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First data on secondary production of *Philodina roseola* (Rotifera, Bdelloidea) grown in laboratory

Raquel Aparecida Moreira*, Adrislaine da Silva Mansano and Odete Rocha

Department of Ecology and Evolutionary Biology, Federal University of São Carlos, Rodovia Washington Luis, km 235, São Carlos, SP CEP 13565-905, Brazil.

* Corresponding author: raquel.moreira88@hotmail.com

ABSTRACT

First data on secondary production of *Philodina roseola* (Rotifera, Bdelloidea) grown in laboratory

The aim of this study was to quantify the secondary production of a benthic rotifer species, *Philodina roseola* grown in the laboratory. The variations in body size of this rotifer and its reproduction were individually determined for nine individual replicates. The measurements of linear dimensions (total length, width, and diameter) of the entire body or individual body parts were performed soon after birth and tracked throughout the individual life cycle. The biomass (μg DW/ind) was estimated by calculating body biovolume, which was determined by using the most suitable equations for the closest geometric shapes of different body parts. The individual production in body growth (Pg) and the reproductive production (Pr) were determined. The average length of the adult rotifer body (429.96 ± 28.12 μm) was about twice larger than the average size of neonates (198.77 ± 25.88 μm). The increase of biomass dry weight at different stages of development occurred at the same proportion; the biomass of an individual neonate (0.0104 ± 0.0014) was around half that of the juvenile (0.0254 ± 0.0029), and the biomass during adulthood (0.0508 ± 0.0071) was approximately twice that of the biomass juvenile. The results related to the body's production were obtained by computing the biomass value related to the mean body growth of the species (0.0425 ± 0.0172 μg DW/ind). The production regarding the increase in body mass between neonatals and juveniles was the highest, corresponding to 1.3 times the increase that occurred when the individual passed from juvenile to adult stages. The reproductive output was much higher (in average 5.8 times) than the body production, and that corresponded to 84.68 ± 3.65% of the total production value of *Philodina roseola*.

Key words: Biomass, dry weight, growth, reproduction, rotifer.

RESUMEN

Primeros datos de producción secundaria de* Philodina roseola *(Rotifera, Bdelloidea) cultivado en el laboratório

El objetivo de este trabajo fue cuantificar la producción secundaria de la especie de rotífero bentónico Philodina roseola *cultivada en laboratorio. Las variaciones en el tamaño del cuerpo de este rotífero y su reproducción fueron determinadas individualmente para nueve individuos. Las mediciones de las dimensiones lineales (longitud total, ancho y diámetro) de las partes del cuerpo fueron realizadas después del nacimiento de los individuos y acompañadas a lo largo del ciclo de vida de los mismos. La biomasa (μg PS/ind) fue calculada por medio del cálculo de biovolumen corporal el cual fue determinado por medio de ecuaciones adecuadas para la forma geométrica más cercana a la forma de las diferentes partes del cuerpo de la especie. Se calcularon también la producción individual en crecimiento corporal (Pg) y la producción reproductiva (Pr). La longitud promedio del cuerpo del rotífero adulto (429.96 ± 28.12 μm) fue cerca de dos veces mayor que el tamaño promedio de los recién nacidos (198.77 ± 25.88 μm). El aumento de los valores de biomasa en peso seco en las diferentes fases de desarrollo, ocurrió en la misma proporción, siendo la biomasa del individuo recién nacido (0.0104 ± 0.0014) aproximadamente, la mitad que la del joven (0.0254 ± 0.0029) y la fase adulta (0.0508 ± 0.0071) aproximadamente el doble de la biomasa del joven. Sumando los resultados de los valores de producción corporal fue obtenido el valor asociado a la producción corporal promedio de la especie (0.042464 ± 0.007242 μg PS/ind). La producción asociada al aumento de biomasa corporal entre el recién nacido y el joven fue mayor, siendo 1.3 veces más el incremento ocurrido cuando el organismo pasó de fase joven a fase adulta. La producción reproductiva en promedio fue mayor (5.8 veces más) que la producción corporal, correspondiendo así a 84.68 ± 3.65% del valor de la producción total del* Philodina roseola*.*

***Palabras clave:** Biomasa, crecimiento, peso seco, reproducción, rotífero.*

INTRODUCTION

Quantification of biomass and secondary production of different populations of aquatic communities provide relevant information of the organic material available in different trophic levels and may further characterize the complexity of the main biotic interactions, such as predation, competition, and natural disturbances (Rodríguez & Mullin, 1986; Echevarría *et al.*, 1990; Ahrens & Peter, 1991; Rossa *et al.*, 2007).

The secondary production of meiofauna and its components, such as rotifers, can be considered an important measure of its functional role, because it represents an estimate of organic matter and energy available to their consumers (Lemke & Benke, 2009). However, many difficulties are related to the estimation of secondary production of benthic organisms, as, for example, regarding sampling methodology, in which several approaches can generate misleading results (Butkas *et al.*, 2011; Dolbeth *et al.*, 2012), due to limitations such as: the requirement for screening in vivo the soft-bodied organisms, identification difficulties of the less-known groups and counting difficulties (Stead *et al.*, 2005). Although there are a few studies covering aspects such as biomass and secondary productivity that contribute to a better understanding of the dynamics of matter and energy in aquatic ecosystems, especially for tropical regions, laboratory studies with rotifers addressing aspects of their entire life cycle are still rare.

In most freshwater environments, the Rotifera is a diversified group at the species level, usually including several congeneric associations (Matsumura-Tundisi *et al.*, 1990) and being one of the most representative group in lakes and reservoirs, worldwide (Segers *et al.*, 1993; Bozelli, 2000; Sharma & Sharma, 2012). They are important components of aquatic biota, because their populations reach high numbers in a relatively short time as a result of having one of the highest reproductive rates among the metazoans (Snell & Janssen, 1995). This happens mainly due to the existence of species with parthenogenetic reproduction and species switching from sexual to asexual reproduction. They also display a short development time (Herzig, 1983), thus quickly responding to changing environmental conditions (Sahuquillo & Miracle, 2010). They can colonize empty environments with extreme speed and convert the primary production into a usable form for other secondary consumers (Dias *et al.*, 2014). They are efficient filter feeders of organic particulate matter feeding mainly on algae and bacteria (Havens, 1991; Arndt, 1993; Gilbert & Jack, 1993) and are the prey of many predators such as turbellarians, larvae and nymphs of insects, Cyclopoida copepods, and fish larvae (Setzler-Hamilton *et al.*, 1981; Williamson, 1983; Stoecker & Egloff, 1987; Telesh, 1993; Conde-Porcuna & Declerck, 1998).

Rotifers have higher turnover rates than microcrustaceans, as they live in some water bodies being dominant not only in numbers but also in biomass and secondary production (Makarewicz & Likens, 1979; Pace & Orcutt, 1981; Hernroth, 1983, Casanova *et al.*, 2009; Assefa & Mengistou, 2011). According to Vareschi and Jacobs (1984), the rotifers *Brachionus dimidiatus* and *B. plicatilis* in lake Nakuru (Kenya), though not especially significant in biomass, had the highest production rates (1.7 kJ m^{-3}/d) due to a very short juvenile phase (ca. 2 days) and also exhibited fast production of very large eggs (approximately 1 per day). Although in this lake the production of copepods almost matched that of the rotifers during 1972–1973 (production 1.5 and consumption 6.5 kJ m^{-3}/d), they vanished from the lake in the following years. In addition, rotifers with a high nutritional value have a vital role in the food chains of freshwater ecosystems (Ruttner-Kolisko, 1974, Das *et al.*, 2012).

Rotifers of sub-class Bdelloidea are found in a variety of habitats worldwide (Mayr, 1963; Bell, 1987); however, little is known about various aspects of the biology and ecology of most species of this subclass. Rotifers of the genus *Philodina* are highly representative of aquatic habitats and are generally found in a wide variety of freshwater bodies, including lakes, ponds, swamps, rivers, streams, and springs. They are also present in mosses, liverworts and lichens, moist organic matter, soil, and even in tanks of wastewater treatment (Snell & Wallace, 2010).

Although the *Philodina* genus includes species that are mostly benthic, some species are also semi-pelagic thriving in the water column for food due to corona lashes that allow them to swim freely (Hochberg & Litvaitis, 2000).

Thus, the objective of this study was to quantify the biomass and secondary production of benthic *Philodina roseola* Ehrenberg, 1830 (Rotifera, Bdelloidea) for individuals grown in the laboratory under controlled conditions of temperature and food, throughout different stages of the life cycle. Such knowledge will be useful in selecting more realistic conversion factors and in providing calculated dry weights data that can be used when direct measurements of such variables are not possible.

MATERIALS AND METHODS

Stock cultures and maintenance of *Philodina roseola*

Specimens of *Philodina roseola* Ehrenberg, 1830 (Rotifera, Bdelloidea) were obtained from experimental tanks with a 10 000-liter capacity, and they were kept in the Aquaculture Station of the Federal University of São Carlos, São Carlos, SP, Brazil (21°58′58,4″S e 47°52′42,6″W). Individuals of *P. roseola* were collected by vertical hauls with a plankton net of 68 μm mesh size. They were identified based on descriptions of Koste and Shiel (1986) and Koste and Terlutter (2001). Species identity was kindly checked by Dr. Nataliia Iakovenko (Schmalhausen Institute of Zoology NASU & University of Ostrava, Ukraine).

The culture medium used was reconstituted fresh water, which was prepared by following the recommendations of the Brazilian Technical Standards Society (ABNT, 2010). The water pH ranged from 7.0 to 7.8; the hardness ranged from 40 to 48 mg $CaCO_3$/L; and the electrical conductivity was around 160 μS/cm.

Stock cultures of *P. roseola* were maintained as described by Moreira *et al*. (2015) and Moreira *et al*. (2016) in 50–250 mL beakers; incubators were set at 25 ± 1 °C, with a photoperiod of 16 h light:8 h darkness. Cultures reached a high den-

sity (around 116 ind/mL). Food and water were replaced every 76 h as proposed by Hagen *et al*. (2009). Rotifers were fed on a live suspension of the alga *Raphidocelis subcapitata* that was grown in CHU-12 medium (Müller, 1972) at $1 \cdot 10^5$ cel/mL concentration.

Size Measurement and Biomass Calculation

The life cycle study was carried out on 9 individual rotifers that were maintained individually. Adults of *P. roseola* were collected in aquaculture tanks, and they were used to establish a stock culture of around 116 ind/mL density. It is not possible to say whether they are clones, since they came from field, and not from a single-parent individual. Each rotifer was maintained in 3.0 mL of culture medium, in a 9-cm-diameter watch glass that was kept inside a Petri dish (110 × 15 mm) with a lid to prevent evaporation. They were observed under a stereo microscope at 50× magnification and were gently handled with Pasteur pipettes. Linear dimension measurements were performed under an optical microscope with a micrometer eyepiece for each body part and total length. Measurements were performed each 3 hours, during three consecutive days. Length and width of each rotifer were measured only in moments of full stretch.

Body volumes of *P. roseola* were calculated by using two geometric shapes (cylinder and cone), as shown in Fig. 1. Linear dimension measurements were used in the equation given next to calculate newborn, juvenile, and adult volumes:
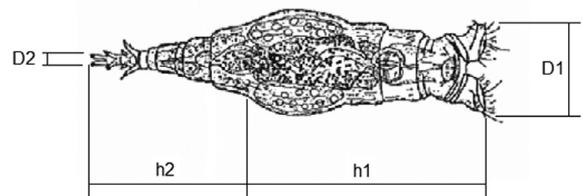
Figure 1. Schematic representation of the shape of *Philodina roseola* body and linear dimensions measured to calculate the biovolume of an individual. *Representación esquemática de la forma del cuerpo de* Philodina roseola *y dimensiones lineales medidas para el cálculo del biovolumen de un individuo.*

$$Vt = \frac{\pi}{4}\left[D1^2 \times h1 + \frac{h2}{3}\left(D1^2 + D1 \times D2 + D2^2 \right)\right],$$

being: Vt = rotifer total volume; $D1$ = width at the upper body portion; $h1$ = height of the upper body portion; $h2$ = height of the lower body portion; and $D2$ = width of finger tip.

Volumes were converted into wet weights, assuming that 10^6 μm^3 correspond to 1 μg of wet weight. Wet weights were transformed into dry weights, assuming a 10% conversion factor following Doohan (1973, in Bottrell *et al.* 1976). Body dimensions are expressed in μm; volumes, in μm^3; and wet and dry weights, in μg.

Secondary production of *Philodina roseola*

Secondary production for neonates, young, and adults of *P. roseola* was carried out by quantifying size increments and calculating corresponding volumes for each developmental phase, as described in Moreira *et al.* (2016). Secondary production allocated for growth (Pg) by juveniles was determined by subtracting dry weight values of juveniles from those of neonates, and the difference was divided by the development time spent from neonate to juvenile stages (in hours). The production yield was subsequently calculated and expressed in μg DW/ind/day. Similarly, the production of adults was determined by subtracting the average dry weight of adults from that of juveniles, and the difference was divided by development time spent from juvenile to adult stages. Average development times from neonate to juvenile stages and from juvenile to adult stages were 15.0 and 33.0 hours, respectively (Moreira *et al.*, 2016). Summing up production values between developmental stages (neonate to juvenile and juvenile to adult), the amount of secondary production allocated to growth by *P. roseola* was assessed.

The secondary production allocated to reproduction or egg production (Pr) was obtained by quantifying the volume of eggs and converting them into fresh and dry weights in similar procedures to those just described for body biomass. To determine the egg biomass, the linear dimensions of eggs were obtained (egg length and width)

from 10 eggs. The total fertility of each experimental individual (9 replicates) was determined over its entire life cycle. Egg biomass volume was calculated by using the following oval ellipsoid formula:

$$\frac{4}{3}\pi\, r_1 r_2^2$$

with r_1 being the largest linear dimension (length) and r_2 being the smallest linear dimension (width).

Egg volume was then converted into wet and dry weights as previously described for body volumes. *P. roseola* reproductive production (Pr) was obtained by multiplying the average egg dry weight by the mean number of eggs produced over the whole life by each individual, divided by embryonic development time.

Total secondary production of *P. roseola* was obtained by summing up daily average body growth production with daily average reproductive production, thus expressed as μg DW/ind/day.

The normality (Shapiro-Wilk) and homogeneity of data (Levene) were tested, and analysis of variance (ANOVA) followed by the post-hoc Fisher LSD test were performed to verify differences between quantities of secondary production invested in growth during the intervals between neonate and juvenile stages and between juvenile and adult stages. Significant differences were accepted at $p < 0.05$. The analyses were performed by using the free Statistica version 7 software (Statsoft, 2004).

In Fig. 2, the *P. roseola* growth curve throughout the life cycle is depicted. It can be observed that the average length of adult *Philodina roseola* was 429.96 ± 28.12 μm, which was more than twice the average size of neonates: 198.77 ± 25.88 μm. The values of biomass (dry weight) of the developmental phases indicate that biomass increments occurred in the same proportion (doubling) between neonates (0.0104 ± 0.0014 μg) and juveniles (0.0254 ± 0.0029 μg) and between the latter and adults (0.0508 ± 0.0071 μg).

Biomass values for *P. roseola* eggs are presented in Table 1, as well as the number of eggs
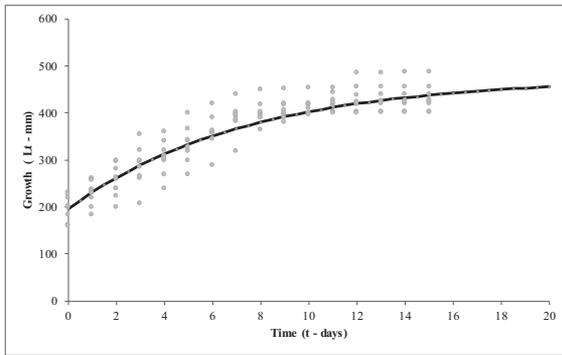
Figure 2. Growth curve for individual *Philodina roseola* (The observed values for 9 replicate animals) cultured at 25 ± 1 °C and fed on the chlorophycean microalga *Raphidocelis subcapitata* at a density of 1 · 10⁵ cells mL⁻¹. Source: Moreira *et al.* (2016). *Curva de crecimiento individual de* Philodina roseola *(valores obtenidos para nueve réplicas de animales) cultivada a 25 ± 1 °C y alimentadas con la microalga clorofícea* Raphidocelis subcapitata *en una concentración de 1 · 10⁵ células mL⁻¹. Fuente: Moreira* et al. *2016).*
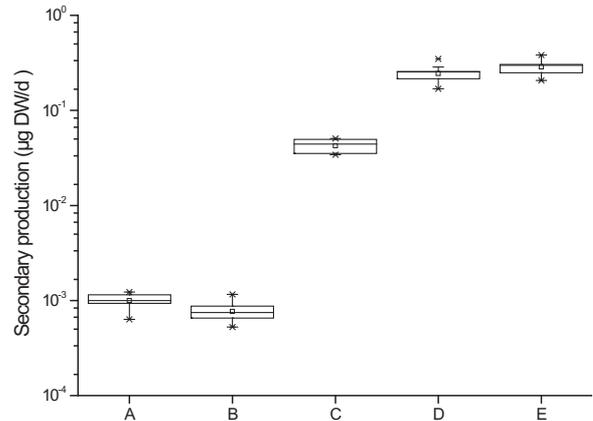
in the first brood and the embryonic development time for each individual cultivated.

RESULTS

Estimates of total secondary production over the life cycle of *Philodina roseola* are depicted in

Figure 3. Secondary production of *Philodina roseola* (Rotifera, Bdelloidea) from laboratory cultures during its whole life cycle. A = production from neonate to juvenile stages (body growth); B = production from juvenile to adult stage (body growth); C = total body growth production; D = total reproductive (egg) production; E = total secondary production. *La producción secundaria de* Philodina roseola *(Rotifera, Bdelloidea) a partir de cultivos de laboratorio durante todo el ciclo de vida. A = producción de neonato a juvenil (crecimiento corporal); B = producción de juvenil a adulto (crecimiento corporal); C = producción total de crecimiento corporal; D = producción total reproductiva (huevo); E = producción secundaria total.*

Fig. 3. Body measurements of neonates were taken soon after birth, and those of mature adults were taken soon after the stabilization in individual sizes (no further growth occurred after

Table 1. Values of the linear dimensions of the egg (length and width) used for calculating the biovolume and biomass in wet weight, dry weight biomass, number of eggs produced throughout the life cycle, embryonic development time, and reproductive production of each cultivated individual of *Philodina roseola* (n = 9). Mean values and corresponding standard deviations for each variable is included. *Valores de las dimensiones lineales del huevo (longitud y anchura) para el cálculo del biovolumen y biomasa en peso húmedo y peso seco, número de huevos en el ciclo de vida, tiempo del desarrollo embriológico y producción reproductiva para cada individuo cultivado de* Philodina roseola *(n = 9). Se incluye valores medios y desviación estándar para cada variable.*

Measures used in the formula		Biovolume (μm⁻³)	WW Biomass (μg/egg)	DW Biomass (μg DW/egg)	No. of eggs	Embryonic development time (hours)	Reproductive production (μg DW/h)	Reproductive production (μg DW/d)
Length (μm)	Width (μm)							
69	41	102207	0.102207	0.0102207	20	29	0.0070488	0.16917
70	40	102625.4	0.102625	0.010262536	24	23	0.0107087	0.25701
70	41	105191	0.105191	0.010519099	23	27	0.0089607	0.215057
71	40	105578.5	0.105578	0.010557846	20	20	0.0105578	0.253388
71	40	105578.5	0.105578	0.010557846	26	26	0.0105578	0.253388
72	40	108573.4	0.108573	0.010857344	22	20	0.0119431	0.286634
72	40	108573.4	0.108573	0.010857344	24	18	0.0144765	0.347435
71	39	102939	0.102939	0.0102939	19	27	0.0072439	0.173853
71	40	105578.5	0.105578	0.010557846	23	25	0.0097132	0.233117
Mean and standard deviation		105205 ± 2342.222	0.105205 ± 0.002342	0.01052 ± 0.000234	22.33333 ± 2.291288	23.88889 ± 3.822448	0.0101345 ± 0.002298	0.243228 ± 0.055151

11 days of age). The secondary production invested in growth during the interval between neonate and juvenile stages was higher than that between juvenile and adult stages ($p < 0.05$). Furthermore, the total production invested in growth by *P. roseola* was 5.8 times less than the total production invested in reproduction (eggs).

DISCUSSION

The secondary production related to growth allocated to the period between neonate and juvenile stages corresponded to a biomass increase that was 1.3 times larger than the increment occurring between juvenile and adult stages. Analyzing the production invested in growth versus the reproduction in *P. roseola*, it can be seen that a larger fraction was channeled to reproduction (84.68 ± 3.65%) than for body growth. The slowdown in the somatic growth of this species coincided with the beginning of reproduction representing an energy trade-off between growth and reproduction that is often found in micrometazoans, which channelizes more energy into the body's growth until the first reproduction and thereafter allocates most matter and energy for reproduction (Snell & King, 1977).

Regarding body structure and growth of species of the Phylum Rotifera, it is important to point out that these invertebrates, despite having an alleged segmentation of the body, are not really segmented, because the apparent segments are not originated from metamerization. The division marks are actually local folds or shrinkage of the organism that possess a body cavity filled with liquid (pseudocoel), as do all blastocoelomates. They have a complete gut and a small and strictly determined number of cells (eutely), or more clearly speaking, a fixed number of nuclei, since many tissues are syncytial (Gilbert, 1983; Barnes *et al*., 2001; Wanninger, 2015). According to Ruppert and Barnes (1996), rotifers, therefore, grow only by enlargement of the syncytium, without multiplication of nuclei.

As reported by Lebedeva and Gerasimova (1985) for *Philodina roseola* and by Ricci and Fascio (1995) for two other bdelloids, *Macrotra-*

chela quadricornifera and *Philodina vorax*, these rotifers begin to reproduce while still continuing to grow (even though growth is not so expressive), thus suggesting that before starting reproduction these rotifers have to reach a minimum size. This has already been documented for other invertebrates, as, for example, for cladocerans (Perrin, 1989; Ebert, 1992).

A number of studies on the secondary production of rotifers and microcrustaceans were published in the 1970s, with the overall objective to review and systematize the techniques used in research on this topic (Winberg *et al*., 1971; Edmondson, 1974; Bottrell *et al*., 1976). There were also studies showing that several factors may influence survival, growth, reproduction, and biomass of a given species, particularly when *in situ* populations are studied (Snell & King, 1977; Duncan, 1984; Stemberg & Gilbert, 1985). Some of these factors are as follows: method of sample preservation, individual species characteristics; physical and chemical environmental factors are as follows: concentration of nutrients, temperature, quality and quantity of food, predation pressure, and variations in the genotype of local populations are very important for rotifers (Rossa *et al*., 2007).

Rotifers are influenced by a wide range of external factors, as already observed for *Philodina roseola* (Lebedieva & Gerasimova, 1985, 1987; Fischer *et al*., 2013). Among these, food availability and temperature are the most important, with temperature being especially relevant for the duration of the developmental stages and food availability for reproduction (Gophen, 1976; Bottrell *et al*., 1976; Pérez-Legaspi & Rico-Martínez, 1998). There are significant relationships between the body mass of rotifers and the concentrations of food to which they are exposed, a fact of great importance when interpreting production results (Duncan, 1984; Stemberger & Gilbert, 1985; Galindo *et al*., 1993). A range of algal concentrations were tested by Pilarska (1977) to evaluate the effects of food quantity on the growth and production of *Brachionus rubens*, finding that the optimum range for this rotifer was between 0.4 and 1×10^6 cells/mL of *Chlorella vulgaris*. The same range of con-

centrations was also used by Halbach-Keup (1974) to cultivate *Brachionus calyciflorus* with a diet of *Chlorella pyrenoidosa*. In our studies, the concentration of food used to grow *P. roseola* was $1 \cdot 10^5$ cells/mL of *Raphidocellis subcapitata*, a species with an average biovolume of 10.5 μm^3 (Fonseca *et al.*, 2014), which is about two times the 5.2 μm^{-3} of *Chlorella vulgaris*. *Brachionus rubens* also has a greater biomass (0.082 μg DW/ind) than *Philodina roseola* (0.050 μg DW/ind) adult individuals, which justifies the adequacy of the food concentration used.

A literature search reveals that information regarding the biomass and secondary production of rotifers cultured in laboratory are yet scarce. Data on the biomass of rotifers are slightly more abundant than in secondary production, so some values can be cited for cosmopolitan species as follows: *Keratella cochlearis*, 0.07 to 0.3 μg/ind (Dumont *et al.*, 1975); *Kellicottia longispina*, 0.04 to 0.1 μg/ind (Margalef, 1983); *Brachionus* sp., 0.11 to 0.4 μg/ind (Doohan, 1973); *Polyarthra* sp., 0.10 to 0.3 μg/ind (Dumont *et al.*, 1975); *Conochilus* sp. and *Conochiloides* sp., 0.10 μg/ind (Margalef, 1983); *Asplanchna* sp., 0.2 to 6.0 μg/ind (Salonen & Latja, 1988); and *Ploeosoma hudsoni*, 1.8 to 2.0 μg/ind (Margalef, 1983).

In relation to the secondary production of laboratory-cultivated organisms, a detailed description of the bionomics of *Brachionus rubens*, including the assessment of its production, was provided by Pilarska (1977), which reported the average value of 0.06199 μg DW/ind·day (value calculated by using her production data, expressed in energy units) for individuals fed with *Chlorella vulgaris*, at the concentration of 10^6 cells/mL.

In freshwater environments, natural or artificial, rotifer biomasses tend to vary widely over short intervals of time or space (Steele & Frost, 1977), being usually related to differences in the body weights according to the trophic state of the environment (Andrew & Fitzsimons, 1992; Baião & Boavida, 2005). Values reported for systems of varying trophic degrees suggest that the production of rotifers is directly related to the trophic state of the system, with more eutrophic environments having higher biomass and production

than oligotrophic systems (Sommer *et al.*, 1986). Nitrogen and phosphorus are known to regulate and significantly increase primary productivity when simultaneously added to the environment (Smith *et al.*, 2006; Elser *et al.*, 2007) by controlling the availability of resources for primary consumers, such as rotifers. Along with the nutrient enrichment, phytoplankton organisms can invest in growth (Pan *et al.*, 2014), not only increasing secondary production but also affecting community properties, such as rotifer and microcrustacean biomass (Sorf *et al.*, 2015).

Most studies carried out *in situ* with rotifers take into account only the secondary production related to the reproduction (number of eggs produced), disregarding the body growth of the organism. Body growth production can, however, be relevant, as shown in the present study for *P. roseola*, and the necessary data could be easily obtained from laboratory studies by following up the development throughout the whole life cycle. From our results, we recommend the use of laboratory experiments to provide the required information to the secondary production estimation of many other micrometazoans.

ACKNOWLEDGMENTS

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