Life history characteristics and production of Ceriodaphnia silvestrii Daday (Crustacea, Cladocera) under different experimental conditions.

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ABSTRACT Life history characteristics and production of Ceriodaphnia silvestrii Daday (Crustacea, Cladocera) under different experimental conditions. Effects of different food sources and culture medium on life history traits of Ceriodaphnia silvestrii were studied under controlled conditions. Animals were submitted to five different treatments: (1) autoclaved natural water filtered through plankton net of 20mm mesh size, supplied with Chlorella lacustris as food source; (2) autoclaved natural water filtered through plankton net of 20 mm mesh size, supplied with Scenedesmus bijugus; (3) soft reconstituted water supplied with S. bijugus; (4) soft reconstituted water supplied with S. bijugus plus humic substances (20 mg L^{-1}) and (5) hard reconstituted water supplied with S. bijugus. Observations were performed at 12 hours intervals from organism birth until its natural death. All treatments were kept at 25 ± 1°C. Results showed longer longevity in treatment 1 and better fecundity on treatment 2. In treatment 3 (with artificial culture medium), fecundity and growing parameters were lower than in natural water treatment (3). Increasing in water hardness did not result in better suitability of reconstituted water to C. silvestrii. The addition of humic substances resulted in greater fecundity and survival values. Body length-dry weight relationship was established: $W = 11.3 \times L^{2.52}$ (R^2=0.91). Secondary production was calculated, for each treatment, based on estimated biomasses increment and egg production. Treatment 2 showed the higher production (1.3 mg DW. $female^{-1}.day^{-1}$). Through the present study, it was possible to conclude that: S. bijugus is a relatively better food source for C. silvestrii than C. lacustris; the addition of humic substances in artificial culture medium resulted in better reproduction and development of C. silvestrii; natural water proved to be a better culture medium for C. silvestrii than artificial reconstituted water; and increase in reconstituted water hardness (recommended by EPA) did not result in a more suitable culture medium to tested animals.

Keywords: Ceriodaphnia silvestrii; Life history; reproduction, secondary production, humic substances, length-dry weight relationship.

RESUMO: Características bionômicas e produção de Ceriodaphnia silvestrii Daday (Crustacea, Cladocera) sob diferentes condições experimentais. Foram estudados os efeitos de cinco diferentes tratamentos nas características bionômicas de Ceriodaphnia silvestrii : (1) água do ambiente natural autoclavada e filtrada através de rede de plâncton (20mm) com fornecimento de Chlorella lacustris como alimento; (2) água do ambiente natural filtrada e autoclavada através de rede de plâncton (20mm) com fornecimento de Scenedesmus bijugus; (3) água reconstituída mole com fornecimento de Scenedesmus bijugus; (4) água reconstituída mole com fornecimento de S. bijugus mais substâncias húmicas (20mg L^{-1}) e (5) água reconstituída dura com fornecimento de S. bijugus. Características de crescimento e reprodução foram observadas a cada 12 horas desde o nascimento dos organismos até o momento de sua morte natural. A temperatura de cultivo para todos os tratamentos foi de 25±1 °C. Os resultados evidenciaram maior longevidade no tratamento 1 e maior fecundidade no tratamento 2. No tratamento 3 (meio de cultivo artificial), os valores dos parâmetros fecundidade e crescimento foram menores que no tratamento com água natural (tratamento 2). O aumento na dureza da água não afetou o crescimento e a reprodução de C. silvestrii. A adição de substâncias húmicas resultou em valores maiores de fecundidade e de sobrevivência. A relação peso-comprimento estabelecida para C. silvestrii foi $W = 11.3 \times L^{2.52}$ (R^2=0.91). A produção secundária foi calculada para cada tratamento a partir de estimativas do incremento em biomassa e da produção de ovos. O tratamento 2 apresentou a maior taxa média de produção total (1,3 mgPS.$fêmea^{-1}.dia^{-1}$). O presente estudo permite concluir

que: S. bijugus é um alimento de maior qualidade para C. silvestrii que C. lacustris; a presença de substâncias húmicas no meio artificial produziu uma melhora nos parâmetros de reprodução e desenvolvimento; a água do ambiente natural mostrou ser mais adequada que o meio de cultivo artificial; e que um aumento na dureza total não tornou o meio de cultivo artificial (recomendado pela EPA) mais adequado para o cultivo de C. silvestrii.
Palavras-chave: Ceriodaphnia silvestrii, historia de vida, reprodução, produção secundária, substâncias húmicas, relação peso seco-comprimento.

Introduction

Zooplankton plays an important role in trophic chains of aquatic ecosystems, significantly contributing to matter and energy flux from the primary producers to the top consumers (Abrantes & Gonçalves, 2003). Cladoceran zooplankters particularly, as efficient filter-feeders, take a relevant part on this flux. As generalist herbivores, these animals do not feed only on algae, but also on bacteria, organic detritus and dissolved organic matter (Arnold, 1971; Sarnelle, 1986; Vijverberg, 1989; Ojala et al., 1995; Boersma & Vijverberg, 1996; Abrantes & Gonçalves, 2003). For these reasons, it is of great interest to study mechanisms affecting its population dynamics and energy allocation strategies.

Some of the most important environmental factors controlling zooplankton growing and reproduction are temperature, food quantity and quality, and the physical environment suitability (Vijverberg, 1989; Fonseca, 1998). Effects of these factors are well known in the literature. Temperature is responsible for decreasing development time, instar intervals, time between clutches and longevity (Bottrell, 1975; Hardy & Duncan, 1994; Amarasinghe et al., 1997; Rietzler, 1998). Vanni & Lampert (1992) verified that Daphnia galeata reacts similarly to resource depression, regardless if it results from low food quantity or low food quality.

The observed effects of relatively lower quality and quantity of food on cladoceran species generally are delayed age and decreased size at maturity, smaller production of eggs and neonates (Hall, 1964; Boersma & Vijverberg, 1996; Díaz-Castro & Hardy, 1998; Rose et al., 2000; Abrantes & Gonçalves, 2003), and also reduced biomass production (Melão, 1997; Melão & Rocha, 2001) Ryther (1954) described toxicity and inhibitory effects of Chlorella species on filtering rates, survival, growth and reproduction of Daphnia magna.

In a classic study dealing with cladoceran life histories, Lynch (1980) pointed out life history strategies evolved by species of different sizes to avoid two main kinds of predators: invertebrates and vertebrates. Certain large species (e.g. large Daphnia) are able to grow to a size at which they are relatively invulnerable to invertebrate predators. The juveniles of these species maximize energy input into growth and postpone reproduction until they have attained a certain size. This strategy is of little value to the small-sized species that remain vulnerable to invertebrates throughout their lives. Instead, small species minimize the impact of invertebrate predation by initiating reproduction at earlier stages. Following the onset of reproduction they continue to grow, and thereby reduce predation by invertebrates.

Food quality and/or quantity can alter life history strategies of cladoceran species, leading to a greater vulnerability of some size classes to predators (Lynch, 1980; Schwartz & Ballinger, 1980). This flexible reproductive pattern may be an adaptation to a variable environment imposed by fluctuations of dominant algal species presenting different food quality characteristics.

For cladocerans, it can be observed that results due to culture medium variations, like water hardness, (Lewis ; Maki, 1981; Girling & Garforth, 1989 ; Abrantes & Gonçalves, 2003) are very similar to those obtained from food quality variations (Schwartz & Ballinger, 1980 ; Vanni & Lampert, 1992).

C. silvestrii, a common neotropical cladoceran species, occurs in natural environments which frequently have an expressive amount of dissolved organic matter (DOM). Gouvêa (2004) reported approximately $10 mgC.L^{-1}$. The importance of DOM on zooplankton diet is also emphasized in Salonen & Hammar (1986), which may consist of a significant source of energy for filter feeders as cladocerans.

However, there is little information about environmental factors' influence on life history traits of C. silvestrii. Fonseca (1991) studied life history traits of C. silvestrii at laboratory cultures, regarding some selected growing and reproduction parameters. Rietzler (1998) evaluated temperature effects on development time, reproduction and survival of C. silvestrii reared in natural water fed on natural seston.

Moreover, this tropical cladoceran species is recently standardized for ecotoxicological bioassays by Brazilian pollution monitoring institutions, CETESB, intending to substitute foreign species such as Daphnia similis and Ceriodaphnia dubia in ecotoxicological studies (ABNT, 2005).

The purpose of this study is to investigate the effects of variations on culture medium (natural or reconstituted water and different water hardness), food quality (two algae species as food source), and the presence of natural DOM, as humic substances, (in an equivalent concentration found in natural environments) on some life history traits of C. silvestrii, such as development times, age and size at first reproduction, clutch size, fecundity, longevity, medium and maximum body size. It was also obtained the body length-dry weight relationship of C. silvestrii, which allows, together with life history information, estimate female biomass and secondary production (growth and reproduction) in the different experimental conditions.

Materials and methods

Natural water and animals, which initiated stock cultures, were collected at Barra Bonita reservoir (22° 29´ S and 48° 34´ W, Barra Bonita, São Paulo state, Brazil).

Algal species used as cladocerans food source in this study (Scenedesmus bijugus and Chlorella lacustris) were cultured in WC medium (Guillard & Lorenzen, 1972), modified by Lombardi & Vieira (2000). Algal cultures were maintained in laboratory controlled conditions, under a 12-h/12-h light/dark cycle and at 25 ± 1°C.

C. silvestrii neonates were harvested from females cultured in laboratory controlled conditions. As soon as they were born, each neonate was measured by a micrometer eyepiece, fitted to a Leica MZ6 optical microscope, and individually placed in test tubes vessels, containing 50 mL of the tested medium. The animals were maintained at a temperature of 25 ± 1 °C, in a 12-h/12-h light/dark cycle. Algae were supplied in a concentration of 10^5 cells mL^{-1}. Daily, the medium was replaced by a fresh one, containing fresh food. The animals were observed every 12 hours since its birth until natural death, except for the treatment with humic substances, which was carried out for a period of 15 days. Stock cultures of C. silvestrii were previously acclimated for a month to each experimental condition (temperature, food concentration, tested culture medium).

Growing and reproduction parameters of C. silvestrii were daily recorded during the time of experiment, and were defined based on Vijverberg (1989) and Bottrell (1975). Life history parameters were: body length, age at primipara, size at primipara, number of instars, instar of primipara, longevity, number of eggs per female, number of neonates per female, percentage of eclosion, embryonic period and time between clutches.

Animals were submitted to five different treatments (Tab. I). Natural water, used in treatments 1 and 2, was previously autoclaved and filtered through plankton net of 20 mm mesh size. For treatment 4, it was used IHSS Suwannee River Humic Substances (7.0% ash, 52.47% C, 4.19% H, 42.69% O, 1.10% N, 0.65% S, and 0.02% P).

Artificial medium used was reconstituted soft fresh water (U.S. EPA, 2002), with hardness of 44.0 mg $CaCO_3$ L^{-1} (treatments 3 and 4, and in stock cultures) and hard water, with hardness of 150.0 mg $CaCO_3$ L^{-1} (treatment 5).

Table I: Experimental conditions of the five tested treatments on C. silvestrii culturing.

Treatment	Culture medium	Algae	Hardness	Humic subst.	Replicates
1	natural	C. lacustris	soft	-	12
2	natural	S. bijugus	soft	-	15
3	reconstituted	S. bijugus	soft	-	17
4	reconstituted	S. bijugus	soft	20mg L^{-1}	9
5	reconstituted	S. bijugus	hard	-	16

Differences among the five treatments were analysed with one-way ANOVA followed by the Tukey multiple comparison test when parameters were parametric and with Kruskal-Wallis (non-parametric ANOVA) followed by Dunn´s multiple comparison test when they were not (Zar, 1999).

To establish body length-dry weight relationship for C. silvestrii, 20-40 non-preserved individuals of similar sizes from stock cultures (kept in similar conditions to treatment 2) were harvested, measured by a micrometer eyepiece fitted to a Leica MZ6 microscope, and placed inside aluminum vials, previously dried (60°C for 24 hours), and weighed on a Mettler Toledo UMT2 microbalance (accuracy of 0.1 mg). Three size classes were chosen: neonates up to 24 hours of age, juveniles and adult females (without eggs). There were at least three replicates for each size class. This method is similar to those described in Bottrell et al. (1976) and Mansudire (1994).

Mean dry weights and body length measurements were used to obtain the length dry-weight relationship, as follows:

$W = a.L^b$, where

W is dry weight (mg), L is body length (mm), ´a´ and ´b´ are constants. Regression equation was derived using Origin 6.0™ software. Animal biomasses of each treatment were estimated, based on length-dry weight relationship obtained by the method described above. Secondary production was estimated following concepts and methods described by Edmondson & Winberg (1971), Winberg (1971) and Downing & Rigler (1984). Total production (P) of the experimental population was calculated by summing production related to growth (Pg) and that related to reproduction (Pr). Pg is the individual biomass increment during the experimental period, while Pr was obtained by multiplying the number of eggs produced by each female during the experiment by each egg weight (0,21mg). Individual growth production (Pg) was calculated for each treatment using mean initial (M_0) and final (M_t) individual biomass after t days, as shown below:

$$Pg = (Ln\ M_t - Ln\ M_0)\ t^{-1}$$

Results

Development, growing and reproduction parameters of C. silvestrii were significantly affected by the five different treatments (as shown in Tab. II).

Animals reared in treatment 2 produced neonates before any other (mean of 2.63 days), followed by treatment 4 (2.89 days), treatment 3 (3.09 days), treatment 5 (3.21 days) and treatment 1 (4.21 days) (Tab. III).

Treatment 2 showed the highest value for mean values of neonates per female (see Fig. 1). During all life cycle, females of

Table II: Statistical analysis of selected life-history parameters of C. silvestrii at five different treatments. Different letters (a, b, c and d) indicate statistically significant differences.

Parameter	Test applied (**)	P	Treatment				
			1	2	3	4	5
Age at primipara (days)	KW / D	‹ 0.0001	c	b	a	ab	a
Size at primipara (mm)	KW / D	‹ 0.0001	ab	cd	bcd	bcd	a
Instar of primipara	KW / D	‹ 0.0001	ab	b	a	a	a
Total number of instars	KW / D	‹ 0.0001	b	b	a	a	a
Longevity (days)	KW / D	‹ 0.0001	b	b	a	nd*	a
Number of eggs / female	KW / D	‹ 0.0001	b	b	a	c	a
Number of neonates / female	KW / D	‹ 0.0001	b	b	a	c	a
Embryonic period (days)	A / TK	‹ 0.0001	b	ab	d	b	bc
Time between clutches (days)	A / TK	‹ 0.0001	b	b	a	b	b
Body length (mm)	KW / D	‹ 0.0001	b	b	a	c	a
Secondary production (P)***	A / TK	‹ 0.0001	bc	a	d	c	d
Growth production (Pg)***	A / TK	‹ 0.0001	a	b	c	b	c
Reproductive production (Pr)***	A / TK	‹ 0.0001	b	a	c	b	c

(*) nd: not determined up to the end of experiment; (**) A = Anova, KW = Kruskal-Wallis; TK = Tukey-Kramer; D = Dunn´s. (***) mg $DW.female^{-1}.day^{-1}$

this treatment produced the highest mean number of neonates, 107.07 (Tab. III). The lowest mean number of neonates per female was verified in treatment 3, with just 7.41 neonates. Treatments 1 and 2 showed the highest values for eclosion percentage, which were very similar (89.30% and 85.65%, respectively). Treatment 5 had an eclosion of 78.06%, followed by treatment 4, with 74.42%, and treatment 3, with 52.92% (Tab. III).

Food source did not result in statistically significant differences, on time between clutches, among treatments 1 and 2: 1.64 and 1.66 days, respectively (Tab. III). Among all treatments, treatment 3 showed a slightly longer time (1.90 days).

Fig. 2 illustrates the influence of environmental conditions on fecundity parameters, such as accumulated number of neonates per female. Treatment 2 showed higher values of neonates per female, followed by treatment 1.

Fig. 3 illustrates the influence of the five tested treatments on proportion of aborted eggs by females of C. silvestrii. Females submitted to treatments 3 and 4 had faster increasing of proportion of aborted eggs early in their lives. Females from treatments 1 and 2 had a similar lower proportion up to old age, when just before senescence and death they had an increasing of aborted eggs. The increasing in hardness postponed high proportion of aborted eggs.

Treatment 1 was responsible for the greatest survival and treatment 3 for the lowest one (see Fig. 4). Mean body lengths per instar are given in Fig. 5. Animals of treatment 2 showed the greatest body length and the fastest growth. Animals reared in treatment 1 grew slower, and showed the greatest mean longevity (36.0 days), followed by those of treatment 2 (28.8 days) (Tab. III).

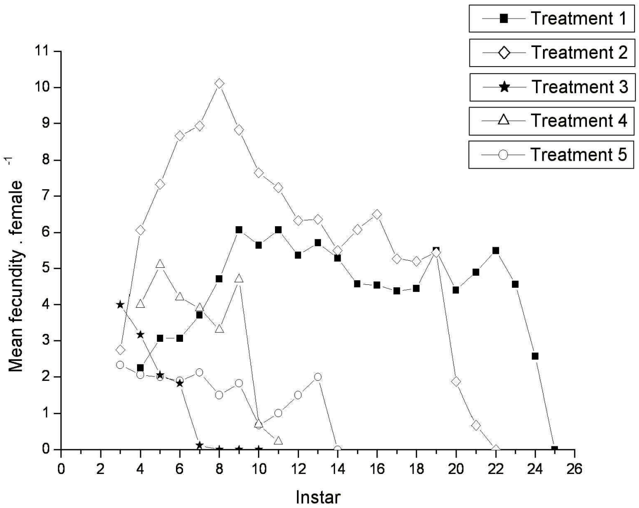

Figure 1: Mean fecundity per female per instar of C. silvestrii from five different treatments: 1-5.

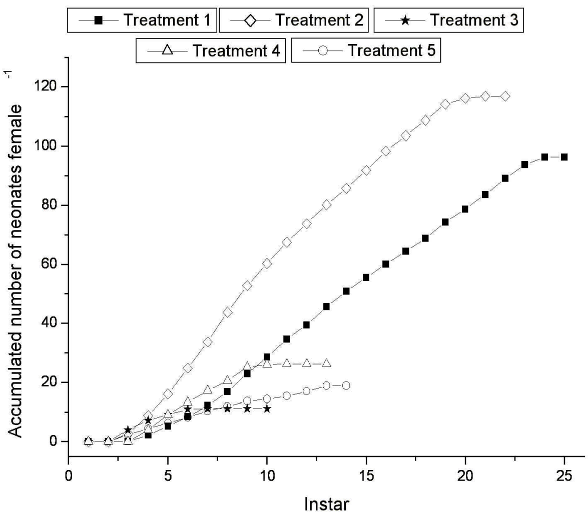

Figure 2: Accumulated number of neonates female^{-1} in relation to instars of individuals of C. silvestrii submitted to five different treatments: 1-5.

Table III: C. silvestrii development, growing, reproduction parameters and production estimates found in life history experiments, from five different treatments (1-5). The last four columns present data from C. silvestrii obtained by other authors

Parameters	Treatments										Other Authors			
	1 (N=12)		2 (N=15)		3 (N=17)		4 (N=9)		5 (N=16)		Fonseca, 1991		Rietzler, 1998	
	Mean values	S.D.	Mean values	S.D.	Mean values	S.D.	Mean values	S.D.	Mean values	S.D.	Mean values	S.D.	Mean values	S.D.
Age at primipara (days)	4.21	0.68	2.63	0.23	3.09	0.32	2.89	0.22	3.21	0.61	-	-	4.60	0.63
Size at primipara (mm)	0.61	0.03	0.72	0.06	0.66	0.03	0.64	0.02	0.66	0.04	0.77	-	0.69	0.04
Instar of primipara	4.17	0.39	3.27	0.46	3.94	0.24	4.00	0.00	4.00	0.61	-	-	-	-
Number of instars	23.00	2.62	18.33	3.06	9.29	0.69	11.33	0.60	10.12	1.66	-	-	-	-
Longevity (days)	36.00	3.64	28.80	4.82	14.76	1.03	› 16.00	-	16.69	3.14	29.8	6.89	17.22	2.86
Number of eggs / female	101.08	41.27	124.63	32.87	14.41	2.66	36.66	6.71	16.26	4.49	-	-	32.00	17.14
Number of neonates / female	91.83	41.42	107.07	29.71	7.41	1.91	27.77	8.70	12.00	4.68	86.00	-	4.13*	2.21
Eclosion (%)	89.30	9.64	86.66	6.90	62.92	16.89	74.42	16.87	78.06	16.01	-	-	-	-
Embryonic period (days)	1.66	0.06	1.62	0.04	1.73	0.09	1.61	0.06	1.64	0.11	-	-	1.33	0.47
Time between clutches (days)	1.64	0.09	1.66	0.06	1.90	0.28	1.60	0.04	1.66	0.12	-	-	-	-
Body length (mm)	0.91	0.06	1.02	0.03	0.74	0.02	0.82	0.06	0.76	0.03	1.04	0.04	0.86	-
Secondary production (P)**	0.81	0.22	1.30	0.18	0.60	0.07	0.90	0.12	0.62	0.06	-	-	-	-
Growth production (Pg)**	0.23	0.03	0.39	0.06	0.30	0.04	0.39	0.06	0.31	0.06	-	-	-	-
Reproductive production (Pr)**	0.58	0.21	0.91	0.18	0.20	0.04	0.62	0.09	0.21	0.04	-	-	-	-
Pg / Pr	0.40	-	0.43	-	1.60	-	0.76	-	1.47	-	-	-	-	-

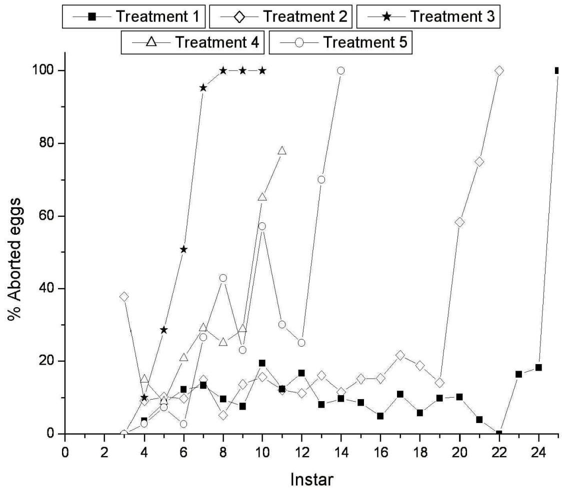

Figure 3: Proportion of aborted eggs (%) in relation to instar of females of C. silvestrii submitted to five different treatments: 1-5.

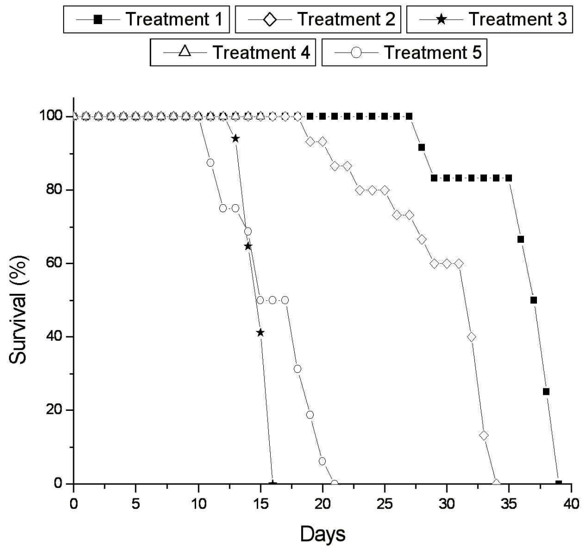

Figure 4: Survival (%) of individuals of C. silvestrii submitted to five different treatments: 1-5.

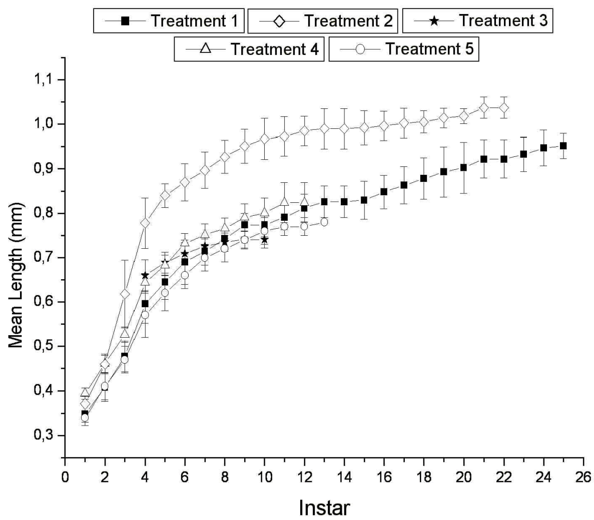

Figure 5: Mean body length (mm) per instar of Ceriodaphnia silvestrii submitted to five different treatments: 1-5. The error bars give the S.D.

The maximum mean body length was registered in treatment 2 (1.02 mm), while the minimum in treatments 3 and 5 (0.74 and 0.75 mm, respectively) (Tab. III). Mean number of molts of these two treatments showed similar values: 23.0 for the first one, and 18.3 for the second one.

Adult females of C. silvestrii, used to length-dry weight relationship determination, varied in mean body length from 0.719 to 0.753 mm and in mean individual dry weight from 4.81 to 5.85 mg. The ranges of similar measurements for other size classes are also given in Tab. IV. The length-dry weight regression equation and plots of dry weight against body length can be seen in Fig. 6.

Through the obtained length-dry weight relationship (Fig. 6), it was possible to estimate the biomass of animals submitted to each treatment, in each instar, throughout its life (Fig. 7). It was also possible to estimate the secondary production (P), constituted by both production related to growth (Pg) and

Table IV: Data used for determination of length-dry weight relationship for C. silvestrii: mean body length (mm) and mean individual dry weight (mg), from three life stages (neonate, juvenile and adult).

	N*	Mean body Length (mm)	Mean indiv. Weight (µg)
Neonates	3(120)	0.341 ± 0.006	1.337 ± 0.169
Juveniles	3(60)	0.561 ± 0.013	2.113 ± 0.159
Adults	5(100)	0.736 ± 0.017	5.335 ± 0.523

* Number of replicates. In parenthesis, total number of individuals measured.

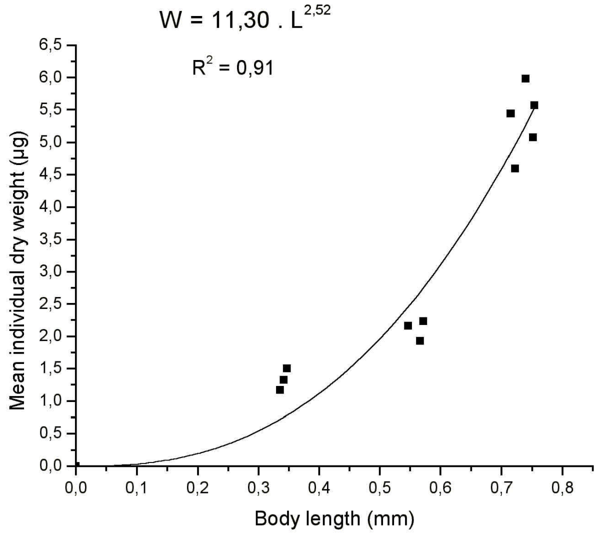

Figure 6: Relationship between body length (mm) and dry weight (mg) of C. silvestrii.

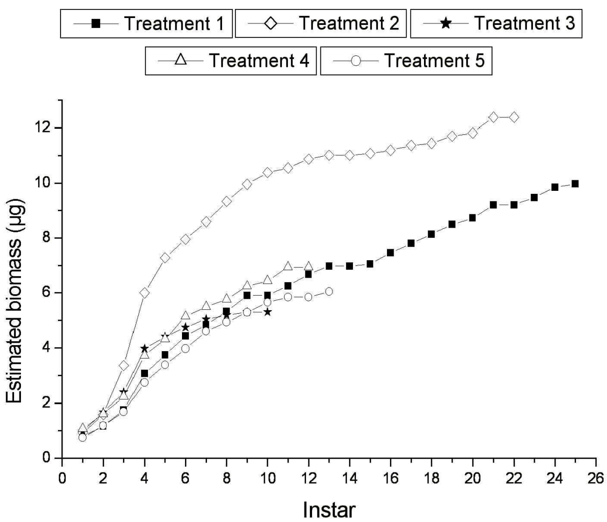

Figure 7: Biomass of animals submitted to the five different treatments (1-5), estimated based on the obtained length-dry weight relationship.

reproduction (Pr) (Tab. II and III). Results of accumulated production also clearly show the effects on females submitted to the five treatments (Fig. 8).

Treatment 2 had the greatest secondary production value (1.30 mg DW female^{-1} .day^{-1}), followed by treatment 4 (0.90), treatment 1 (0.81), treatment 5 (0.52) and treatment 3 (0.50).

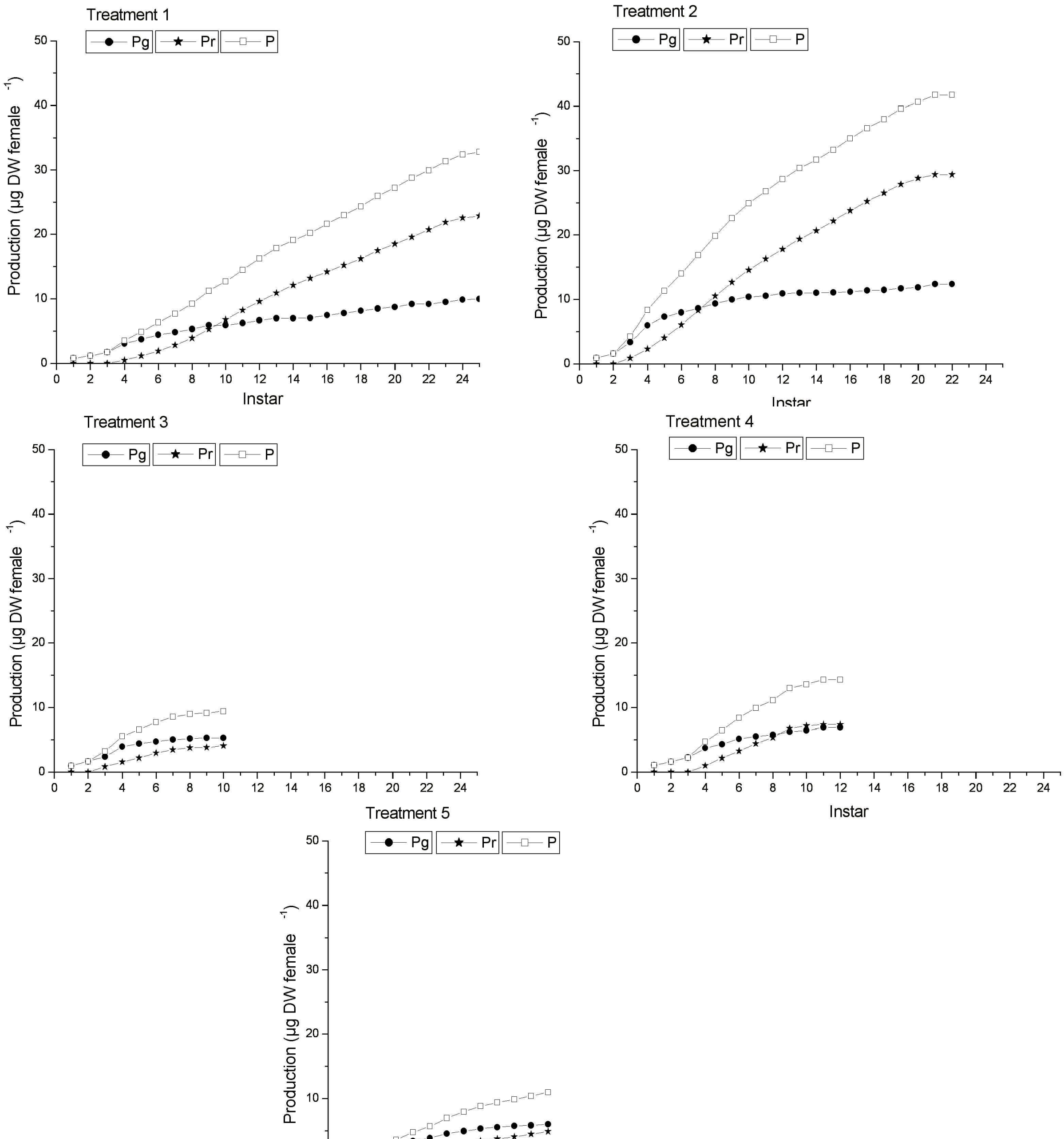

Figure 8: Secondary production of females submitted to all treatments (1-5). P = Total secondary production, Pg = Growth production, and Pr = Reproductive Production (□g DW.female^{-1}.instar^{-1}).

Discussion

In all treatments of the present work, in the same way as described by Bottrell (1975) for eight cladoceran species, adult females of C. silvestrii passed through the following cycle of events: development of eggs and release of fully developed neonates from the brood chamber; molting accompanied by an increase in body size; and release of a new set of eggs from the ovaries into the brood chamber. Moreover, the duration of adult instars was very similar to time between clutches and embryonic period.

Effects of humic substances

The addition of humic substances in reconstituted water (treatment 4) resulted in higher values of reproduction and survivorship, such as neonates per clutch (Fig. 1), survival (Fig. 4), time between clutches, embryonic period, mean longevity, number of eggs and neonates per female during all its life, percentage of eclosion, and body length (Tab. II and III), in comparison with treatment 3. Other parameters such as age at first reproduction, size at primipara, instar of primipara, and total number of instars, showed statistically similar values. The addition of humic substances also shifted the relation Pg/Pr from 1.5 in treatment 3 to 0.75 in treatment 4, thus showing an inversion on energy allocation strategy (Tab. III). Females reared in treatment 3 showed the most accentuated curve of aborted eggs (Fig. 3), which was slightly attenuated by the presence of humic substances (treatment 4).

The addition of DOM was sufficient to obtain better growing and reproduction results for C. silvestrii, since it can also be utilized as food source for cladocerans (Arnold, 1971; Vijverberg, 1989; Boersma & Vijverberg, 1996). This result could also be explained by an addition of nutrients (7.0% ash present in humic substances samples) in the synthetic medium (Girling & Garforth, 1989). Salonen & Hammar (1986) demonstrated the importance of DOM in the nutrition of zooplankton in highly humic lakes, frequently found in tropical and subtropical regions. They showed that several cladocerans species are able to utilize allochthonous organic matter as food source, either directly or via other organisms (e.g., bacteria). They obtained good growth and reproduction results for planktonic crustaceans in the absence of photosynthetic production. Although allochthonous DOM is generally considered refractory or of little value as energy source, it is composed of numerous molecules. Since the concentration of allochthonous DOM is very high in some lakes, even a small biologically available proportion may be quantitatively important as a source of carbon for food webs, either directly or indirectly.

Effects of food quality

Considering treatments 1 and 2, whose difference was only the algae supplied as food, some parameters showed significant differences. Females fed on S. bijugus (treatment 2) had its first reproduction at earlier age and earlier instar, and showed a greater primipara size (Tab. III). Fecundity parameters were affected by food quality, although statistical analysis did not show significant differences. Although the relation Pg/Pr was similar (0.40 and 0.43), differences on P, Pg and Pr values of treatments 1 and 2 were statistically significant (Tab. II).

It is well established that some species of Chlorella can liberate toxins in the water, thus altering growth and reproduction of cladocerans (Ryther, 1954; McMahon & Rigler, 1965; Infante & Litt, 1985). Greater longevity and lower fecundity are some of the responses in reaction to the presence of toxic substances in the water (Vijverberg, 1989). Results of treatment 1 in comparison with treatment 2 suggest a possible toxic effect. The relatively high quality of Scenedesmus bijugus permitted animals to have more eggs per clutch, more neonates per clutch, greater body length, earlier primipara and a greater size at first reproduction (as shown in Tab. III). Lower longevity is adopted in favor of a greater reproduction when organisms are fed on high quality food (Vijverberg, 1989). Other authors obtained similar results when feeding cladoceran species with Chlorella. Lundstedt & Brett (1991) concluded that Scenedesmus acutus is a higher quality food than Chlorella homosphaera to Daphnia longispina, Bosmina longispina and Chydorus sphaericus. Infante & Litt (1985) obtained poor growth and reproduction culturing Daphnia pulicaria and Daphnia thorata with Chlorella sp. These results were attributed to inhibitory effects and decreasing of filtering rates caused by Chlorella toxins (Ryther, 1954; McMahon & Rigler, 1965; Infante & Litt, 1985).

Two patterns of reproduction became apparent in treatments 1 and 2. In the first one, reproductive maturity was delayed and brood size was smaller, but a longer longevity was observed. On the other hand, early maturity with high fecundity and shorter longevity were observed in animals fed on S. bijugus. Thus, C. silvestrii, in a rich environment, would be able to take advantage of high quality sources available. In a less favorable environment (lower quality food), C. silvestrii may not reproduce at the same rate, but its life is lengthened perhaps due to decreased reproductive stress. Similar results were found by Schwartz & Ballinger (1980) for Daphnia pulex fed on Pediastrum duplex (relatively high quality) and Melosira ambigua (relatively low quality), and by Vanni & Lampert (1992) to Daphnia galeata fed on Scenedesmus acutus (relatively high quality) and Oocystis lacustris (relatively low quality).

Females reared in treatment 2 showed low proportion of aborted eggs until its senescence and natural death, as well as females from treatment 1 (see Fig. 3). In spite of a longer longevity, which delayed the increasing aborted eggs rate of females from treatment 1 by some instars, this parameter was similar for both treatments. According to Vijverberg (1989), this is a typical reproductive behavior of Cladocera, which generally produce large broods up to old age, and before senescence and death there may be a short period with smaller broods and a high proportion of aborted eggs. Such phenomena can be observed also in Fig. 1.

Effects of culture medium

Culture medium comparisons produced the most pronounced differences on both growing and reproduction parameters of C. silvestrii submitted to treatments 2 and 3 (Tab. III). All parameters showed statistically significant differences (Tab. II): females of treatment 2 reproduced at an early age and instar, had a greater size at primipara and mean body length, had greater longevity, production of eggs and neonates per female, eclosion percentage, number of instars, and had lower time between clutches and embryonic period in comparison to females of treatment 3. Females reared in treatment 3 showed the most accentuated curve of aborted eggs (Fig. 3), when compared with treatment 2 curve. Secondary production values also indicate these pronounced differences.

The major differences revealed in this study were between treatments 2 and 3. Even after acclimation period, reconstituted water was less adequate to culturing this cladoceran species in experimental conditions, in spite of hardness adjustment. Abrantes & Gonçalves (2003) found similar results when culturing Ceriodaphnia pulchella both in natural and synthetic medium. C. pulchella reared in ASTM hard water fed on Selenastrum capricornutum had lower mean body length and grew more slowly. Moreover, animals delayed to reach the first reproduction and showed the smallest size at first reproduction. Girling & Garforth (1989) tested the influence on variations in culture medium on the survival and reproduction of Daphnia magna. These authors compared EPA reconstituted water with filtered river water for D. magna during 28 days. They registered a greater number of neonates per female and survival rate on river water treatment. When blends of both waters were tested, better results for these two parameters were obtained in treatments which had the highest percentages of natural water. Vijverberg (1989) also pointed out that artificial waters prepared using distilled water can give poor culture results.

However, there are many arguments for the use of a synthetic medium for culture zooplankton at laboratory-controlled conditions: the most important is that a synthetic medium has a known and reproducible composition. This cannot be guaranteed when natural waters (surface-, spring-, well, tap-water) are used for zooplankton culturing. Each laboratory has its own natural water, which further complicates the interpretation of results. Moreover, a synthetic medium is more certainly free from pesticides, pollutants and DOM than most of natural waters, which can be undesirable to the purposes of zooplankton cultures.

Effects of hardness

The increasing in water hardness produced statistically significant differences on size at primipara (0.66 mm and 0.56 mm), embryonic period (1.73 d and 1.54 d) and time between clutches (1.90 d and 1.65 d) for females of C. silvestrii submitted to treatments 3 and 5, respectively (see Tab. II and Tab. III). All other observed parameters did not show significant differences, such as all secondary production values (Tab. II).

Increases in water hardness, as an attempt to improve the suitability of artificial culture medium did not result in significant differences on growth and reproduction parameters (Tab. II) between treatments 3 and 5. Fonseca (1998) observed better survival and fecundity of C. silvestrii reared in soft water (4.0 mg $CaCO_3$ L^{-1}) than in hard water (143.0 mg $CaCO_3$ L^{-1}). Fonseca & Rocha (2004), culturing C. silvestrii on natural hard water (143.0 mg $CaCO_3$ L^{-1}), obtained higher values for fecundity and similar values for body growth. Treatments tested in the present study suggest that greater water hardness did not result in better reproduction and growth of C. silvestrii reared in laboratory cultures, nor in an improvement of the suitability of U.S. EPA (2002) reconstituted water to this cladoceran species.

Biomass and Secondary Production

Length-dry weight regression equation was in agreement with other studies (Dumont et al., 1975; Bottrell et al., 1976; Persson and Ekbohm, 1980; Vijverberg, 1989). As expected, b constant varied near 3.00 (2.52 in this study), indicating that body weight increases exponentially with length. Dumont et al. (1975) reported similar results for Ceriodaphnia quadrangula ($W = 1.7 \times L^{2.26}$) and Ceriodaphnia reticulata ($W = 5.91 \times L^{2.02}$), and Maia-Barbosa & Bozelli (2005) for Bosmina hagmanni ($W = 11.93 \times L^{2.68}$) and Ceriodaphnia cornuta ($W = 4.23 \times L^{1.89}$). The separation of individuals in size classes for further weighting was a good method for length-dry weight determination, since size is closely related to the age of animals, and animals of similar sizes present similar weights. Moreover, the same equation fitted well to all treatments, as demonstrated by the agreement between secondary production values (which were calculated based on such equation results) and the obtained life history parameters.

Secondary production values clearly show the amount of energy allocated by C. silvestrii between growth and reproduction, among the different environmental conditions tested in the present study. It was clear that animals reared in good food and medium conditions allocated a greater amount of energy on reproduction, while when they were in a poor environment, energy was primarily allocated in surviving and maintenance (Tab. III). Duncan (1989) describes the same behavior, when studying planktonic rotifers and cladocerans. The present results are in accordance with life history strategies described by Lynch (1980) for large-sized cladoceran species: when in good environmental conditions, they initially invest a greater amount of energy in growth and, after reaching maturity, they allocate nearly all of their energy to reproduction (Fig. 8, treatments 1 and 2).

Finally, Tab. III also shows a comparison of results of the present study with other authors, which also investigated life history of C. silvestrii under controlled conditions. Rietzler (1998) studied life histories responses of C. silvestrii under the following conditions: water from the natural habitat of the tested organisms (Barra Bonita Reservoir, São Paulo State, Brazil), fed on natural seston, at 25°C. Water hardness and pH were not considered by this author. C. silvestrii fed on natural seston had its primipara at later instars, were smaller at first reproduction, had smaller body length, and production of eggs and neonates per female than in natural water treatments (1 and 2) of the present study.

Fonseca (1991) cultured C. silvestrii in natural water (pH ~7.0, water hardness of ~150.0 mg $CaCO_3$ L^{-1}), fed on Monoraphidium dybowskii (10^5 cells mL^{-1}), at 25°C. Females submitted to this culture condition had greater fecundity, size at primipara and body length when compared to Rietzler (1998) data. Fonseca (1991) growth and longevity data were similar to data from treatment 2 of the present study, except that fecundity parameter was lower (85 neonates per female against 107 neonates per female in treatment 2). Abrantes & Gonçalves (2003), studying Ceriodaphnia pulchella, also demonstrated that food conditions altered all these life history parameters, as can be seen in Tab. III, comparing results of treatments 1 and 2 from the present study with Rietzler (1998) data.

Conclusion

Through the present study, it was possible to conclude that S. bijugus is a relatively better food source for C. silvestrii than C. lacustris in laboratory cultures, based on the observed life history parameters. The relatively higher quality food provided earlier primipara, a greater size at primipara, and a shorter longevity, while the relatively lower quality food resulted in longer longevity, later primipara, and a smaller size at maturity. It was also verified that the addition of humic substances in artificial reconstituted water resulted in better development, growing, reproduction and survival values for C. silvestrii. Natural water proved to be better culture water for C. silvestrii than artificial reconstituted water, as shown by the life history parameters. Increasing water hardness did not improve U.S. EPA reconstituted water for C. silvestrii.

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