

Effects of culture medium and food quantity on the growth, fecundity and longevity of the cladoceran *Daphnia similis* Claus.

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ABSTRACT: Effects of culture medium and food quantity on growth, fecundity and longevity of a cladoceran *Daphnia similis* Claus. *Daphnia similis* was maintained in laboratory under constant temperature (20 ± 1 °C). Two different kinds of culture medium (distilled water and natural water from a headspring) and food concentrations (1×10^5 cells and 5×10^5 cells mL⁻¹ from a algae solution of the Chlorophyceae *Monoraphidium dybowskii*) were tested. The individuals were daily measured and transferred to a new algal suspension and individual growth rates, fecundity and longevity of the animals exposed to the four different treatments were determined. Headspring water with food concentration of 5×10^5 cells mL⁻¹ of the algae *Monoraphidium dybowskii* was the treatment that resulted in the best performance of *Daphnia similis* at 20 °C in laboratory conditions. *D. similis* achieved the highest growth rate, total egg production and average longevity under this treatment.

Key-words: *Daphnia similis*, culture media, growth, longevity, fecundity.

RESUMO: Efeitos do meio de cultura e quantidade de alimento no crescimento, fecundidade e longevidade do cladóceros *Daphnia similis* Claus. *Daphnia similis* foi mantida em laboratório sob temperatura constante (20 ± 1 °C). Dois tipos diferentes de meios de cultura (água destilada e água natural de um riacho) e concentrações de alimento (1×10^5 células e 5×10^5 células mL⁻¹ da suspensão algal da Cloroficea *Monoraphidium dybowskii*) foram testados. Os indivíduos foram medidos diariamente e transferidos para uma nova suspensão algal observando-se o crescimento individual, fecundidade e longevidade dos animais expostos aos quatro diferentes tipos de tratamento. A água natural com a concentração de 5×10^5 células.mL⁻¹ da alga *Monoraphidium dybowskii* foi o tratamento que resultou em melhores condições para o cultivo de *Daphnia similis* a 20 °C. *Daphnia similis* alcançou a maior taxa de crescimento, a maior produção total de ovos e a maior longevidade média neste tratamento.

Palavras-chave: *Daphnia similis*, meio de cultura, crescimento, longevidade, fecundidade.

Introduction

Cladocera are zooplanktonic microcrustacea present in freshwaters and represent the primary consumers in the trophic web. According Rocha & Matsumura-Tundisi (1990) few species of the genus *Daphnia* are known to occur in Brazil. Only three species, *Daphnia laevis* Birge, *D. gessneri* Herbst and *D. ambigua* Scourfield have been recorded at the latitude range 0° – 23°S (Matsumura-Tundisi, 1984).

Rearing these organisms in laboratory makes their utilization possible in toxicity tests. Among the advantages pointed out for the utilization of these crustaceans in ecotoxicologic studies are the easy maintenance of laboratory cultures, short generation time, and a predominantly partenogenic reproduction, allowing the easy production of clones acclimatized to experimental conditions.

Zagatto & Goldstein (1984) compared the reproduction of *Daphnia magna* and *Daphnia similis* in waters of different hardness. Although this variable did not interfere with reproduction of these species, to *D. similis* the reproduction rate was approximately three times higher than that of *D. magna*, which results in a higher number of organisms available for bioassays.

According to EPA (1991), the Cladocera are the most sensitive organisms to toxic agents. Patrick et al. (1981), Buikema et al. (1982) and other authors have demonstrated that algae and macroinvertebrates are frequently more sensitive to toxic agents than fish.

The optimization of laboratory cultures of Cladocera has been largely involved with the aim to establish an ideal medium for rearing the organisms. Banta (1921) showed that a culture medium for rearing Cladocera can be easily obtained by using water from dams, enriched with horse manure.

Jana & Pal (1985) compared six different culture media for *D. carinata* and found that in medium enriched with cattle manure the animals presented highly increased abundance and production. On the other hand, Elendt & Bias (1990), found that *D. magna* reared in a standard medium proposed showed low reproduction, growth reduction, and high numbers of non-developed partenogenic eggs as well as high mortality of neonates and adults.

Adequate food for laboratory reared daphnids has also been the aim of many investigations. Taub & Dollar (1964) concluded that algae of the genera *Chlorella* and *Chlamydomonas* are not adequate as food for *D. pulex* because they cause a reduction in longevity and ovulation, and an increase in the percentage of eggs that do not complete embryonic development. The quantity of algae as food are dependent upon nutrient concentration and on the presence of bacteria.

The aim of the present study was to test some different conditions to keep cultures of *D. similis* in laboratory for their ultimate utilization in toxicity tests. This species comes being used in bioassays for the CETESB (Companhia de Saneamento Ambiental, SP) since 1978 in substitution to the *Daphnia magna* that is recommended by restricted international standard organization to the temperate regions (Venkataraman, 1980). *Daphnia similis*, even so without register of occurrence for Brazil, is adapted to our waters, mainly in the moving one to the total hardness.

Material and methods

Cultures of *D. similis* were maintained in the Limnology Laboratory, at the Center of Ecology of the Federal University of Rio Grande do Sul (UFRGS), between January, 1993 and March, 1994. The specimens were obtained from CETESB (Companhia de Tecnologia de Saneamento Ambiental, SP) proceeding from water samples of the Station of Treatment of Sewers of the city of Valinhos, SP.

Acclimatization started with the offspring of a partenogenetic female reared in a 100 mL glass jar. When the culture density increased, it was subdivided and transferred to two jars with the same characteristics. The acclimatization period of the individual cultures was at least two generations, according to the scheme of Goulden et al. (1982).

The organisms were acclimatized in a incubator at 20 (\pm 1°C) and fed with an algal solution of *Monoraphidium dybowskii*, cultivated by the Laboratory of Zooplankton of UFRGS.

After acclimatization of the organisms, four different treatments were established, enabling an analysis of the effect of two culture medium types (natural spring and distilled reconstituted water) and two different food concentrations (1×10^5 cells mL⁻¹ and 5×10^5 cells mL⁻¹).

Culture Medium

Natural water was weekly collected at the headsprings of Sabão stream, a water body without anthropogen influences situated in the "campus" of the Universidade Federal do Rio Grande do Sul. Water quality parameters (Tab. II) such as dissolved oxygen, hardness, total phosphorous, total nitrogen, COD (Chemical Oxygen Demand), BOD₅ (Biochemical Oxygen Demand) and alkalinity were measured in the laboratory following the methods in APHA (1985). During each sampling event, water was filtered in a 45 mm

plankton net, after having measured pH, conductivity and temperature in the field with WTW equipment. Both different kinds of water (natural and distilled) had their hardness reconstituted to 45 mg CaCO₃.L⁻¹.

Growth, fecundity and longevity

Growth, fecundity and longevity were the life cycle parameters of *D. similis* observed in this experiment. In each treatment were reared 10 neonates less than 8 hours old, obtained from females previously acclimatized to laboratory conditions. These neonates were kept individually in 30 mL of water and were fed daily. Water was renewed daily and the animals were daily measured under a stereoscopic microscope. The length of the animals was measured from the top of the head until the base of the tail spine. The data were used to estimate the growth curves following the model of Bertalanffy (1938), and were fitted by the method described in Santos (1978):

$L = L_{max} (1 - e^{-k(t-t_0)})$ where:

L = Length at a given time t, in mm;

L_{max} = Mathematical asymptote of the curve and maximum average length that individuals can reach, in mm;

k = Parameter related to the growth rate;

e = Base of natural logarithm;

t₀ = Parameter related to total average length of individuals at the time of hatching (L₀), in days.

The number of off-spring produced daily was counted. The longevity of the organisms was obtained by daily checking each individual until its death.

Analysis of Variance (ANOVA) was used to analyse the data. We tested for normality of the distribution and homogeneity of variance using the χ^2 -square procedure. Tukey test were employed to detect differences among the four treatments for each of the biological variables observed. These test were performed by the software TOXSTAT 3.3 by Gulley et al. (1991).

Results and discussion

Different rearing media, as well as different algae species and artificial diets are recommended to feed *Daphnia*, but the impact of these food items have not been cautiously studied.

Standardized methods advise the utilization of reconstituted and distilled water as dilution water for the rearing of daphnids to be used in toxicity tests. According to APHA (1985) natural non-polluted water or synthetic water of constant quality and favourable to aquatic life is to be used as dilution water when the aim of the test is to determine the effluent toxicity.

Individual growth

Daphnia similis showed no significant difference regarding female length at first maturity when exposed to different rearing conditions, as demonstrated by the similar mean lengths values attained (Tab. II). However, the test of Tukey showed significant differences for the length at the age of 21 days between group 2 specimens and the other groups ($p < 0,05$). Considering the mean values, it can be observed that group 2 specimens reached a greater mean length, corresponding to 3.18 mm.

Group 2, where neonates of *D. similis* were reared in headspring water and fed on 5×10^5 cells mL⁻¹ of *M. dybowskii*, showed the highest L_{max} value, corresponding to 3.44 mm (Tab. II and Fig. 1b). Their mean length was significantly greater than that of group 1, 3 and 4. (Tukey, $p < 0,05$, Tab. II). In group 4, where the females were fed on the same food concentration, the L_{max} value was lower (3.13 mm, Fig. 1d). Groups 1 and 3 showed either

lower L_{max} values, corresponding to 2.76 and 2.68 mm, respectively. It can be observed that group 1 shows a slightly higher growth than group 3 (Fig. 1a and c, respectively).

Table II: Length (mm) of primiparous females and length at the age of 21 days for *D. similis* submitted to the four treatments (Groups 1, 2, 3 and 4).

Treatment	Primiparous length			Length at 21 days		
	Mean	min.	max.	Mean	min.	max.
Group 1	1.75	1.34	2.00	2.68	2.00	2.91
Group 2	1.73	1.56	2.15	3.18	1.98	2.75
Group 3	1.67	1.50	1.93	2.38	1.00	2.75
Group 4	1.75	1.44	2.09	2.28	0.73	3.15

* = statistically significant difference ($p < 0.05$)

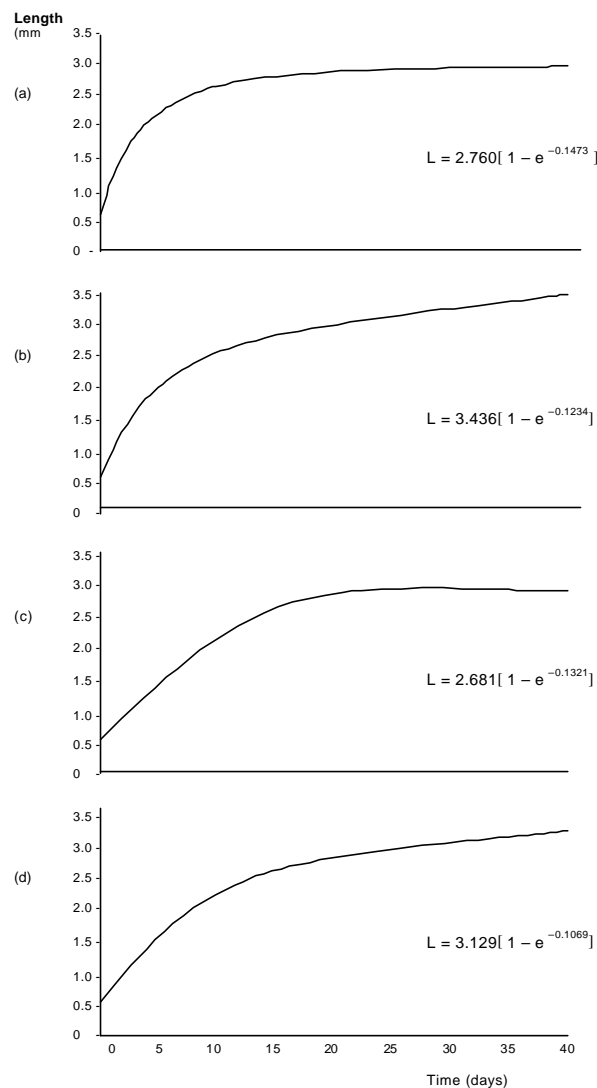


Figure 1 a, b, c, d.: Growth curves for females of *D. similis* reared in different culture medium and food concentration: (a) natural spring and 1×10^5 cells.mL⁻¹ of algal solution, (b) natural spring and 5×10^5 cells.mL⁻¹ of algal solution, (c) distilled and reconstituted water and 1×10^5 cells.mL⁻¹ of algal solution, (d) distilled and reconstituted water and 5×10^5 cells.mL⁻¹ of algal solution).

Table III: Life history characters of *Daphnia similis* in culture medium and food concentration experiment.

	Group 1	Group 2	Group 3	Group 4
Mean length at birth (mm)	0.69 ± 0.054	0.69 ± 0.031	0.69 ± 0.045	0.69 ± 0.025
Minimum size at first reproduction (mm)	1.34	1.56	1.50	1.44
Mean size at first reproduction (mm)	1.75 ± 0.102	1.73 ± 0.066	1.72 ± 0.108	1.88 ± 0.085
Maximum adult size (mm)	2.91	3.53	2.76	3.15
Earliest age at first reproduction (days)	4	4	4	5
Mean life span (days)	27.5 ± 10.448	30.6 ± 10.012	26.4 ± 14.826	19.9 ± 14.791
Maximum longevity (days)	43	40	42	37
Minimum longevity (days)	4	5	2	1
Maximum clutch size (number of eggs)	21	57	18	27
Mean fecundity	13.8 ± 5.426	28.5 ± 11.022	10.4 ± 3.422	13.2 ± 7.065
Mean total fecundity (neonates/female)	136 ± 34.372	342 ± 86.302	118 ± 57.0236	58 ± 17.775
Total egg production	1249	3275	1056	410

Daphnia may grow during its whole life cycle (Mac Arthur & Baillie(1929), may show a distinct plateau (Richman, 1958) or reduce its body length in later stages (Ingle et al., 1937). The results of Hall (1964) apud Vijverberg (1989) suggest that there is a strong relationship between body length and the food, as there is a pause in the growth process under low food concentrations. The results of the present study presented a similar pattern, showing a plateau in the treatments that used lower food concentrations (Groups 1 and 3) and growth until death in the treatment with higher food concentrations (Group 2), probably because animals that were better fed continued to allocate energy to growth in addition to reproduction.

Fecundity

Natural water, even after being filtered through a 45µm mesh plankton net, contained a 15.0 mg.L⁻¹ O₂ load of organic matter as measured by COD, and a BOD of 2.5 mg.L⁻¹ O₂, indicating the presence of bacteria which allowed for a complementation to the diet provided in the laboratory. The animals reared in natural water and fed with higher algae concentrations attained a high offspring production.

A Tukey test on the total fecundity data obtained from the four groups (Tab. III) showed a significant difference between group 2 and the remaining groups. *Daphnia similis* showed a distinct behaviour regarding fecundity in each treatment. In group 1 mean total fecundity was low (13.8). In group 2 the animals showed a mean fecundity of 28.5 and groups 3 and 4 attained the lowest mean values (10.5 and 13.2 respectively). Cowgill et al. (1986) also observed higher values of fecundity, number of clutches and mean clutch size in populations reared in natural waters.

Taub & Dollar (1964) concluded that *D. pulex* did not reproduce normally when fed with *Chlorella pirenoidosa* and *Chlamydomonas reinhardtii* when cultivated in a determined inorganic (chemical) medium. The daphnid showed shorter longevity, ovulation and the embryos did not complete their development. However, when the algae were cultivated in a natural medium, the longevity of *D. pulex* and its reproduction increased, although not to maximum levels.

The Chlorophyte *Monoraphidium dybowskii* used as food in the present investigation demonstrated to be quite efficient, as the reproduction of adult females of *D. similis* was more intense when algae concentration was 5×10^5 cells.mL⁻¹.

This species showed continuous growth, although the increments were small and reproductive rate was low. Rocha & Matsumura-Tundisi (1990) observed similar results for *D. gessneri* and *D. ambigua*. For *D. similis* the values of body growth and fecundity in group 2 are similar to those observed by the above mentioned authors. The animals exhibited indeterminate growth for almost its whole life-cycle. There was a significant number of neonates in the first adult instars. A reproductive peak was reached at approximately halfway the life-cycle, with an average of 45 neonates per female. The remaining groups presented a different pattern with respect to fecundity. Groups 1 and 3 did not show a reproductive peak and group 4 showed an initial increase in the number of neonates, decreasing steadily before reaching half of the life-cycle.

There is a strong correlation between the body size of the mother and its offspring. According to Rocha (1983), the size of the body can be a limiting factor to the number of eggs and embryos carried by the mother when food is abundant. This relationship was also observed in the present experiment. The larger the females, the higher the production of neonates. *D. similis* female from group 2 reached the largest maximum adult size (3.5 mm) and also presented the highest number of neonates (57) of all group.

Longevity

Data on longevity show that the highest mean value (in days) was observed for animals of group 2, where headspring water and a food concentration of algae 5×10^5 cells mL⁻¹ were employed. Groups 3 and 4 presented the lowest values for minimum longevity (2 and 1, respectively). Animals from group 1 showed the higher value for maximum longevity.

According to Hardy & Duncan (1994), the quantity of food is as important factor as temperature for the duration of development of tropical planktonic cladocerans as of temperate species.

Conclusion

Although *Daphnia similis* is considered an exotic species in Brazil, its use in toxicity tests has been spread out in all the Country. Therefore is an alternative species to the use of *Daphnia magna*, known of tempering regions and less adapted to our ambient conditions. Of this form, the knowledge of the biology of *D. similis* becomes very important when comparisons between a natural standard and a substance that wants to test are necessary.

The life history characteristics of *D. similis* observed in our experiment indicate that animals reared in natural water and at a high food concentration (group 2) gave the best result with respect to adult size, mean fecundity per female, the maximum number of eggs produced, total egg production, total number of clutches and mean longevity. Of this form, natural water in the concentration of 5×10^5 cells mL⁻¹ demonstrated to be best medium to culture *D. similis* in laboratory.

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