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Nutritional quality of prey (*Brachionus calyciflorus*) affects the population growth of predatory rotifers (*Asplanchna sieboldi*) (Rotifera)

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RESUMEN

La calidad nutricional del rotífero presa *Brachionus calyciflorus* cultivado en dos tipos y tres combinaciones de alimento, fue probado usando al rotífero depredador *Asplanchna sieboldi*. *B. calyciflorus* crecio utilizando su alimento tradicional *Clorella vulgaris* y (nejayote) diluido y ajustando el pH, obtenido del nixtamal y su mezcla. La talla más pequeña de los adultos (6.24 x 10^5 æm^3) fue observado en las poblaciones de *B. calyciflorus* cultivado en nejayote, mientras que los individuos más grandes (7.98 x 10^5 æm^3) se obtuvieron de los que se alimentaron de alga. El tamaño del cuerpo de los braquionidos alimentados de la mezcla muestran resultados intermendios. Las curvas de crecimiento poblacional de *A. sieboldi* en diferentes concentraciones de presa (alga, nejayote y su mezcla) y en densidades (2.5 - 20 ind/ml/24 hr) muestran oscilaciones en la abundancia. La abundancia de *A. sieboldi* alimentado con presas que se alimentaron con nejayote fue significativamente alta; comparado con aquellos alimentados con presas que se alimentaron de alga o alga más nejayote, lo que implica una mejor calidad nutricional de los rotíferos alimentados del nejayote, a pesar de haber tenido el tamaño del cuerpo más pequeño comparado con la población de *B. calyciflorus* que se alimentó con algas y tubo el tamaño más grande. En los resultados se muestra que la tasa de crecimieto poblacional más alta fue 0.95 ñ 0.01 y la más baja fue 0.33 ñ 0.04 para *A. sieboldi* alimentado con *B. calyciflorus* alimentado con nejayote y con nejayote más alga respectivamente. Cuando los individuos de *A. sieboldi* fueron alimentados solamente con algas, algas y nejayote ó nejayote, no hubo reproducción lo que indicó que la energía obtenida se utilizo para sobrevivir más no para la reproducción.

Palabras clave: Depredación, calidad nutricional, crecimiento poblacional, rotíferos, tamaño corporal.

ABSTRACT

The nutritional quality of prey rotifer *Brachionus calyciflorus* grown on two types and three combinations of food was tested using the predatory species *Asplanchna sieboldi*. *B. calyciflorus* was grown using *Chlorella vulagaris*, diluted and pH-adjusted waste water from *tortilla* industry (*agua de nejayote*) and their mixture. The smallest sized adults (6.24 X 10^5 μm^3) were observed in *B. calyciflorus* populations grown in *nejayote* water while the largest individuals (7.98 X 10^5 μm^3) were obtained from those fed on algae. The body size of brachionids raised on the mixture of alga and *nejayote* water showed intermediate results. Population growth curves of *A. sieboldi* grown on different prey combinations (algae vs *nejayote* water and their mixture) and densities (2.5-20 prey/ml/24 hr) showed oscillations in predators´ abundance. In general, an increase in the abundance of prey resulted in higher densities of prey populations, regardless of prey nutritional status. However, there was a significantly higher abundance of *Asplanchna* fed on prey grown in *agua de nejayote* as compared to those fed on either only algae or algae plus *agua de nejayote*. A consistently higher population growth in *A. sieboldi* fed on *B. calyciflorus* raised on *nejayote* water implied better nutritional quality of prey rotifers, despite their smaller body size. The population growth rate (r) increased significantly with increasing prey availability regardless of the diet on which the prey was cultured. The highest population growth rate recorded in the study was 0.95±0.01 and the lowest was 0.33±0.04 for *A. sieboldi* fed on *B. calyciflorus* grown on *nejayote* water and *nejayote* water plus algae, respectively. When *A. sieboldi* individuals were maintained (in the absence of prey brachionids) in algae, algae+*nejayote* water or *nejayote* water alone, no reproduction occurred, although the asplanchnids continued to live for a few more days in medium with nejayote water with or without addition of algae.

Keywords: Predation, nutritional quality, population growth, rotifers, body size.

INTRODUCTION

Most members of the genus *Asplanchna* are predatory, their diet predominantly comprising not only of ciliates (Arndt, 1993) and rotifers (Ejsmont Karabin, 1974), but also of cladocerans (Guiset, 1977) and sometimes even copepods (Williamson, 1983). Numerous field and laboratory studies have indicated a large number of prey types and in various densities in the gut of *Asplanchna* (Green and Lan, 1974; Sarma, 1993; Iyer and Rao, 1996). These have implied that the predation pressure on small-sized zooplankton community by *Asplanchna* is considerable.

Rotifers, particularly of the genus *Brachionus* are extensively used in aquaculture and in Mexico their cultivation techniques are steadily improving (Ramírez-Sevilla *et al.*, 1991; Castellanos-Páez *et al.*, 1994a,b; Anon. 1996, 1997). In aquaculture practices, not only the prey size and type but also its nutritional quality to the predator is of paramount importance. Extensive research indicates that the nutritional value of *Brachionus* depends to a large extent on the diet provided (Hamza and Robin, 1992). Rotifers cultured on yeast alone for instance, are known to be deficient in certain fatty acids than those cultured on green algae such as *Chlorella* (Rodriguez *et al.*, 1996). It is therefore essential to test the nutritional quality of the prey grown in a particular medium on the predator, in terms of population growth, before recommending large scale use in aquaculture.

In a previous study Stevenson (1997) has shown that *Brachionus calyciflorus* can easily be cultured on *agua de nejayote* which is wastewater arising as a by-product of the large scale *tortilla* (a kind of soft dry thin, plate-like bread made of corn or wheat floor) industry in Mexico. The high production of *B. calyciflorus* on this effluent can be converted into fish production if the nutritional quality of the rotifers grown in this medium is good. In order to test this, we used *Asplanchna sieboldi* as a bioassay tool and compared its growth rate when fed on *B. calyciflorus* which was cultured exclusively on *Chlorella*, on a mixture of *Chlorella* + *agua de nejayote*, and on *agua de nejayote*.

MATERIAL AND METHODS

We used two rotifer species for this investigation: *Brachionus calyciflorus* Pallas, 1766 as prey and *Asplanchna sieboldi* (Leydig, 1854) as predator. The former was isolated from Chapultepec lake (Lago Viejo) and the latter from a freshwater pond in Caseta 7, close to Sosa Texcoco (both ponds in Federal District, Mexico City). Both the rotifer species were cloned for at least 6 months prior to experimentation.

B. calyciflorus was mass cultured separately in 50 l capacity glass aquaria using three combinations of two types of food. We used green algae (*Chlorella vulgaris*) for rearing *B. calyciflorus* which acted as control diet, and waste water from *tortilla* industry (*agua de nejayote*). In the laboratory aquaria, we were able to grow *B. calyciflorus* to densities of about 200±20 ind/ml using the *agua de nejayote* as exclusive medium and diet. The conditions that favoured the maximal population increase of *B. calyciflorus* were *agua de nejayote* diluted to 84% with EPA medium (Anon., 1985) after being filtered through a 20μm mesh (to remove large organic particles and ciliates) and pH and temperature adjusted to 7.00 and 25°C, respectively (see Stevenson, 1997). This acted as a second prey combination. In the third combination, we grew *B. calyciflorus* in 50% *Chlorella* (at a density of 2 X 10^6 cells/ml) and 50% *agua de nejayote* (of 84% dilution).

The predator *Asplanchna sieboldi* was mass cultured in 1-5 L glass containers in EPA medium using *Chlorella*-fed *B. calyciflorus* as exclusive food. Although we were able to grow *Asplanchna* to densities higher than 10 ind/ml, we always maintained their density to less than 2 ind/ml as higher densities could induce sexual reproduction.

For the experiment, we used 25 ml transparent vials containing 20 ml of EPA medium with prey grown in one of the three food combinations. To the test vials addition of small quantity (3 ml) of dilute algae (0.5 X 10^6 cells/ml) and *nejayote* water (90% dilution) became necessary to keep prey rotifers active during the experiment. We used four prey concentrations (2.5, 5, 10 and 20 ind/ml). For each prey type and concentration three replicates were maintained. In all, we used 36 test vessels (3 prey nutritional types X 4 prey densities X 3 replicates = 36). Into each of the test vessels containing prey of one particular nutritional status and density, we introduced young (2±2 hr age following birth) *A. sieboldi* at an initial density of 0.1 ind/ml (2 individuals in 20 ml medium). The density of both prey and the predators was individually counted by picking the organisms with a finely drawn pasteur pipette, under a stereomicroscope.

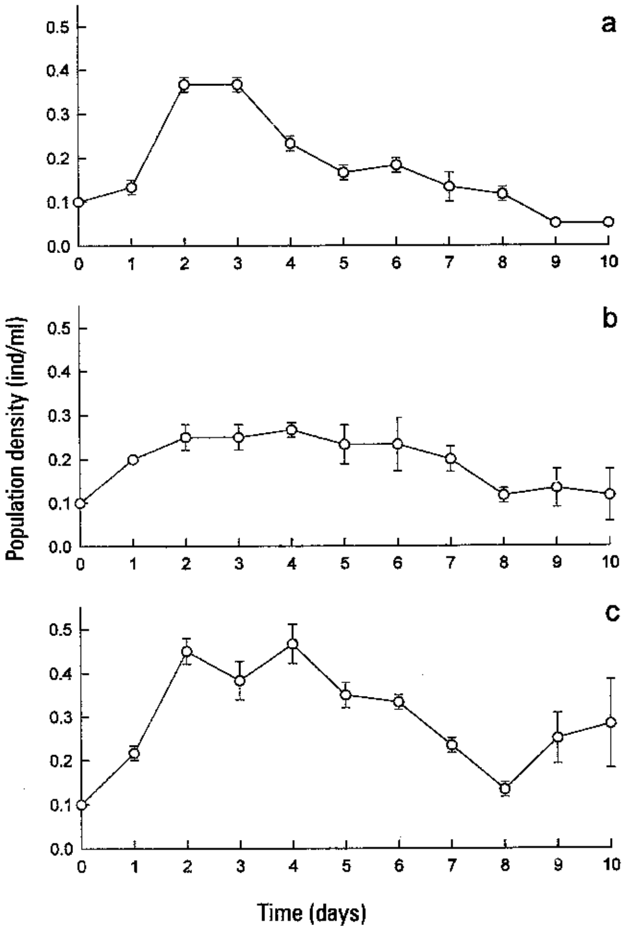

Fig. 1. Population growth curves of *Asplanchna sieboldi* in relation to prey (*Brachionus calyciflorus*) density of 2.5 ind/ml/24 hr. Shown are the values mean±1 standard error, n=3. The legends a, b and c shown on the figure represent the food conditions viz. only algae, algae+*nejayote* water and only *nejayote* water, respectively, used to grow the prey populations.

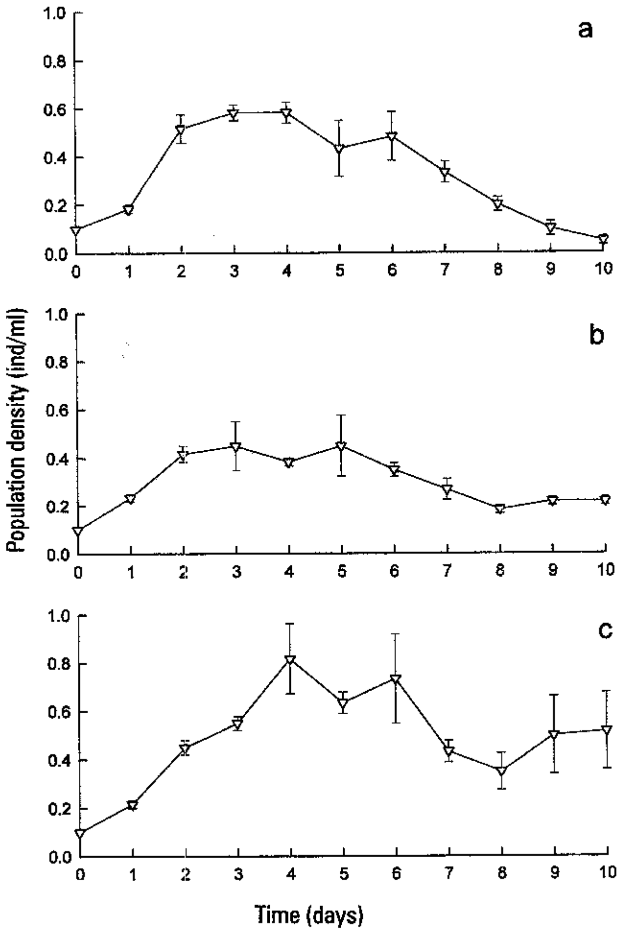

Fig. 2. Population growth curves of *A. sieboldi* in relation to prey (*B. calyciflorus*) density of 5 ind/ml/24 hr. Shown are the values mean±1 standard error, n=3. Other details as in Fig.1

Following initial inoculation, we monitored the density of the predator after every 24 hr. At the end of each observation, uneaten brachionids and dead *Asplanchna* were removed. The surviving predators were transferred to a fresh set of containers with appropriate food type and density. Males were rare and occurred in low densities towards the population peak. They were removed whenever encountered and were not included in estimating *Asplanchna* density. The experiment was terminated after day 10 when most populations completed one population cycle or showed declined population growth. From the data, the instantaneous rate of population increase per day (r) was estimated using the following equation during the exponential growth phase.

$$r = (\ln N_t - \ln N_0)/t$$

where,

N_0 is the initial population density of *Asplanchna*

N_t is the final population density and

t is the time in days.

In order to understand the role of food types on the body size of *B. calyciflorus*, we measured only egg-bearing females from the exponential phase of their growth. For measuring body size, we separated 50 ovigerous females from each of the culture tanks and fixed in 5% formalin. The measurements of *B. calyciflorus* included both the width and lengths. Anterior (posterior and posterolateral spines if present) spines were not included. We used ocular micrometer for measuring rotifer size which was later calibrated using stagemicrometer. Rotifer body size was expressed in volume (μm^3) following Ruttner-Kolisko (1977) and Walz *et al.* (1995).

RESULTS

Population growth curves of *A. sieboldi* grown on different prey combinations and densities showed oscillations in predators´ abundance (Figs1-4). In general, most populations showed a very short lag phase, an exponential phase and a retardation phase. The mean plateau densities of *A. sieboldi* varied from 0.24±0.01 ind/ml to 2.24±0.17 ind/ml in the lowest density (2.5 ind/ml) of prey grown in a mixture of alga and *agua de nejayote* and those at the highest density (20 ind/ml) raised on *nejayote* water only, respectively.

The nutritional effect of prey grown in different food types was evident in terms of the maximum densities reached by *Asplanchna*. In general, there was a significantly

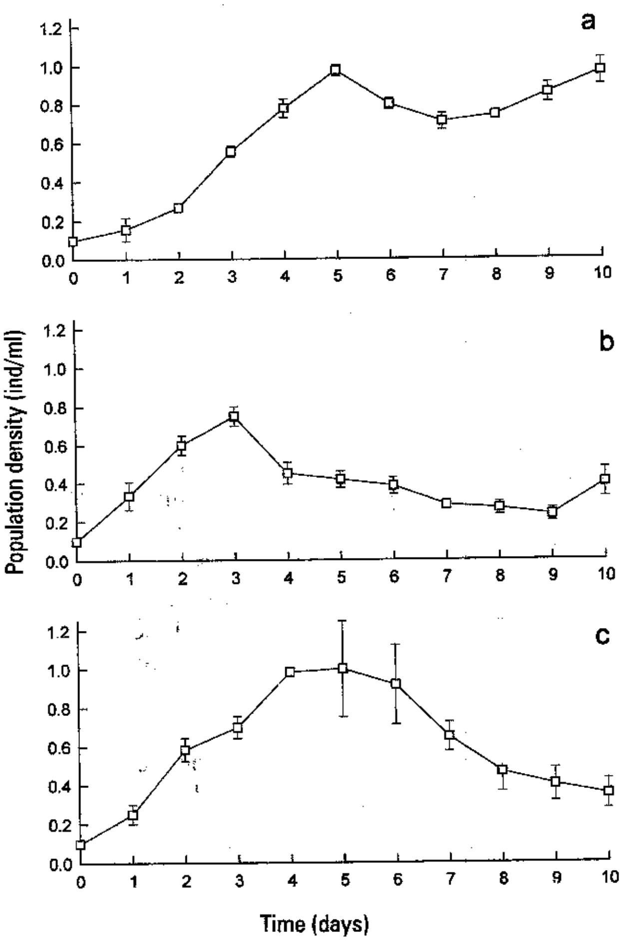

Fig. 3. Population growth curves of *A. sieboldi* in relation to prey (*B. calyciflorus*) density of 10 ind/ml/24 hr. Shown are the values mean±1 standard error, n=3. Other details as in Fig.1

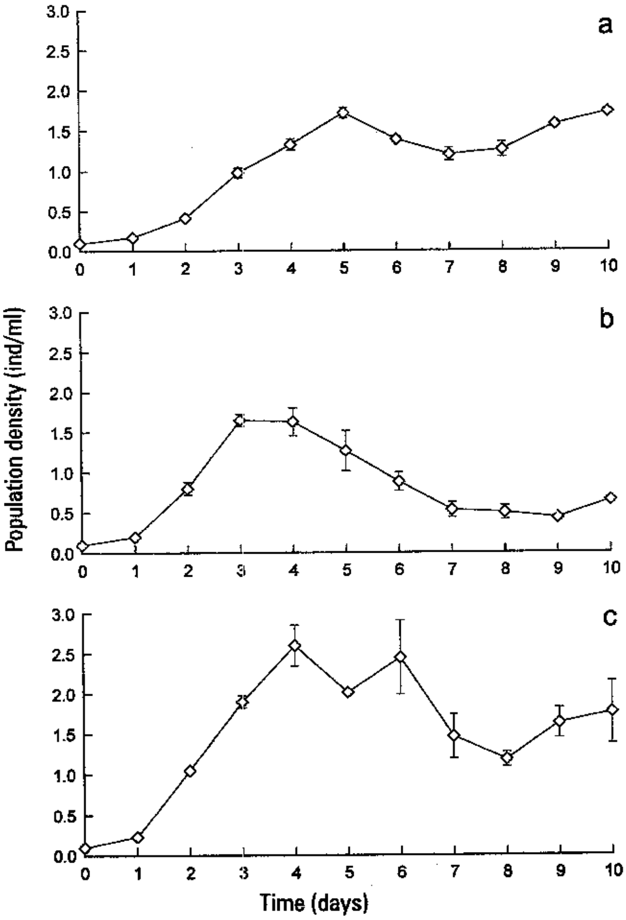

Fig. 4. Population growth curves of *A. sieboldi* in relation to prey (*B. calyciflorus*) density of 20 ind/ml/24 hr. Shown are the values mean±1 standard error, n=3. Other details as in Fig.1

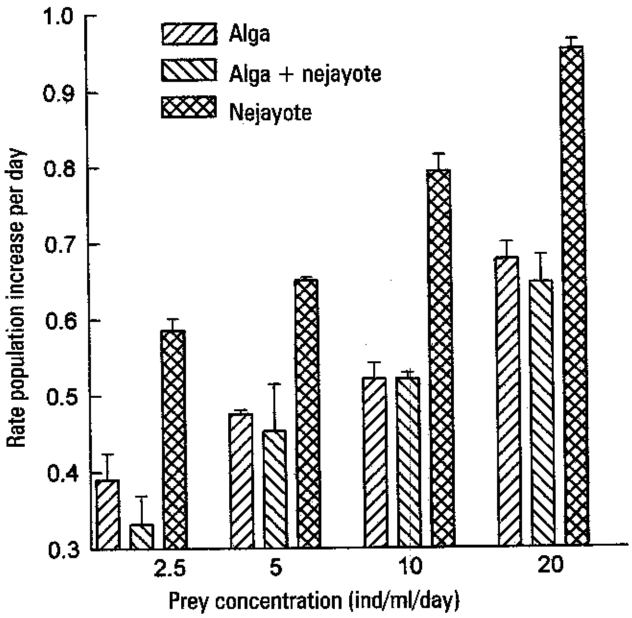

Fig. 5. Rate of population increase per day (r) in *A. sieboldi* in relation to prey density and nutritional quality. Each value represents the mean and standard error based on 3 replicates. The r values were derived for each replicate separately during the exponential phase using growth equation shown in the text.

higher abundance of *Asplanchna* fed on prey grown in *agua de nejayote* as compared to those fed on either only algae or algae plus *agua de nejayote* ($P<0.05$, ANOVA, Table 1, Sokal and Rohlf, 1981). The rate of population growth (r) calculated during the exponential phase of population also showed similar trends (Fig. 5, Table 1). The r value increased significantly with increasing prey availability regardless of the diet on which the prey populations were cultured ($P<0.05$, ANOVA, Table 1). The highest growth rates recorded in the study were 0.95 ± 0.01 and the lowest were 0.33 ± 0.04 for *A. sieboldi* fed on *B. calyciflorus* grown on *nejayote* water and *nejayote* water plus algae, respectively.

Table 1. ANOVA Table of selected population varibles observed in the study.

Variable	DF	SS	MS	F	P
Max. Population density					
Food type	2	2.345	1.17	43.63	0.001
Food density	3	13.809	4.6	171.28	0.001
Interaction	6	0.0878	0.15	5.44	0.01
Error	24	0.06455	0.03	-	-
Rate of population increase					
Food type	2	0.495	0.25	104.89	0.001
Food density	3	0.519	0.17	73.31	0.001
Interaction	6	0.014	0.0023	0.96	ns
Error	24	0.057	0.0024	-	-
Body volume					
Among food types	2	85200X10^7	42600X10^7	22.87	0.001
Within	147	273856X10^7	1863X10^7	-	-

The body sizes of *B. calyciflorus* grown on different food types showed significant differences ($P<0.05$, F-test, Table 1, Fig. 6). The smallest sized adults ($6.24 \times 10^5\ \mu m^3$) were observed from populations grown in *nejayote* water while the largest individuals ($7.98 \times 10^5\ \mu m^3$) were obtained from those fed on algae. In terms of dry weight these values are equivalent to 0.0624 and 0.0798 μg per individual, respectively (Ruttner-Kolisko, 1977). The body size of brachionids raised on the mixture of alga and *nejayote* water showed intermediate results.

DISCUSSION

The population growth curves of *Asplanchna sieboldi* obtained here are typical for opportunistic zooplankton (r-strategists) (Downing and Rigler, 1984; Rothhaupt, 1988; Rothhaupt and Lampert, 1992; Rico-Martínez and Dodson, 1992). Differences in population abundance of *A. sieboldi* in relation to prey grown on different diets are largely due

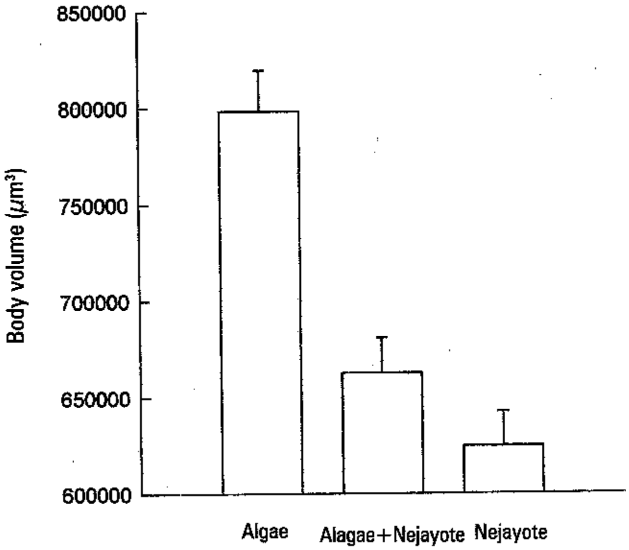

Fig. 6. Rotifer (*Brachionus calyciflorus*) body volume (μm^3) relation to the three food types, viz., only algae (offered a daily ration of about 2X10^6 cells/ml of *Chlorella*), algae plus nejayote water (50% of 84% dilution) and only nejayote water (84% diluted with EPA medium). Values shown are the mean$\pm$standard error based on 50 observations measured in different stages of the population growth.

to differences in the nutritional quality of the prey. For example, Maly (1975) has shown that *A. brightwelli* fed on *Euglena* showed poor growth and survival when compared to those fed on *Paramecium*. For herbivorous rotifers, Rothhaupt (1995) has shown that *Brachionus rubens* ingested non-nutrient-limited and nutrient-limited *Scenedesmus* at comparable rates, but the highest growth rates were observed when grown on non-nutrient-limited algae. This implies that nutritional quality of the diet controls the abundance of zooplankton.

It has been well established that the nutritional quality of prey brachionids is largely determined by the type of food they are fed with (Watanabe *et al.*, 1983). This is also true for the next trophic level, i.e. carnivorous organisms such as fish feeding on brachionids (Rodriguez et al.,1994). The chemical composition of most of the cultured algal species including *Chlorella vulgaris* is well-documented under a variety of culture conditions and media (Kuhl and Lorenzen, 1964). However, the chemical composition of *nejayote* water is not thorougly studied (Pedroza, 1985). There are indications though that the quality of *nejayote* water in terms of major chemical constituents does not vary significantly (Durán de Bazúa, 1988). A biochemical study on the chemical composition of *B. calyciflorus* grown on different food types used here could not be made. However, a bioassay using a predator could confirm the population level effects of prey differing in nutritional quality. Because of the commercial importance, traditionally fish larvae are used to assess the nutritional status of prey rotifers (Fukusho, 1989). *Asplanchna* however, can also be a sensitive bioassay tool to test this, since its numerical response is known to change in relation to prey size, density, movement and accumulated toxins in the prey (Snell and Janssen, 1995; Iyer and Rao, 1996). Our study confirms that in terms of numerical response of *Asplanchna*, the nutritional quality of *B. calyciflorus* raised on *nejayote* water is superior to those grown on *Chlorella*.

The range of body sizes observed in *B. calyciflorus* here (6.24×10^5 - 7.98×10^5 μm^3) are lower than those (18.15×10^5 - 23.94×10^5 μm^3) reported by Walz *et al.* (1995). Nevertheless, the strain level differences in the body size of this species in nature appears to be high and our measurements for this species fall in this range (4.78×10^5 - 150.62×10^5 μm^3 (calculations based on the data of Kutikova and Fernando, 1995). When *B. calyciflorus* were fed on *Chlorella*, the body size of the adults was significantly higher than those fed on *nejayote* water ($p<0.01$, F-test, Table 1). Yet, the mean population densities or growth rates of *A. sieboldi* were lower when fed on *B. calyciflorus* reared on *Chlorella*. This could be possibly due to better nutritional quality of prey rotifers cultured on *agua de nejayote*. An ealier study also shows that *B. calyciflorus* shows an improved growth rate when cultured on *agua de nejayote* than on *Chlorella* alone (Stevenson, 1997). However, we would like to document the influence of overriding factors directly or indirectly affecting the body size of herbivours rotifers even under controlled culture conditions. These include the phase of population growth (Yúfera, 1982; Sarma, 1996), food density (Duncan, 1989) and, the composition and density of bacterial load in *nejayote* water (Pedroza, 1985; Durán de Bazúa, 1988). The last factor may also be assumed to supply food for starving *Asplanchna* in our test vessels. To assess this, we conducted an experiment separately by introducing *A. sieboldi* individually into test vials containing 20 ml medium with algae, algae+*nejayote* water or *nejayote* water but without *Brachionus*. It was observed that no reproduction occurred in *A. sieboldi* in the absence of prey brachionids, although the test individuals continue to live for three more days in medium with *nejayote* water with or without addition of algae (in algae alone *A. sieboldi* individuals died after 24 hrs). This alone could not have enhanced significantly the population density of *Asplanchna* in our test because we used only very small volumes of diluted *nejayote* water. The poor growth of *Asplanchna* fed on *B. calyciflorus* grown on a mixture of algae and *nejayote* water can be attributed to the loss of nutritional quality of *Chlorella* since unlike fresh algae grown on defined media, they probably do not get sufficient nutrients, especially micronutrients, from waste water. A consistently higher population growth in *A. sieboldi* fed on *B. calyciflorus* raised on *nejayote* water implied better nutritional quality of prey rotifers.

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