by the decline in rotifer populations in their presence.
Conclusions: Variations in susceptibility of algal species to rotifer predation could be a result of their individual morphology, cell walls structure, or the biochemical composition of individual species.

1. Introduction

The world has been confronted with an energy and water crisis associated with the depletion of fossil fuels and freshwater, coupled with an atmospheric accumulation of greenhouse gases that is connected to rising temperatures and global warming [1]. Carbon neutral sources of energy and sustainable water management are required to address these challenges. Microalgae have attracted a great deal of attention as a biofuel feedstock due to their high oil yield (5000–100,000 L/ha/y) and their ability to rapidly convert carbon dioxide (CO_2) into hydrocarbon biomass consisting of proteins, amino acids, lipids, polysaccharides, carotenoids and other biologically-active molecules [2,3]. They are also capable of growing under diverse environmental conditions [4,5]. In addition, microalgae are important biological resources that have a wide range of biotechnological applications [6].

Commercial production of microalgal biomass at low cost through large-scale cultivation is a prerequisite for realizing the potential of microalgae. Presently, large-scale production of microalgal biomass uses suspended cultures in outdoor raceways and photobioreactors [7,8]. Outdoor open raceways are estimated to be an order of magnitude less expensive than closed systems, however their overall productivity is typically lower than in closed systems. In addition, open systems suffer from many problems, most important of which is that cultures are not axenic and contaminants may out-compete the desired algal species. Growing algae in an open raceway system has its drawbacks, but because of the significantly lower cost, it is still considered the most practical method of large-scale algal cultivation [9]. Sustained open raceway cultivation has been successful only for a limited number of organisms like *Spirulina* and *Dunaliella* that thrive in extreme conditions such as high pH or high salinity [3], which discourage contamination by predators. With expansion of microalgal

* Corresponding author.
E-mail address: singhm@engr.uga.edu (M. Singh).
[1] Present address: Environmental Biotechnology Dept., City of Scientific research and Technology Applications, New Borg El Arab City, Alexandria 21934, Egypt.
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cultivation to additional species, biomass production is increasingly challenged by biological contaminations [10] of which rotifers are among the most common and most harmful of algal grazers.

Grazing of algal cells by rotifers is an especially serious threat in large-scale microalgal biomass production because it can lead to rapid crash of healthy cultures [11]. Rotifers can survive in extreme environments and reproduce rapidly owing to their sexual and asexual reproduction capabilities [12]. The rotifer (*Brachionus calyciflorus*) can graze on *Chlamydomonas reinhardtii* at a feeding rate greater than 500 cells/h [13]. The high grazing capacity of rotifers leads to a rapid rise of rotifer density, resulting in the inevitable clearing of an algal suspension in a few days [14]. The grazing activity of rotifers also leads to the over-growth of non-target microalgae and the development of bacteria-algae-flocs [15]. Consequently, large-scale cultivation of microalgae usually fails due to rotifer contamination. Although grazing is a widespread problem in the algal biotechnology field, to date relatively little has been published on this topic [16]. In the present study, we use a digital flow cytometer (FlowCAM) to measure the changes in cell density (number of cell/mL), cell size and shape of microalgal species in the culture medium, and quantify the effects of the presence of rotifers on growth characteristics (such as cell densities and biomass productivity) of eleven different microalgal species (green and cyanobacteria).

2. Materials and methods

2.1. Algal strains and growth conditions

Ten green algae and a cyanobacteria species were selected based on their different sizes and shapes for use in the current study (Table 1). The microalgal strains were individually inoculated in 250 mL Erlenmeyer flasks containing 100 mL BG11 growth medium [17] at 10% concentration ($V_{inoculum}/V_{media}$). The pH of the BG11 culture medium was adjusted to 7.5 ± 0.2 before microalgal inoculation and subsequently kept in a temperature controlled growth chamber with agitation at 100 rpm, at 25 ± 1°C under 100 ± 10 μmol/m^2/s light intensity provided by cool white fluorescent T-8 bulbs (6500 K) operated on a 12:12 h light–dark regime for 3 weeks. Specific algal species used include *Botryococcus braunii* UTEX 572 (Bb), *Chlamydomonas augustae* UTEX SNO134 (Ca), *Chlamydomonas globosa* UTEX 2982 (Cg), *Chlorella minutissima* UTEX 2981 (Cm), *Chlorella sorokiniana* UTEX 2805 (Cs), *Chlamydomonas yellowstonensis* UTEX SNO155 (Cy), *Haematococcus pluvialis* (Hp), *Monoraphidium* spp. (Mo), *Scenedesmus bijuga* UTEX 2980 (Sb), *Synechococcus elongatus* (Se), and *Scenedesmus dimorphus* (Sd). The microalgal strains *C. augustae* and *C. yellowstonensis* were known to be cold tolerant (snow algae) and were therefore incubated at 15°C under the same light conditions. However, due to unavailability of an agitation system inside the cold chamber, these strains were incubated under static conditions.

2.2. Rotifer culture conditions and inoculum preparation

The rotifer (*Brachionus rubens*) was originally isolated from an outdoor freshwater raceway at the Bioconversion Laboratory of the University of Georgia, USA. Mass culture of *B. rubens* was grown and maintained under laboratory conditions using *C. sorokiniana* (UTEX 2805) as the food source. The conditions that promoted sustained dense cultures of rotifers were an algal cell density of ~2×10^6 cells/mL and a stable temperature of 25 ± 1°C. For rotifer inoculum preparation, rotifers were separated from *C. sorokiniana* by filtering the algal-rotifer suspension through a 40-μm nylon mesh (Fisher brand), which retained rotifers and allowed algae to pass through. Rotifer culture was washed four times with EPA medium [18], which was prepared by adding 0.9 g of $NaHCO_3$, 0.6 g of $CaSO_4$, 0.6 g of $MgSO_4$ and 0.04 g of KCl per liter of distilled water, and then filtered through the nylon mesh (40-μm) to wash out remaining algal cells. Purities of the rotifer cultures were ensured by repeated washing and regular observation under a microscope. Rotifer inoculum was prepared in the EPA medium by appropriate dilutions so as to obtain a final rotifer density of 20 rotifer/mL in the 100-mL algal culture after inoculation. Incubation was carried out for 21 d under conditions mentioned earlier.

2.3. Cell counting and characterization

To measure growth rate, algal cell densities (cells/mL) were measured using an imaging cytometer FC300 FlowCAM (Fluid Imaging Technologies, Scarborough, ME, USA). Samples were pumped at 0.5 mL/min through the FlowCAM imaging chamber where image-data were acquired by a 20× magnification lens. Images were taken in auto-trigger mode and using image analysis, the average cell size, diameter, length, width and other parameters were determined. The specific growth rate ((SGR), cells/mL/d) was calculated according to Guillard [19] [$SGR = 3.322 \times \log (N_1 / N_0) / t$, where, N_0 is the initial cell number and N_1 is the final cell number at time t in days].

Algal biomass (g/L) was determined by filtering 25 mL of algal culture through a pre-weighed Whatman GF/C filter (4.7 cm diameter; 1.2 μm pore size). The filter was washed with 10 mL of 0.65 M ammonium formate solution to remove excess salts and dried overnight at 60°C in a forced draft oven. Dried filter with biomass was cooled in a desiccator and weighed again to determine the final dry weight. Measured values of algal biomass dry cell density (g/L) were related to the measured algal cell counts (cell/mL) using a linear regression. Data analyses were conducted to obtain the relationships for each algal species and are given by equations shown in Table 2.

B. rubens density was estimated from a 1-mL sample, after fixing with 5% formalin, using a Sedgewick-Rafter counting chamber at 10× magnification. To calculate density of rotifers, averages of 5 to 10 counts were made for each sample and results expressed as numbers

Table 1
Morphological characteristics of microalgal and rotifer species used in the study.

Organism	Morphological characteristics[a] (μm)		
	Length	Width	Diameter (ESD)
B. braunii UTEX572 (Bb)	15.1 ± 3.5	10.8 ± 1.9	11.7 ± 1.8
C. augustae UTEXSNO134 (Ca)	12.8 ± 3.8	9.0 ± 3.5	11.3 ± 3.3
C. globosa UTEX2982 (Cg)	8.5 ± 0.9	6.9 ± 0.9	7.8 ± 0.9
C. minutissima UTEX2981 (Cm)	7.0 ± 0.6	6.1 ± 0.6	6.5 ± 0.5
C. sorokiniana UTEX 2805 (Cs)	6.79 ± 0.8	5.3 ± 0.61	6.1 ± 0.6
C. yellowstonensis UTEXSNO155 (Cy)	12.8 ± 3.4	10.1 ± 3.1	11.6 ± 3.1
H. pluvialis (Hp)	22.5 ± 2.8	19.9 ± 2.6	21.3 ± 2.6
Monoraphidium spp. (Mo)	7.7 ± 0.9	5.0 ± 0.9	6.5 ± 1.7
S. bijuga UTEX2980 (Sb)	14.5 ± 1.6	7.4 ± 1.0	12.7 ± 1.4
S. elongatus (Se)	11.07 ± 2.1	1.4 ± 0.6	6.3 ± 0.4
S. dimorphus (Sd)	21.7 ± 3.6	14.0 ± 3.3	18.5 ± 3.3
B. rubens (R)	112.1 ± 5.1	62.5 ± 21.1	90.8 ± 7.0

[a] FlowCAM parameters; ESD = equivalent spherical diameter.

Table 2
Equations used for dry weight calculation (g/L) of different microalgae species.

Microalgae species	Equation	R^2
B. braunii (Bb)	0.0038 * Cell count + 0.0336	0.9969
C. augustae (Ca)	0.0106 * Cell count - 0.168	1.0000
C. globosa (Cg)	0.0018 * Cell count - 0.2869	0.9768
C. minutissima (Cm)	0.0018 * Cell count - 0.0053	0.9998
C. sorokiniana (Cs)	0.0014 * Cell count - 0.0384	0.9988
C. yellowstonensis (Cy)	0.0031 * Cell count + 0.0026	0.9997
H. pluvialis (Hp)	0.0171 * Cell count + 0.0225	0.9999
Monoraphydium spp. (Mo)	0.0004 * Cell count - 0.1084	0.9988
S. bijuga (Sb)	0.0013 * Cell count + 0.2387	0.9628
S. elongatus (Se)	0.0003 * Cell count + 0.5841	1.0000
S. dimorphus (Sd)	0.0013 * Cell count + 0.9587	0.9623

of individuals/mL of sample. Population growth rate (r) for each replicate of the treatment was calculated using the exponential formula: $r = (1n\ N_t - 1n\ N_0) / T$, where, N_0 is the initial number of rotifers and N_t is the final number at time t days [20]. The average r was calculated from three replicates in each case. Rotifer length, width and diameter were determined using FlowCAM at the 10× magnification and FlowCAM image processing analytics.

2.4. Statistical analysis

Since our primary goal was to quantify the impact of the presence of *B. rubens* on the growth characteristics of various algae, all studies were performed in triplicate and statistical comparison of means was done using a t-test for unpaired data on specific growth rate of each algal species in the presence and absence of *B. rubens*. The significance level of the test was set at $\alpha = 0.05$ and it was concluded that there was a difference in means between treatment (algae + rotifer) and control (algae alone) when the p-value was less than the significance level α. We also ran the same unpaired t-test at a second significance level of $\alpha = 0.01$ for further verification. A second analysis was conducted to evaluate the growth rate of *B. rubens* in the presence of each algal species, which was done using a one-way ANOVA to determine if there were differences. The significance level of the test was set at $\alpha = 0.05$. Once differences were seen, we conducted a Tukey-HSD multiple comparison to identify which treatments were significantly different. All statistical analyses were conducted using JMP Pro v11 (SAS Corporation, Cary NC).

3. Results and discussion

3.1. Phenotypic characterization of rotifer and microalgae species

FlowCAM has the capacity to detect and quantify planktonic organisms and has been employed successfully to estimate the size and morphology of the plankton community in a range of environmental studies [16,21]. The system clearly differentiated between particles of different diameter as equivalent spherical diameter (ESD), length, and width. The mean particle diameters, length, and width of different species showed considerable variation (Table 1). The mean values of diameter, length, and width of different microalgal species were in the range of 6.1 to 21.3, 6.79 to 22.5, and 1.4 to 19.9 μm, respectively. Based on mean values of diameter, length, and width, Hp was identified as the largest among those tested. Both Cs and Se were among the smallest algal species in this study, with Cs having the smallest length and diameter and Se the smallest width among the eleven species in this study. Cyanobacteria may be unicellular, colonial or filamentous, with cell sizes varying from less than 2 to 40 μm in diameter [22]. Cultures of zooplankton species, mainly cladocerans and rotifers, are generally raised on green algae (*Chlorella vulgaris* and *Scenedesmus acutus*) as food because of their small size which is generally lower than 20 μm [23].

3.2. Effect of B. rubens on microalgal growth rate and biomass

Under suitable conditions and sufficient nutrients, microalgae biomass usually doubles within 3.5–24 h during the exponential growth phase [7]. In our study the net growth rates differed among the examined species. Under similar environmental conditions, average growth rates in the absence of *B. rubens* (based on the log number of cell/mL during 21 d incubation) of Mo, Sb, Cm, Cg, Se, Sd, Cs, Bb, and Hp were 2.40 ± 0.01, 2.31 ± 0.02, 2.24 ± 0.02, 2.24 ± 0.01, 2.22 ± 0.01, 2.20 ± 0.01, 2.17 ± 0.04, 2.09 ± 0.03, and 1.97 ± 0.01 cell/mL/d, respectively. In contrast, growth rates in the presence of *B. rubens* were 2.18 ± 0.02, 2.25 ± 0.02, 2.22 ± 0.02, 2.19 ± 0.01, 2.07 ± 0.02, 2.18 ± 0.02, 1.94 ± 0.02, 2.02 ± 0.02, and 1.93 ± 0.02, respectively. Average growth rates of Cy and Ca were 2.16 ± 0.01, 2.14 ± 0.02 and 1.92 ± 0.03, 1.85 ± 0.02 cell/mL/d in the absence and presence of rotifers, respectively, under static culture at 15°C (Fig. 1). Comparison of means showed that there were significant differences in specific growth rates of algae in the presence and absence of *B. rubens* ($\alpha = 0.05$). In this test, all 11 calculated p-values were less than 0.05 with the highest p-value occurring in Sd (p-value = 0.036). In the remaining 10 treatments, calculated p-values ranged between 1.32×10^{-3} and 1.34×10^{-8}. Although the presence of *B. rubens* affected growth rate in all algal species tested, the difference varied among species as indicated by the range in p-values presented above (Fig. 1). At a lower significance level ($\alpha = 0.01$), all algal species tested showed significant difference between treatment and control, with the exception of Sd (p-value = 0.036).

In the presence of *B. rubens*, the specific growth rate of Cs decreased from 6.04 to 4.38 and Mo from 6.63 to 5.09 cell/mL/d, respectively, after 21 d incubation. The highest specific growth rates (6.65 and 6.63 cell/mL/d) were achieved by Se and Mo, respectively, after 21 d culture in the absence of rotifer. While, the lowest growth rate (4.38 cell/mL/d) was achieved by Cs in the presence of *B. rubens* (Fig. 1). As percentage, compared to the specific growth rate (SGR) of

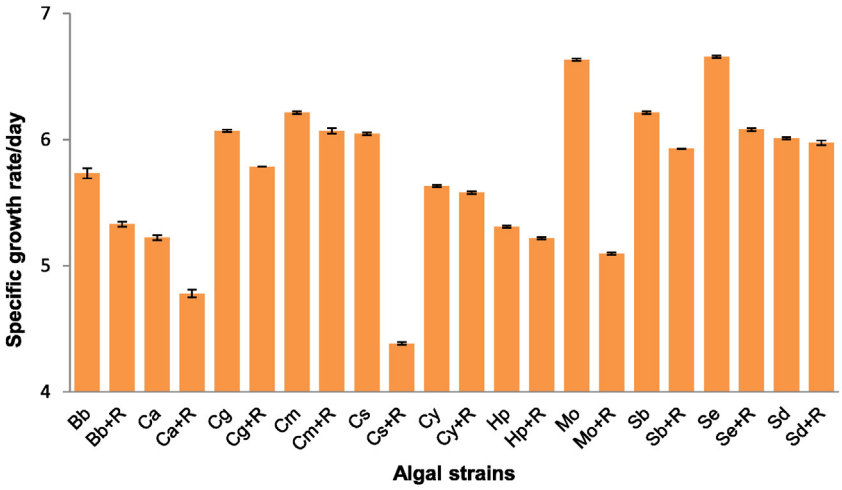

Fig. 1. Specific growth rate of different microalgae species [*B. braunii* (Bb), *C. augustae* (Ca), *C. globosa* (Cg), *C. minutissima* (Cm), *C. sorokiniana* (Cs), *C. yellowstonensis* (Cy), *H. pluvialis* (Hp), *Monoraphydium* spp. (Mo), *S. bijuga* (Sb), *S. elongatus* (Se), and *S. dimorphus* (Sd)], grown in the presence (R+) and absence of rotifer (*B. rubens*) after 21 d cultivation. Values represent mean ± standard error based on three replicates.

Table 3
Variation of cell dry weight of different microalgal species grown in the presence (R+) and absence (R-) of rotifer.

	Incubation day															
	0		3		7		10		14		17		21			
	R+	R-	R+	R-	R+	R-	R+	R-	R+	R-	R+	R-	R+	R-		
Algal strain	Dry biomass (g L^{-1})															
B. braunii	0.277 (0.017)	0.252 (0.019)	0.365 (0.021)	0.354 (0.016)	0.546 (0.022)	0.623 (0.020)	0.765 (0.049)	0.880 (0.078)	0.515 (0.074)	1.260 (0.079)	0.434 (0.021)	1.331 (0.117)	0.707 (0.043)	1.739 (0.195)		
C. augustae	1.20 (0.008)	0.105 (0.017)	0.131 (0.006)	0.137 (0.004)	0.253 (0.007)	0.338 (0.030)	0.355 (0.005)	0.495 (0.016)	0.250 (0.008)	0.670 (0.013)	0.196 (0.018)	0.920 (0.024)	0.294 (0.022)	1.117 (0.067)		
C. globosa	0.049 (0.001)	0.039 (0.003)	0.173 (0.006)	0.150 (0.005)	0.335 (0.013)	0.740 (0.039)	0.553 (0.043)	0.958 (0.024)	0.655 (0.004)	1.190 (0.005)	0.588 (0.013)	1.377 (0.006)	0.790 (0.003)	1.696 (0.045)		
C. minutissima	0.336 (0.018)	0.229 (0.018)	0.299 (0.002)	0.381 (0.010)	0.384 (0.031)	0.456 (0.020)	1.010 (0.048)	1.480 (0.032)	1.456 (0.021)	1.790 (0.068)	1.987 (0.040)	2.150 (0.073)	2.070 (0.123)	2.721 (0.055)		
C. sorokiniana	0.090 (0.004)	0.074 (0.001)	0.114 (0.012)	0.136 (0.015)	0.242 (0.002)	0.411 (0.085)	0.097 (0.008)	0.512 (0.052)	0.020 (0.001)	0.650 (0.122)	0.005 (0.00)	0.859 (0.031)	0.003 (0.00)	1.263 (0.036)		
C. yellowstonensis	0.527 (0.029)	0.575 (0.024)	0.560 (0.031)	0.622 (0.024)	0.787 (0.026)	0.913 (0.016)	0.890 (0.006)	1.063 (0.059)	0.993 (0.090)	1.190 (0.034)	1.050 (0.034)	1.290 (0.031)	1.140 (0.037)	1.335 (0.029)		
H. pluvialis	0.544 (0.023)	0.634 (0.022)	0.625 (0.027)	0.824 (0.007)	0.779 (0.096)	1.216 (0.012)	1.122 (0.082)	1.517 (0.026)	1.516 (0.035)	1.910 (0.173)	1.780 (0.052)	2.229 (0.069)	2.090 (0.042)	2.666 (0.088)		
Monoraphydium spp.	0.030 (0.002)	0.042 (0.001)	0.110 (0.003)	0.153 (0.011)	0.150 (0.010)	0.290 (0.011)	0.166 (0.006)	0.940 (0.007)	0.035 (0.003)	1.170 (0.003)	0.020 (0.001)	1.755 (0.042)	0.014 (0.001)	1.997 (0.048)		
S. bijuga	0.746 (0.039)	0.748 (0.025)	0.670 (0.017)	0.867 (0.025)	0.770 (0.019)	0.900 (0.027)	0.920 (0.037)	1.402 (0.150)	1.200 (0.074)	1.695 (0.003)	1.250 (0.143)	2.220 (0.082)	1.450 (0.007)	2.504 (0.070)		
S. elongatus	0.591 (0.024)	0.590 (0.016)	0.596 (0.009)	0.610 (0.037)	0.618 (0.052)	0.685 (0.013)	0.665 (0.041)	0.831 (0.023)	0.682 (0.036)	1.234 (0.040)	0.738 (0.021)	1.467 (0.025)	0.844 (0.024)	1.531 (0.048)		
S. dimorphus	1.105 (0.116)	1.122 (0.063)	1.170 (0.065)	1.231 (0.059)	1.260 (0.065)	1.302 (0.030)	1.420 (0.158)	1.453 (0.040)	1.758 (0.043)	1.921 (0.042)	1.873 (0.010)	2.050 (0.053)	2.070 (0.110)	2.158 (0.071)		

Values represent mean of three replicates and numbers in parenthesis represent the standard deviation (SD).

microalgal species in the absence of rotifer, the sequence of SGR of microalgal species in the presence of rotifer is: Sd (99.3%), Cy (99.1%), Hp (98.3%), Cm (97.6%), Cg (95.2%), Sb (95.3%), Bb (93%), Ca (91.4%), Se (91.4), Mo (76.8%), and Cs (72.5%).

For zooplankton feeding and population growth studies, two genera of green algae are widely used, *Chlorella* for rotifers [23] and *Scenedesmus* for culturing cladocerans [24]; a few others, such as *Chlamydomonas*, *Desmodesmus*, and *Monoraphidium* [25] have also been used. This is due to the differences in cell size (*Scenedesmus* is often twice as large as *Chlorella*) and nutritional quality (*Scenedesmus* has more lipids, proteins and carbohydrate per unit dry weight than *Chlorella*) [26]. There has not been much agreement among different researchers on the nutritional adequacy of *Chlorella* or *Scenedesmus* for zooplankton studies.

Dry weights of eleven microalgal species cultivated in BG-11 in the presence and absence of *B. rubens* were determined over a period of 21 d (Table 3). The highest average dry weights (0.53 and 0.52 g/L/d) were achieved by Sd and Hp, grown in the absence of *B. rubens*, while, the lowest average dry weight (0.02 g/L/d) was achieved by Mo in the presence of rotifers over 21 d incubation. The biomass yield of Cm, Hp, Sb, and Sd accounted for higher dry weight of 2.72, 2.66, 2.50, and 2.15 g/L, respectively, compared to the other seven microalgal species under optimal conditions (in the absence of rotifer) after 21 d cultivation. The lower dry weights (0.003 and 0.014 g/L) were achieved by Cs and Mo, respectively, as a result of rotifer (Table 3). The biomass yields of Cs and Mo were reduced by 99.8% and 99.3%, respectively, as a result of rotifer presence, while a very slight effect was observed on Sd (4.1% biomass decrease) after 21 d cultivation. The influence of rotifer on algal productivity (measured as mg/L/d) is presented in Fig. 2. The lowest algal biomass productivity (-4 mg/L/d) was achieved by Cs and was negative as a result of rotifer feeding. The biomass productivity of Sd (46 mg/L/d) was slightly affected by the presence of rotifer compared with the biomass productivity of Sd (49 mg/L/d) in the absence of rotifer. The resistance of Sd to rotifer could potentially be a result of its size and cell morphology. *Scenedesmus* is known to be highly polymorphic, particularly in response to zooplankton grazing pressure [27]. Phytoplankton often develop various defense mechanisms in response to zooplankton grazing, such as spine length, motility, chemical constituents such as toxin, the presence of gelatinous substances, or the formation of colonies and filamentous structures, helps in reducing zooplankton filtering rates [28]. Various studies have demonstrated that the formation of colonies by green alga offers considerable protection against grazing by zooplankton [29]. Rotifers are the common predatory species, among these zooplankton, in mass cultivation of microalgae [25]. Their presence can reduce algal populations up to 90% and in some cases cause total loss of the culture [27].

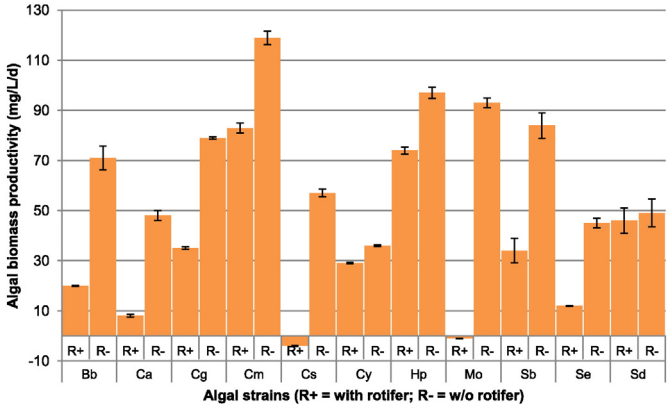

Fig. 2. Algal biomass productivity in the presence and absence of rotifer.

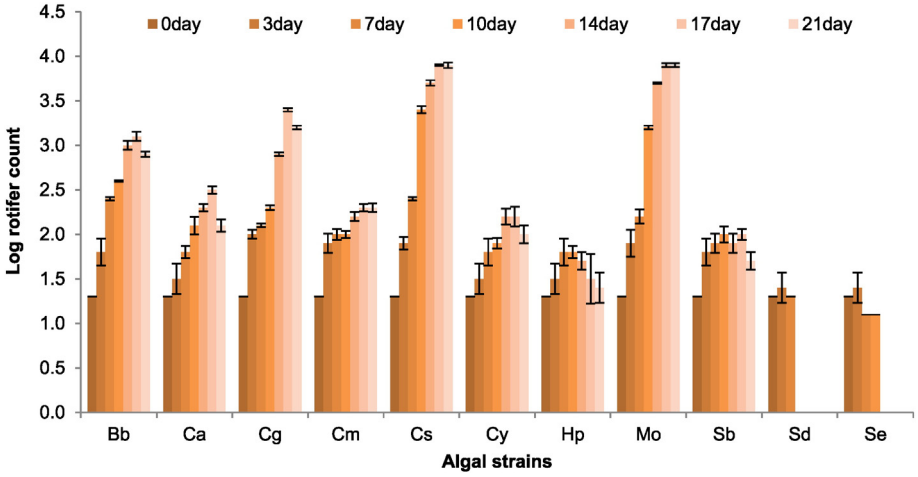

Fig. 3. Log rotifer count raised on different microalgae species at different cultivation time.

3.3. Growth rates of *B. rubens* feeding on different microalgae species

The growth rates of *B. rubens* fed on eleven different microalgae species for 21 d are shown in Fig. 3. The different species exerted distinct effects on the population growth of *B. rubens*. With the exception of Se and Sd, the other 9 microalgal species support good to excellent growth of *B. rubens*. In 17 d of culture, the maximum numbers of rotifer (based on the log number) (3.9 ± 0.02, 3.9 ± 0.01, 3.4 ± 0.02 and 3.1 ± 0.05 individual/mL) were achieved when they were fed on Mo, Cs, Cg, and Bb, respectively. In 21 d of culture, the population growth of *B. rubens* fed on Cs and Mo was higher than those of the other experimental groups at 3.82 ± 0.02 (highest density = 7633 ± 451 individual/mL) and 3.81 ± 0.02 (highest density = 7433 ± 391 individual/mL), respectively (Fig. 3). The highest growth rates of *B. rubens* were observed when Cs was offered as food. Growth as a percentage of the maximum growth rate (that of Cs) for other microalgae varied between -1.6% for Sd and Se and 99.7% for Mo (Fig. 4). A one-way ANOVA confirmed that there were significant differences in growth rate of rotifer in the presence of different algal species (p-value < 0.0001). Additionally, Tukey-HSD confirmed significant differences between growth rates of *B. rubens* in the presence of different algal species (Fig. 4). The calculated p-values for treatments that were significantly different had calculated p-value < 0.0001, with the exceptions of Sb–Hp (p-value = 0.0110), Cm–Cy (p-value = 0.0033), and Cy–Sb (p-value = 0.0023). Treatments Cs, Mo, Cm, Ca, and Cy had overlaps indicating lack of significant differences which are shown by differing letters in Fig. 4. The lowest rotifer population (-0.06 individual/d) was obtained with cyanobacteria (Se). Both Se and Sd had similar effect on *B. rubens* growth, and this effect was significantly different (p-value < 0.001) from effect of other algal species tested (Fig. 4). Lack of essential compounds is considered one of the factors that determine the quality of cyanobacteria as food to zooplankton [28]. Those studies do not support the finding that certain cyanobacteria might be valuable supplements in combination with other green algal food species [29]. Many freshwater cyanobacteria are toxigenic and the most frequently encountered cyanobacterial toxins are microcystins [30]. Cyanobacteria are known for being inadequate as a food source for zooplankton, whether by their toxicity, size, and lack of essential compounds or due to feeding inhibitors [31].

The results of the current study clearly show that although green algae may be closely related and most of them cultured under similar conditions, their susceptibility to *B. rubens* contamination and subsequent grazing may differ considerably. One of the major variables that influence growth of rotifers is the morphology of the food algae, which acts through changes in feeding efficiencies over the range of ingestible food size [32]. That study also showed that the optimal algal size for consumption by the rotifers was about 8 μm ESD and that the lower size for retention was 1 μm. The particle grazing by

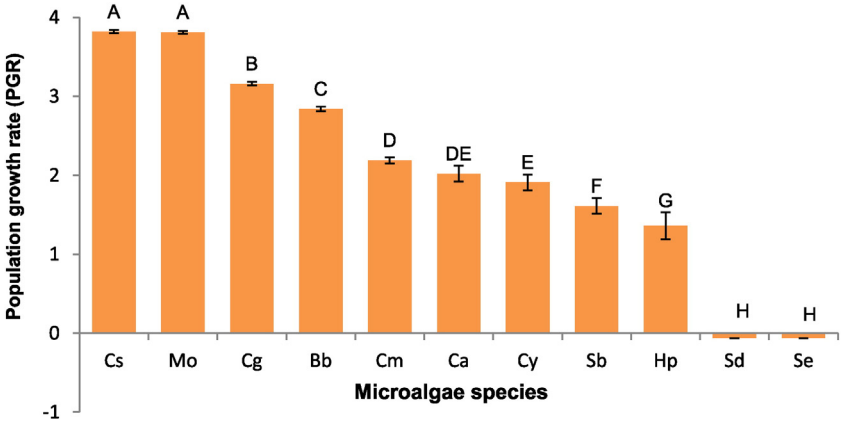

Fig. 4. Population growth rate of *B. rubens* raised on different microalgae species (*Monoraphydium* spp. (Mo), *C. sorokiniana* (Cs), *B. braunii* (Bb), *C. globosa* (Cg), *C. augustae* (Ca), *C. yellowstonensis* (Cy), *C. minutissima* (Cm), *S. bijuga* (Sb), *S. elongatus* (Se), *S. dimorphus* (Sd), and *H. pluvialis* (Hp)) after 21 d cultivation. Values represent mean ± standard error based on three replicates. Treatments with statistically significant differences are indicated by different letters.

suspension feeding rotifers has been reported to be governed by additional factors than particle size and is suggested to be behaviorally influenced, i.e. capability to select or reject particles according to their quality. The behavior is due to the presence of chemo- and mechanoreceptors in connection to the corona [33]. Selectivity has among other things been linked to particle characteristics such as algal cell surface, physiological conditions, and motility [34]. Actively moving prey-microalgae in this case may increase their encounter rates with predators [35].

4. Conclusion

Rotifers contamination and subsequent culture crashes in large-scale microalgal cultivation is a serious threat to the functioning of the algaculture industry. Due to the severity of rotifer grazing problems, significant research efforts are required to study the physical, chemical and biological interactions between algal and rotifer species for developing long term and sustainable solutions to control rotifer contamination. Although most green algae are closely related and are cultured under similar conditions, our results show that their susceptibility to rotifers contamination (*B. rubens* in particular) vary considerably. We also show that different algal species have different effects on the population growth rate of *B. rubens*. Prey size, shape, and motion are implicated as probable causes for these differences in feeding behavior. In our study, *B. rubens* achieved the highest population density when fed with *C. sorokiniana* or *Monoraphidium* spp. leading to complete algal culture crash. In contrast, *S. dimorphus* and *S. elongatus* completely inhibited the rotifer growth within 10 and 14 d of *B. rubens* incubation, respectively. This study shows that a better understanding of the predator–prey interactions can lead to developing microalgal consortia with enhanced abilities of biomass production and rotifer resistance, which can be a natural and sustainable solution for the algaculture industry.

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Conflict of interest

It's hereby declared that all authors of this publication have no financial, personal or intellectual conflict of interest that could affect objectivity of this publication.

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