The life-cycle of Ceriodaphnia silvestrii Daday, 1902, a Neotropical endemic species (Crustacea, Cladocera, Daphnidae).

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ABSTRACT: The life-cycle of Ceriodaphnia silvestrii Daday, 1902, a Neotropical endemic species (Crustacea, Cladocera, Daphnidae). Considering the scarcity of detailed information on the life cycle of Neotropical cladocerans, a study was conducted to get the necessary information to maintain cultures of Ceriodaphnia silvestrii in laboratory, aiming its use as a standard test-organism in ecotoxicological tests. Additionally, the life history information may help to understand the functional role of the species in natural communities, since it has a wide geographical distribution and sometimes occurs in high densities. The cladoceran culture was kept under constant conditions (Monoraphidium dybowskii at 10^5 cells.mL^{-1}, photoperiod 12h light : 12h dark and water temperature of 25 ± 2ºC). The parameters observed were: individual growth, population growth, biomass, fecundity and longevity. The following results were obtained: final body length of 1.0mm; intrinsic natural rate of increase (r) of 0.34 d^{-1}; fecundity of 9.46 ± 4.17 eggs per female; longevity of 29.8 ± 5.89 days; size at maturity was 0.57 ± 0.04mm, and dry weights for neonates and primipara were 3.8 ± 1.69 and 9.7 ± 3.3 mg, respectively. This species can be readily cultured in the laboratory, and had excellent performance in the conditions tested. It can therefore be used for ecotoxicological tests.

Key-words: Zooplankton, life cycle, Cladocera culture, Ceriodaphnia silvestrii.

RESUMO: Ciclo de vida de Ceriodaphnia silvestrii, Daday, 1902, espécie endêmica Neotropical (Crustacea, Cladocera, Daphnidae). Considerando a escassez de informações sobre o ciclo de vida de cladóceros da região Neotropical, o presente estudo foi realizado com a espécie Ceriodaphnia silvestrii Daday,1902, com objetivo de manter o cultivo desta espécie em laboratório, visando a sua padronização como organismo-teste nos ensaios ecotoxicológicos. Além disso, visou contribuir com informações relevantes sobre seu ciclo de vida que sirvam de subsídio para a avaliação do seu papel funcional nos ecossistemas em que ocorre, visto que é uma espécie de ampla distribuição geográfica. As culturas de Ceriodaphnia silvestrii foram mantidas sob condições constantes: concentração alimentar de 10^5 céls.mL^{-1}, da alga clorofícea Monoraphidium dybowskii, fotoperíodo de 12 : 12h claro/escuro e temperatura de 25 ± 2ºC. Os parâmetros do ciclo de vida quantificados foram: crescimento individual, crescimento populacional, biomassa, fecundidade e longevidade. O comprimento máximo do corpo foi de cerca de 1,0mm, 0,34 d^{-1} para taxa intrínseca de aumento natural (r), fecundidade de 9,46 ± 4,17 ovos/fêmeas, longevidade de 29,8 ± 5,89 dias, comprimento de primeira maturação de 0,57 ± 0.04mm, e peso seco individual para neonatas e primíparas foi de 3,8 ± 1.69 e 9,7 ± 3,3 mg, respectivamente. Esta espécie é de fácil cultivo em laboratório, e tem excelente desempenho nas condições testadas, podendo portanto ser utilizada em ensaios ecotoxicológicos.

Palavras-chave: Zooplâncton, Ciclo de vida, cultivo de Cladocera, Ceriodaphnia silvestrii.

Introduction

Ceriodaphnia silvestrii, was described by Daday, 1902 and named in honour of the collector of the material, Dr. Fillipo Silvestri (Santa Cruz and Chubut – Patagonia, Argentina; during 1899-1900). This species can be rapidly diagnosed among Ceriodaphnia species by the presence of an accentuated cervical sinus, large eye almost filling the whole anterior portion of the head, 9-12 anal spines, and a claw with a pecten (Olivier, 1962; El–Moor-Loureiro, 1997). It is a very common species in Brazilian freshwaters, especially in reservoirs of the states of São Paulo, Rio Grande do Sul, Goiás and Distrito Federal (El-Moor-Loureiro, 1997; Rocha & Guntzel, 2000). Information on species life-cycle is important for the evaluation of population functional role in the community, and is also essential for the calculation of secondary production.

Zooplankton life cycles, including data on temperature and food influences are very common for the temperate region and have increased substantially in tropical region (Bandu-Amarasinghe et al., 1997), for several species of Cladocera occurring in Brazilian freshwaters (Rietzler, 1998; Diaz-Castro & Hardy, 1998; Sipaúba-Tavares & Bachion, 2002; Bunioto & Arcifa, 2003; Guntzel et al., 2003). These studies have significantly contributed to better understanding the ecological interactions among species playing important roles in aquatic food-chains.

C. silvestrii was chosen for this study due to some bionomical characteristics, such as its wide geographical distribution throughout South America, its abundance, short life-cycle and culture facility. Chronic toxicity tests should be assessed rapidly. Since the test organism must have a short life-cycle, Ceriodaphnia is more frequently used than Daphnia (Lynch, 1980). Endemic species are preferable to alien ones, since the latter can be accidentally introduced in natural ecosystems, causing serious damage to native species. This problem is nowadays considered a primary factor of biodiversity losses in many countries. Therefore, the replacement of C. dubia by C. silvestrii is interesting in the sense of preventing the use of the former, an exotic and introduced species. The use of C. silvestrii primarily in chronic toxicity tests, instead of C. dubia, is envisaged based on the fact that preliminary studies have shown its sensibility to be equal or greater than that of the latter species, when exposed to a number of reference substances (Oliveira-Neto & Botta-Paschoal, 2000).

The aim of this investigation is to contribute with detailed information on the life cycle of Ceriodaphnia silvestrii, by culturing the species under controlled laboratory conditions, and monitoring the individual growth, population growth, biomass, fecundity and longevity. This knowledge is fundamental for the establishing standard procedures in ecotoxicological bioassays. Being an endemic Neotropical species with wide geographical distribution and common occurrence in Brazilian freshwaters, there are many advantages in using C. silvestrii as a test-organism.

Materials and methods

Zooplankton sampling

Ceriodaphnia silvestrii individuals were collected from plankton tanks in the Experimental Reserve of the Federal University of São Carlos Campus. This population has been maintained isolated in a mesocosm, for more than 10 years. Individuals were kept in 2L glass flasks with water from the same source (1,000L concrete tank), previously filtered through a 68 mm plankton net. They were fed with an algal suspension of Monoraphidium dybowskii at a concentration of 10^5 cells.mL^{-1}, taken from exponentially growing cultures. Organisms were maintained at 25°C, under a light/dark regime of 12 : 12h, during four generations (approximately 15 days) before starting the life cycle experiments.

Food supply

The inoculum of Monoraphidium dybowskii. (strain 005 CH) was obtained from the Laboratory of Algae Physiology and Biochemistry in the Botany Department of the Federal

University of São Carlos, and cultured in Chu 12 medium (Rocha, 1983), previously autoclaved at 100ºC for 20 min. Cultures were maintained at 25±1ºC and under 5,200 lux illumination from fluorescent lamps. A photoperiod of 12 : 12h light/dark and continuous aeration were used.

Life Cycle Parameters

The fecundity (eggs/female), of a recently collected sample, was determined by counting the eggs in the brood chamber. Fifty ovigerous females (approximately 10eggs/female) were isolated and transferred to a beaker containing three litres of filtered water (mesh of 68mm). Each day the total number of individuals was counted, and food added (a suspension of 10^5 cells.mL^{-1} of Monoraphidium dybowskii). Water and food suspension were totally renewed every two days. The experiment lasted 20 days, under controlled conditions of 25±1ºC and 12/12h light/dark photoperiod. Population growth curves were plotted and adjusted. The intrinsic rate of population natural increase was calculated according to the following equation (Odum, 1985):

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

where: N_t = Population size at time t

N_0 = Population size at time zero.

One hundred neonates and fifty pre-adults were placed in aluminium paper recipients previously weighed and dried in an oven at 60ºC. After 24 hours drying, the recipients were weighed again, using an analytical balance (Metler Toledo, model AG245) with readibility on one hundredth of a milligram, in order to determine dry weight biomass. Calculated weights were expressed in mg.

Growth

Ovigerous females were isolated and kept in filtered water with food. After birth of the neonates, six individuals, all from the same female, were isolated and transferred individually to Erlenmeyer flasks containing 150 mL of filtered water, with 10^5 cells.mL^{-1} of the green alga Monoraphidium dybowskii.

Measurements of total body length were taken daily, making drawings in a camera lucida and a calibrated reticulum. Growth curves were adjusted using the von Bertallanffy equation, as follows (the initial parameter of which was obtained by the Ford-Walford transformation) (Santos, 1978):

$$L = L_{\infty} \left(1 - e^{-k(t-t_0)}\right)$$

where:

L = size at a certain time interval t, expressed in mm; L_{∞} = maximum length, expressed in mm); K = constant related to the growth rate; e = base of neperian logarithm; t_0 = parameter related to the initial length of individuals at birth (L_0), expressed in days.

Fecundity was evaluated daily. The experiment was conducted until the death of the ultimate organism, thus allowing a longevity evaluation.

Physical and Chemical Variables

Chemical oxygen demand (COD), pH, water hardness, ammonium, sulphate and potassium concentrations in the culture water were measured according to APHA (1995) in order to keep conditions adequate, avoiding possible environmental stress.

Results

The individual growth curve is presented in Fig.1. It can be described by the logistic function, since after a rapid exponential period, there is a reduction in the growth rate, increasing slowly until reaching the asymptotic maximum. Maximum body length was approximately 1.0mm.

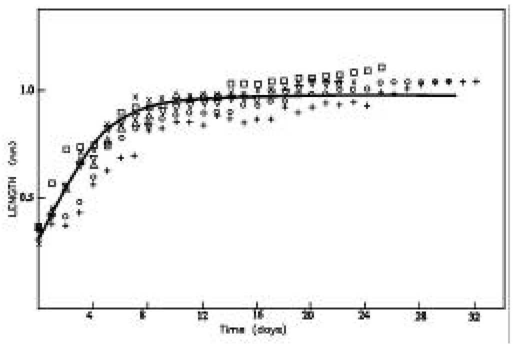

Figure 1: Growth of Ceriodaphnia silvestrii, in laboratory, at 25°C and fed with Monoraphidium dybowski 10^5 cellsmL^{-1} and 12:12 h light-dark photoperiod.

Population growth is shown in Fig. 2. This curve indicates that in a short period of time (19 days), the population increased from 17 ovigerous females. L^{-1} to a total of 5,469 individuals. L^{-1} with an intrinsic growth rate (r) of 0.34 d^{-1}.

The mean values of fecundity got from field and cultured populations, as well as values of longevity, body lengths of neonates, primiparous female, and adult senescent individuals at the last clutch formation are presented in Tab. I. Fecundity of field and laboratory populations were practically identical. Individual dry weight for the neonates and primipara are also presented in Tab. I. Primipara is approximately 2.5 times heavier than neonates, and about 1.5 times larger.

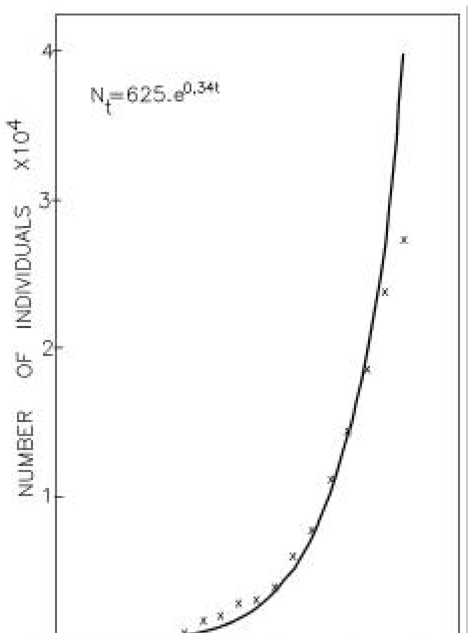

Figure 2: Population growth curve of Ceriodaphnia silvestrii cultured at 25°C and fed with Monoraphidium dybowski 10^5 cellsmL^{-1} and 12:12 h light-dark photoperiod in the laboratory.

Table I: Life cycle parameters of Ceriodaphnia silvestrii. (Cladocera, Daphnidae) cultured at 25ºC and fed with a suspension of 10^5 céls.mL^{-1} Monoraphidium dybowskii.

Variable	Mean ± standard deviation
Field fecundity (eggs/female)	9.79 (±2.55)
Laboratory fecundity (eggs/female)	9.46 (±4.17)
Longevity (days) $_{(n=6)}$	29.8 (±5.89)
Neonate length (mm)$_{(n = 100)}$	0.37 (±0.03)
Primipara length (mm) $_{(n=50)}$	0.57 (±0.04)
Length of senescent adult female (mm) $_{(n= 6)}$	1.04 ±0.04
Dry weight of neonates (mg) $_{(n = 100)}$	3.8 ±1.69
Dry weight of primipara (mg) $_{(n = 50)}$	9.7 ±3.3

Fig. 3 shows the relationship between fecundity and longevity. The highest fecundity was 15 eggs per female, reached at an age of 19 days.

In order to determine if the culture conditions were adequate, the main physical and chemical variables were monitored along the experiments. The values for the concentrations of the main ions and other characteristics of the water culture indicate that the water used as medium had moderate hardness (143mgL^{-1} $CaCO_3$), nearly neutral pH (7.2), high conductivity (172 mScm^{-1}) and low ammonium nitrogen (0.28 mgL^{-1}), sulphate (9.17 mgL^{-1}) and potassium (7.36 mgL^{-1}) concentrations, throughout the experimental period.

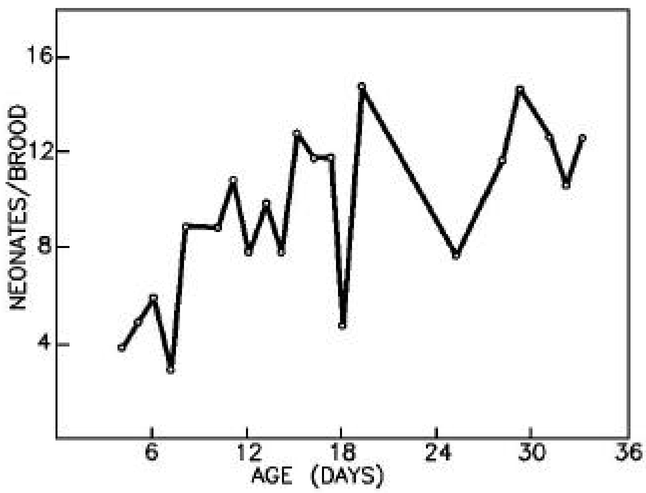

Figure 3: Relationship between fecundity and longevity of Ceriodaphnia silvestrii cultured at 25°C and fed with Monoraphidium dybowski 10^5 cellsmL^{-1} and 12:12 h light-dark photoperiod in the laboratory.

Discussion

In the present work, Ceriodaphnia silvestrii, a species considered to be small in comparison with other cladocerans, showed greatest growth during the initial phase of the life-cycle, with quite accelerated growth until the seventh day, reaching a size of 0.9mm in length, close to the maximum length attained (1mm). Lynch (1980) obtained a similar pattern of growth for Ceriodaphnia cornuta and Daphnia schoedleri and interpreted this data as a strategy of energy allocation, that is to say that certain species invest more energy in the initial period of the life cycle, rapidly reaching a size close to maximum, after which energy is continuously devoted to reproduction. Vijverberg (1980) observed the same pattern of growth for Ceriodaphnia pulchella grown at 25ºC, in the laboratory.

In the present study, this strategy is further evidenced by the increase in fecundity after the seventh day of age. Although this is the most common pattern of energy allocation among cladocerans, different patterns can also be found. For example, Diaz-Castro & Hardy (1998) observed that Moina micrura collected from fish ponds in Manaus, Amazonian region and reared at temperatures of 26 – 28ºC, had a continuous body growth throughout the life cycle.

In nature values of natural rate of increase (r in d^{-1}) which vary from negative to positive can be observed, as a consequence of intrinsic characteristics of the population, such as genetic, age structure and related responses to environmental factors. However, in the case of laboratory cultures, most limiting factors are controlled and experimental animals are usually maintained without competition or predation and under optimal physical and chemical conditions (near optimum conditions of temperature, pH, oxygen, food concentration). Under such conditions, organisms will grow in their maximum potential, and the growth rate measured corresponds to their natural rate of increase (Boughey, 1973).

The value of the natural rate of increase (r) obtained in the present study (0.34 d^{-1}) was relatively high, indicating that the experimental conditions were near optimum, corresponding to a population turnover-time of only three days. Rocha & Sipaúba-Tavares (1994) have found for the same species, an r value of 0.28 d^{-1} in cultures maintained at 24 ± 3ºC, without control of food concentration (Tab. II). Probably the differences observed are influenced by the initial fecundity, since experiments in that case started with a fecundity of 1.1 eggs/female, while in the present study this value was 12.0 eggs/female. Rose et al. (2000) obtained a natural rate of increase for Ceriodaphnia dubia even higher than for Ceriodaphnia silvestrii. They obtained a value of 0.54 d^{-1} in cultures maintained at 25ºC and fed on a mixture of Ankistrodesmus sp. and Pseudokirchneriella subcapitata at the concentration of 1.5 10^5cellsmL^{-1}.

In relation to the influence of temperature on the values of intrinsic rates of increase, Boersma & Vijverberg (1996) found a value of r of 0.25 d^{-1} for Ceriodaphnia pulchella, in a culture maintained at 17.5ºC with a food concentration of 0.50mgC L^{-1} of Chlamydomonas globosa, while Lynch (1992) found an r of 0.26 d^{-1} at 20ºC for Ceriodaphnia quadrangula, fed with a mixture of Scenedesmus and Chlamydomonas.

Body growth rate varies with age. The majority of growth curves show three different rates, namely a high rate for juveniles, followed by an intermediate rate, and lastly, a low rate for ageing adults. Sometimes, growth stops at maturity, but normally continues until death (Lynch, 1980). Growth rate is directly related to temperature (Vijverberg, 1980). Individual growth assays and measurements of egg, juvenile, and adult development rates in the laboratory generally indicate a greater potential for growth than that accomplished in the field (Vivjerberg, 1989).

The mean maximum length reached by C. silvestrii in the present study was 1.04mm ± 0.04. This value is similar to the value 1.02mm ± 0.03 found by Santos et al. (2004), and much larger than the value of 0.66mm recorded by Rietzler (1998). In a review of the species taxonomy, El-Moor Loureiro (1997) presents a variation between 0.86 and 0.88mm as the adult size from field collections. It appears therefore that there is great variation in size both between field animals from different places and between laboratory cultures. It must pointed out that biotic interactions as predation by vertebrates can have a strong effect on cladoceran maximum sizes, as shown by Hrbacek et al. (1961), Hrbacek (1962) and Brooks & Dodson (1965). In the present study, Ceriodaphnia silvestrii individuals collected from an isolated mesocosm have been long-term maintained without vertebrate predation (for more than 10 years) although subject to the strong pressure of invertebrate predators (planktonic cyclopoids, Chaoborus sp, Hydracarina and turbellarians). Smaller individuals from the field (Barra Bonita reservoir) can be probably the result of a process of size-selection by predation.

In the present study, first maturation of C. silvestrii occurred between the age of three to four days, indicating a fast development, probably determined by both, good food conditions (quantity and quality) and by high temperature. Temperature is a factor

Table II: Summary table on life history parameters of Ceriodaphnia species reared in the laboratory under specified conditions.
Lmax= maximum length; Lprim=primípara length; Lneo=neonate length; prim age=primípara age; r (d^{-1})= growth rate; L (days)=longevity.

Specie	Lmax (mm)	Lprim. (mm)	L neo (mm)	Prim age (days)	r (d^{-1})	L (days)	Fecundity egg/female	Dry weight (neo)	Dry weight (prim.)	Food	T(°C)	Author
Ceriodaphnia silvestrii	0.66	0.59±0.04	0.31±0.03	4	-	17.22±2.86		-	-	Natural	25±2	Rietzler, 1998
Ceriodaphnia Silvestrii	0.64	0.44	0.36		0.28			1.8	8.4	Ankistrodesmus gracilis	24±3°C	Rocha & Sipaúba -Tavares, 1994
Ceriodaphnia silvestrii	1.02±0.03	0.74	0.34	3.94	0.53	36	5.61	1.33 ±0.17	2.11±0.16	Natural + Chlorella silvestrii 10^5 cel..mL^{-1}	25±1	Santos et al, 2004
Ceriodaphnia silvestrii	1.04 ± 0.04	0.57± 0.04	0.37±0.03	3 to 4	0.34	29.8±5.89	9.46±4.17	3.8 ±1.69	9.7 ±3.3	10^5 cel..mL^{-1} Monoraphidium dybowskii	25±2	Present study
Ceridaphnia richardi	-	-	-	-	-	-	≅ 5	0.50	4.9	0.2mgC.l^{-1} Scenedesmus spinosus	23.0-24.0	Bunioto & Arcifa, 2003
Ceriodaphnia dubia	-	-	-	4	-	37.5	7.64	-	-	› 10^4 cel. mL^{-1} Ankistrodesmus convolutus	24	Cowgill et al, 1985

strongly influencing the age at first maturation (Shau & Bercau, 1962). An increase in temperature causes a decrease in the duration of all phases of the cladoceran life-cycle, as a result of accelerated metabolism changing the velocity of biochemical reactions, especially those related to protein synthesis (Shau & Bercau, 1962).

Besides the quantity of food being important in controlling growth and reproduction of zooplankton species, food quality is also important, being reflected directly in fecundity.

The experimental conditions established in the present work resulted in high mean fecundities of C. silvestrii of around 10.0 eggs per brood per female. Rietzler (1998) obtained a mean fecundity of 4.13 eggs/brood ± 2.21 per female for C. silvestrii cultured at 25°C and fed on natural phytoplankton from Barra Bonita reservoir. Santos et al. (2004) also culturing C. silvestrii in similar conditions to those used by the previous author, recorded a fecundity of 5.61 eggs/brood per female (Tab. II). Fecundity attained by C. silvestrii in the present work was nearly double of that obtained by those authors, thus evidencing good culturing conditions. The fecundity of C. silvestrii from the field were also very high (9.79 eggs/brood ± 2.55), because they are maintained in isolated concrete tanks, receiving external food supply.

Adult longevity is expressed as the mean duration of life-span. This is inversely related to temperature (Vijverberg, 1980). In the present study, the mean longevity of 29.8 days ± 5.89 obtained for C. silvestrii was lower than the value of 36.0 days by Santos et al. (2004) and higher than 17.22 ± 2.86 days recorded by Rietzler (1998), for the same species, at the temperature of 25°C (Tab. II). It is well known that there is a trade-off between longevity and fecundity in many species. Animals with accelerated growth and high fecundity have a shorter life span (Pianka, 1981; Sommer, 1989).

Cowgill et al. (1985), in a study on Ceriodaphnia dubia found a longevity of 36.0 days at 24ºC, close therefore to the value recorded in the present study, evidencing similarity in life cycle parameters among species from the same genus.

Organism weight or individual elements are used to express biomass in studies on the production of planktonic crustaceans and rotifers, but dry weight is the most commonly used (Bottrell et al., 1976). Evidence exists that dry weight can vary between animals of the same size, depending on the nutritional state, developmental stage and reproductive condition (Rocha, 1983). Lemcke & Lampert (1975) showed experimentally that Daphnia pulex under conditions of starvation, presented values of dry weight and carbon content considerably lower than those well-fed.

Values of dry weight and body length for neonates and primipara found in the present study were comparable to those found Rocha & Sipaúba-Tavares (1994) (Tab. II). The dry weight obtained is higher than those found by Santos et al. (2004). However the culture conditions in this case were not optimal, as revealed by low values of fecundity and also the occurrence of egg abortion. Other species of Ceriodaphnia as C. richardi were found to have smaller size and weight (Bunioto & Arcifa, 2003).

Comparing the maximum size, fecundity and dry weight reached by C. silvestrii in the present study with those obtained by Rocha & Sipauba-Tavares (1994), Rietzler (1998) and Santos et al. (2004), it appears that the conditions offered allowed a much better performance regarding the life cycle parameters, which are probably close to the maximum attainable in the laboratory. The ease of laboratory maintenance, as well as the organisms wide distribution in Brazilian water-bodies indicate that C. silvestrii is a potentially suitable organism to be used in toxicity testing.

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