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Impact of algae and their concentrations on the reproduction and longevity of cladocerans

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Cladocerans are used in a wide range of applications (aquatic toxicology, experimental hydrobiology, and aquaculture). Although known to be successfully cultivated with microalgal food sources, cladoceran demographic parameters tend to be different, depending on the kind of cladoceran and concentration of food supplied. For most species, finding precise instructions on culture conditions and expected outcomes is difficult. The aim of this study was to determine the most favorable feeding conditions for the development of three cladoceran species frequently used as test organisms in aquatic toxicology. For this purpose, *Ceriodaphnia dubia, Daphnia pulex*, and *Simocephalus mixtus* were fed three concentrations (6, 12, and 18 mg L^{-1} dry weight) of the green microalgae *Ankistrodesmus convolutus, Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*), and *Scenedesmus incrassatulus*. Reproduction and survival were evaluated during the entire life cycle. For *C. dubia*, the *P. subcapitata* and *S. incrassatulus* diets produced the best reproductive results. For *D. pulex*, the best overall results were obtained with *S. incrassatulus* at 6 mg L^{-1} and results with *P. subcapitata* were similar at all concentrations. For *S. mixtus* fed *P. subcapitata*, results were similar at all concentrations. With *S. incrassatulus*, a decrease in longevity and an increase in fecundity occurred as the amount of food increased; the best reproductive response was achieved with the highest (18 mg L^{-1}) concentration. The lowest reproduction and survival in the three cladocerans were recorded with *A. convolutus*. For the cladoceran species tested, the same microalga produced different reproductive and survival results, depending on the concentration; some of the tested foods did not support adequate cladoceran development, because they were nutritionally deficient, despite the similar conditions used for their production. Feeding and culture conditions here described could be useful for a controlled propagation of these cladocerans.

Keywords: Daphnidae, Microalgae, *Ceriodaphnia dubia, Daphnia pulex, Simocephalus mixtus*.

Introduction

Cladocerans are ecologically relevant because they participate in the main energy flow between primary producers and higher trophic level consumers in aquatic ecosystems (De Bernardi et al. 1987, Hanazato & Dodson 1995). Under natural conditions, they feed on phytoplankton, bacteria, protozoans, and fine detritus (Sterner et al. 1993, Hanazato & Dodson 1995, Sanders et al. 1996). Some species develop around aquatic vegetation along with high amounts of bacteria and yeasts, which they consume (Sanders et al. 1996). In turn, cladocerans are consumed by several small vertebrate species, such as juvenile fish and zooplankton feeders (Conklin & Provasoli 1978, De Bernardi et al. 1987).

Cladocerans are adequate as biological material for a number of applications, such as aquaculture, hydrobiology and ecology studies, and as test organisms in aquatic toxicity assessment; features important for these purposes are their short life-cycle, high fecundity, multiple reproductions (mainly asexual), diversity in size (as adults and for the progeny), wide geographical distribution, easy maintenance and propagation in laboratory conditions, and easy management during the tests, among others (Martínez-Jerónimo 1995). From the toxicological point of view, many species are sensitive to a wide spectrum of chemical pollutants, and a substantial database is available for some species.

In natural conditions, cladocerans depict alternate cycles of sexual and asexual reproduction. Asexual mode is through parthenogenesis and, by this, females produce clutches consisting of female neonates that can, once

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matured, reproduce parthenogenetically in a process that can be endless, depending on the environmental conditions; this is the main reproductive mechanism under constant and adequate environmental factors (Fryer 1996) and in the presence of abundant food (De Bernardi et al. 1987). Although variable (depending on the cladoceran species and strain), high population densities, low temperatures, and short photoperiods are the factors frequently recognized as promoting male production and triggering sexual reproduction (Korpelainen 1986), which ends in the production of a resistant and dispersal structure called ephippium, containing one or two diapausing eggs, depending on the species. Because sexual reproduction is frequently related with deteriorated or adverse environmental conditions, the presence of males and/or ephippial eggs in a culture are indicators of stress conditions and, for some application purposes (e.g., production of test organisms for ecotoxicological studies), these organisms are unacceptable, because they reflect inappropriate maintenance conditions in laboratory cultures (U.S. EPA 2002). On the other hand, asexual reproduction reduces genetic variability in test organisms and their response in toxicological assays could be more uniform. From a practical point of view, reproduction by parthenogenesis simplifies cladocerans culture, because no sex proportion must be maintained for successful reproduction.

Regarding nutrition, the filtering apparatus in Daphnidae is the most complex and advanced among branchiopods. Suspended-particle filtration is a mechanical process with no feeding particle selectivity (Ivleva 1973); however, they maintain selective consumption of food because the post-abdominal claw separates feeding components from minerals. Burns (1968) defined a formulaic relationship between cladoceran size and the feeding particle, making it possible to infer what food items can be consumed under natural conditions; this information can be applied to cladocerans when cultivated under controlled conditions.

As feeding is a basic process in the growth and development of any organism, we evaluated the effects of three microalgae species, used as food, on the survival and reproduction of selected cladocerans. Algae species were chosen according to the following two criteria: (1) Chlorophycean algae are a source of nutrients suitable for cladocerans growth and development and, if supplied in adequate amounts, they can increase cladoceran fecundity (Price et al. 1990, Martínez-Jerónimo et al. 1994, Martínez-Jerónimo 1995). (2) Size of algae that can be readily assimilated is between 8 and 15 μm, according to the Burns equation (Burns 1968), and the selected species are within this range. On the other side, the differences in their shape and size and their permanence in suspension in the test solution are additional factors to be considered.

Detailed instructions on culture conditions and expected outcomes in controlled cultures are not available. Selection of food and food dosages are particularly critical. Hence, our study was aimed at determining the best feeding conditions for laboratory cultivation of three wide-spread cladoceran species used as test organisms in toxicological assays or experimental hydrobiology.

Material and methods

Three cladoceran species were selected; they were fed three Chlorophycean microalgal species, each supplied at three concentrations, for a total of nine treatments per cladoceran species. The selected Cladocera were *Daphnia pulex* Leydig 1860, *Ceriodaphnia dubia* Richard 1894, and *Simocephalus mixtus* Sars 1903; they were obtained from the collection of the Experimental Hydrobiology Laboratory (ENCB-IPN). These species belong to the Daphnidae family, they are different in size and are found distributed in lentic systems: *D. pulex* is larger than *C. dubia* and both are planktonic, whereas *S. mixtus* is the largest in size and has a littoral distribution, thriving in sediments, merging plants, and roots of floating macrophytes. The first two are regularly used as test organisms in aquatic toxicology assays.

The culture medium was reconstituted hard water (U.S. EPA 2002), with 0.001 mg L^{-1} selenium in the form of sodium selenite added to the medium to: reduce the possibility of damage or malformation of the second pair of appendages (Winner 1984), reduce massive abortions in a medium at 25 ºC (Winner & Whitford 1987), increase organism's strength (Cooney et al. 1992a), and increase survival and reproduction rates (Cooney et al. 1992b).

The progeny for the experiments (neonates aged ≤ 12 h) was obtained from the third clutch of previously acclimated specimens of each cladoceran species to the selected food regimens for one generation.

Microalgal diets were either *Ankistrodesmus convolutus* (**AC**), *Pseudokirchneriella subcapitata* (**PS**, formerly *Selenastrum capricornutum*), or *Scenedesmus incrassatulus* (**SI**), each supplied at three cell concentrations that were equivalent in dry weight, as shown in Table 1 (6, 12, and 18 mg L^{-1}, dry weight, respectively **low**, **med**, and **high**). These algae were chosen because the last two species are frequently used as food for filter-feeding zooplankters in laboratory cultures, and

Table 1. Concentrations used in algal diets supplied to *Daphnia pulex, Ceriodaphnia dubia*, and *Simocephalus mixtus*. The corresponding soluble proteins (mg mL^{-1}) and total phosphorus contents per cell (pg $cell^{-1}$) are also shown.

	Ankistrodesmus convolutus			*Pseudokirchneriella subcapitata*			*Scenedesmus incrassatulus*		
Treatment	AC_{low}	AC_{med}	AC_{high}	PS_{low}	PS_{med}	PS_{high}	SI_{low}	SI_{med}	SI_{high}
Cellular density*	1.4	2.2	3.3	0.75	1.35	1.95	0.65	1.3	1.95
Dry weight (mg L^{-1})	6.0	12.0	18.0	6.0	12.0	18.0	6.0	12.0	18.0
Proteins	0.111	0.174	0.238	0.208	0.375	0.542	0.183	0.365	0.548
Total P		0.43			4.84			5.50	

* 10^6 cells mL^{-1}

A. convolutus is a small size alga that is easily ingested; all of them are readily available from natural sources and several suppliers. The tested concentrations were established according to Martínez-Jerónimo et al. (1994), based on a complete study on the effect of food in the development of the cladoceran *Daphnia magna*.

Microalgae were obtained from the collection of the Experimental Hydrobiology Laboratory, National School of Biological Sciences (ENCB-IPN), and were mono-specifically grown in autoclaved Bold's basal medium (Stein 1973) under near-constant temperature conditions (23 ± 1.5 ºC) and constant and continuous illumination with fluorescent "day light" lamps at approximately 5,000 luxes. To obtain stable quality feed, microalgae were harvested during the exponential growth phase. The biomass was concentrated and kept refrigerated at 4 ºC until used. Soluble protein of the cultured algae was determined using the protein dye binding method of Bradford (1976); total phosphorus content per cell was determined by the technique described by Ames (1967).

Experiments were conducted with 10 neonates individually and randomly distributed in each test container for each treatment (Table 1). For *D. pulex* and *S. mixtus*, 50-mL Pyrex® borosilicate beakers containing 40 mL test volume were used, and for *C. dubia*, 7.5 mL assay tubes containing 5 mL test volume were used. Replacement of the culture medium and food was performed daily for *C. dubia* and every other day for *D. pulex* and *S mixtus*. Experiments were performed at 25 ± 0.5 ºC with artificial illumination using "daylight" fluorescent lamps with an intensity of ~1200 luxes; a 16:8 photoperiod (light: darkness) was used.

Observations were made throughout the life cycle; longevity, fecundity, age at first reproduction, time between broods, and number of broods were recorded. All these parameters are important to assess the biological effects of treatments, and they provide valuable information for the management of controlled cultures. Responses were compared by two-way ANOVA for the tested factors (alga species and food concentration) (Sokal & Rholf 1982, Zar 1999); Tukey´s or T test for *post hoc* comparisons (Sokal & Rholf 1982, Zar 1999) was performed when necessary. Life table and fecundity analyses were made according to Krebs (1985).

Results

Reproductive responses and longevity are presented for each tested cladoceran. This approach was used to ease observation of the differences produced by the applied treatments.

Ceriodaphnia dubia

The age for first reproduction was significantly affected by the concentrations of each microalga ($P < 0.01$), but not by the algal species ($P>0.05$). With the AC_{high} treatment, first reproduction occurred when females were older (7.8 ± 3.5 days) than with the other two concentrations of the same diet and, in general, with the other treatments (Fig. 1). With PS_{med} females began reproduction when they were 4-day old.

The average number of clutches per female was higher with PS diets (12.1 to 13.9), although no significant differences existed between PS and SI concentrations ($P > 0.05$). However, there were significant differences among the three AC treatments and the PS and SI diets ($P < 0.001$), except SI_{high}, which did not differ from the AC_{low} treatment (Fig. 1). Females fed PS and SI diets reproduced more than females fed AC diets (average 1.5 to 3.6).

The longest inter-clutch period (4.3 days) occurred with the AC_{med} treatment, whereas the others showed lower but similar values (Fig. 1); two-way ANOVA and comparison intervals (*post hoc* T test), confirmed this. In general, the inter-brood period was *ca*. 2 days.

The largest clutch sizes occurred with PS diets (10.3 to 12 neonates in average), followed by SI and AC diets, respectively (Fig. 1). There were no significant differences among concentrations of the microalgae ($P > 0.05$), but significant differences existed among algae species ($P < 0.001$). The *post hoc* comparisons showed that the PS_{high} treatment promoted the highest fecundity per clutch as compared to all SI and AC concentrations.

The highest total progeny occurred with diet PS_{high} for an average of 166.8 neonates per female (Fig. 1). There were no significant differences among concentrations ($P > 0.05$), but animals fed on *A. convolutus* had clearly the lowest fecundity (4.6 to 11.6 neonates),

Table 2. Main parameters from the Life Table and Fecundity Analysis for *Ceriodaphnia dubia, Daphnia pulex,* and *Simocephalus mixtus* fed with three concentrations of three microalgae. r = intrinsic population growth rate (days^{-1}), R_o = net reproductive rate (neonates), G = generational time (days), E_x = Life Expectancy at birth (days).

	A. convolutus			*P. subcapitata*			*S. incrassatulus*		
	AC_{low}	AC_{med}	AC_{high}	PS_{low}	PS_{med}	PS_{high}	SI_{low}	SI_{med}	SI_{high}
C. dubia									
r	0.329	0.288	0.218	0.557	0.536	0.580	0.470	0.478	0.379
R_o	11.6	6.9	4.6	135.7	125.2	166.8	112.4	89.3	74.2
E_x	11.4	15.9	13.6	24.8	24.9	26.3	22.6	20.3	19.6
D. pulex									
r	0.457	0.429	0.426	0.529	0.511	0.524	0.506	0.490	0.491
R_o	178.7	155.9	100.2	169.5	179.4	218	250.1	220.2	134.1
E_x	33.2	36.9	28.7	25.3	26.5	30.2	29.6	25.5	21.3
S. mixtus									
r	0.492	0.458	0.283	0.432	0.430	0.423	0.415	0.484	0.487
R_o	127.5	86.2	22.3	228.4	240.8	261.5	231.5	314.6	367.8
E_x	37.3	32.2	22.1	37.7	38.6	41	39.9	29.9	32.2

as compared with the other two microalgae. *Post hoc* comparisons showed that total fecundity with PS_{high} was significantly higher than with treatments PS_{low}, PS_{med}, and SI_{high} ($P = 0.05$).

The highest longevity was recorded for the PS diet (25.3 to 26.8 days in average). Two-way ANOVA showed distinct differences among the three treatments ($P < 0.001$). The T method for *post hoc* comparisons showed that only the longevity recorded with the AC_{low} treatment was significantly lower than that recorded for organisms fed PS ($P = 0.05$).

Life table analysis showed that the highest values for intrinsic population growth rate (r), life expectancy at birth (E_x), and net reproductive rate (R_o) occurred with the PS diets (Table 2), whereas the lowest values were obtained with the AC treatments; this result confirms the inadequacy of using *A. convolutus* for *C. dubia*, despite that this was the smallest tested cladoceran and this alga is the smallest in size of the three supplied as food.

Daphnia pulex

The age at first reproduction was similar in all treatments (5.1 to 6.2 days) and there were no differences among microalgal concentrations ($P > 0.05$), although there were significant differences among the algae ($P < 0.001$). Females fed SI_{high} had their first brood before those fed AC diets, and females fed PS, SI_{low}, and SI_{med} had their first brood at the same age, in average (Fig. 2).

The largest number of clutches (14.1) was recorded for females fed AC_{med} (Fig. 2), and the smallest occurred with females fed SI_{high} (8.3), although the effect of a particular alga or of concentration was not significant on this reproductive response ($P > 0.05$ for both factors), probably due to the variability among replicates in each treatment.

Inter-clutch time was similar among diets, ranging from 1.9 to 2.5 days, and there were significant differences ($P < 0.001$) only between PS_{med} and AC_{high} (Fig. 2).

The smallest clutch size was for females fed AC_{high} (10.0 neonates in average), and the greatest brood size occurred with SI_{med} (20.5 neonates), as can be seen in Fig. 2. Two-way ANOVA demonstrated significant effects due to both the type of microalgae ($P < 0.001$) and the concentration at which they were supplied ($P < 0.05$), but no *post hoc* comparison was made because the interaction between both factors was also significant ($P < 0.001$).

Total progeny was the greatest with the SI_{low} diet (average 250.1 neonates), whereas the smallest was obtained with AC_{high} (100.2 neonates) (Fig. 2). The tendencies shown in this variable were similar to those found in the average size per clutch, and significant effects were detected for both the type of algae ($P < 0.001$) and the concentration at which they were supplied ($P < 0.01$), with a significant interaction between both variables ($P < 0.001$); because of this, no *post hoc* comparison was performed.

Females fed AC diets showed the greatest longevity (29.2 to 33.7 days) (Fig. 2). Two-way ANOVA demonstrated that there were no significant differences among concentrations of the same microalga ($P > 0.05$), although there was a difference among algae ($P < 0.001$) and a significant interaction algae-concentration ($P < 0.05$). It is notorious that females fed AC had, in general, a longer longevity and a greater number of clutches during their lifespan, but each brood was comparatively smaller and, due to this situation, the total progeny recorded with this microalga is not high; these are important facts to consider in culture management of this cladoceran.

The results of the life table analysis are shown in Table 2. The highest r value occurred with PS diets (0.51 to 0.53 days^{-1}), whereas the highest E_x value was obtained with AC (28.7 to 36.9 days in average) and the highest R_o value with SI. Again, it is important to analyze as a whole the different parameters in a life table analysis to choose the best condition, depending on culture needs.

Simocephalus mixtus

For the age of first reproduction, significant differences were determined among concentrations ($P < 0.001$) but not among microalgae ($P > 0.05$). The interaction algae-concentration was also significant ($P < 0.001$), because of this, no *post hoc* tests were performed. Nevertheless, females fed AC_{high} diet had their first reproduction at 8.7 days, whereas with all the other treatments the first clutch was released when females were, in average, less than 7 days old (Fig. 3).

The PS_{high} treatment yielded the highest number of clutches (16.6 in average) whereas the lowest number of broods occurred with the AC_{high} treatment (2.7). Significant differences were determined among concentrations ($P < 0.001$) and among algae ($P < 0.001$). The lowest number of clutches was recorded with AC_{med} and AC_{high}, and the best results (in terms of the number of reproductive events) occurred with PS diets (Fig. 3).

The shortest inter-clutch time occurred with the SI_{high} diet (2.0 days) and the longest with the AC_{high} diet (4.6 days) (Fig. 3). Significant differences were determined among algae ($P < 0.001$) but not with respect to algal concentration.

The average progeny per clutch was similar for all PS concentration (15.5 to 15.9 neonates) and did not differ significantly from SI_{low} (16.2 neonates). In treatments SI_{med} and SI_{high}, the average values were significantly higher ($P < 0.001$) with respect to other treatments (Fig. 3). The lowest values occurred with AC diets, AC_{high} yielded the lowest fecundity (7.9 neonates), but these results are not conclusive because the interaction algae-concentration was highly significant ($P < 0.001$).

For the total progeny, the two-way ANOVA determined significant effects for microalgae ($P < 0.01$) and for concentrations ($P < 0.001$), but the interaction algae-concentration was also significant ($P < 0.01$). The highest total progeny occurred with specimens fed SI_{high} and SI_{med} diets (367.8 and 314.6 neonates, respectively). The lowest values were recorded in females fed AC diets (22.3 to 127.5 neonates in average) (Fig. 3).

The longest average longevity was recorded for PS_{high}-fed females (41.5 days), whereas AC_{high} yielded the lowest longevity value (Fig. 3). Significant differences were determined for effects produced by microalgae ($P < 0.001$) and concentrations ($P < 0.001$). Females fed AC_{high} and SI_{med} had shorter life-spans (22.6 and 30.0 days, respectively) than those fed any of the three PS concentrations and SI_{low}. The highest average longevity occurred with PS_{high} (41.5 days). No *post hoc* comparisons were determined, because the interaction algae-concentration was significant ($P < 0.001$).

With respect to the life table analysis, the intrinsic population growth rate (***r***) values were similar for almost all treatments, except for AC_{high}, in which the lowest value was recorded (Table 2). Although experimental populations grew at similar rates, clear differences were detected in the net reproductive rate (R_o) and in the life expectancy (E_x) among treatments. The highest R_o values were observed with the SI diet (231.5 to 367.8 neonates in average), and the highest E_x (37.7 to 41 days) with the PS diet.

Figures 1, 2, and 3 show all the reproductive responses, as well as the longevity for the three cladocerans assayed with all the tested diets. These figures enable the comparison among species with respect to the algal species and the concentrations at which they were supplied.

Nutritional content analysis of the microalgae used as food

Differences were detected in the analyzed cladoceran responses with respect to diet, and the analytical comparison of food contents helps to explain these results. The determined nutrient content of the used microalgae is shown in Table 1. PS and SI diets showed similar results, but the poorest values (from the nutritional point of view) were found with AC diets. It is important to remark that although the three microalgae were grown under similar culture conditions, there were noticeable differences in their nutrient composition. Most significantly, proteins in PS and SI diets were about twice the amount found in AC diets, and total phosphorus content per cell was 6- to 10-times the amount found in AC diets; these differences help to explain the results observed in the reproduction and survival of the cladocerans here tested.

Discussion

The *Ankistrodesmus convolutus* (AC) diet was poorly accepted by the three cladocerans here tested, yielding the poorest results in most of the reproductive responses. These results seem to be related to the low nutritional quality of this alga, since culture conditions and feed biomass (in dry weight) were the same for the three algal diets. It is quite unlikely that cell dimensions influenced consumption because the size varied only 7 μm among the three species. The similar results obtained with the PS and SI diets are best explained by the similarity in nutritional content of these two species.

The nutritional quality of the consumed food items determines the zooplankton's biomass in nature (Hessen 1992). Low phosphorus content in food particles

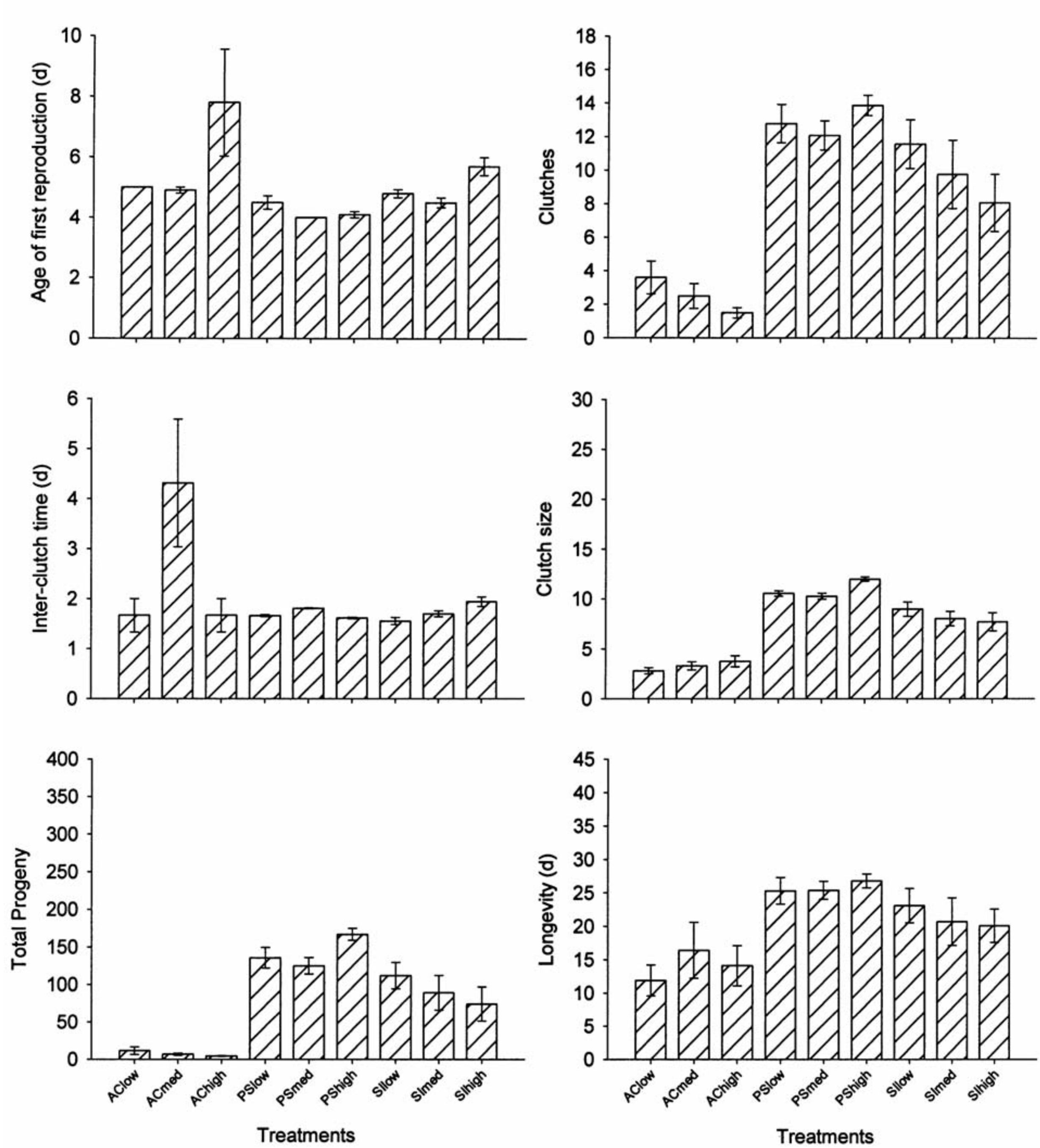

Fig. 1. Reproductive responses and longevity of *Ceriodaphnia dubia* fed with three different concentrations (low, med, and high) of the green microalgae *Ankistrodesmus convolutus* (AC) *Pseudokirchneriella subcapitata* (PS), and *Scenedesmus incrassatulus* (SI). Average values and standard error limits.

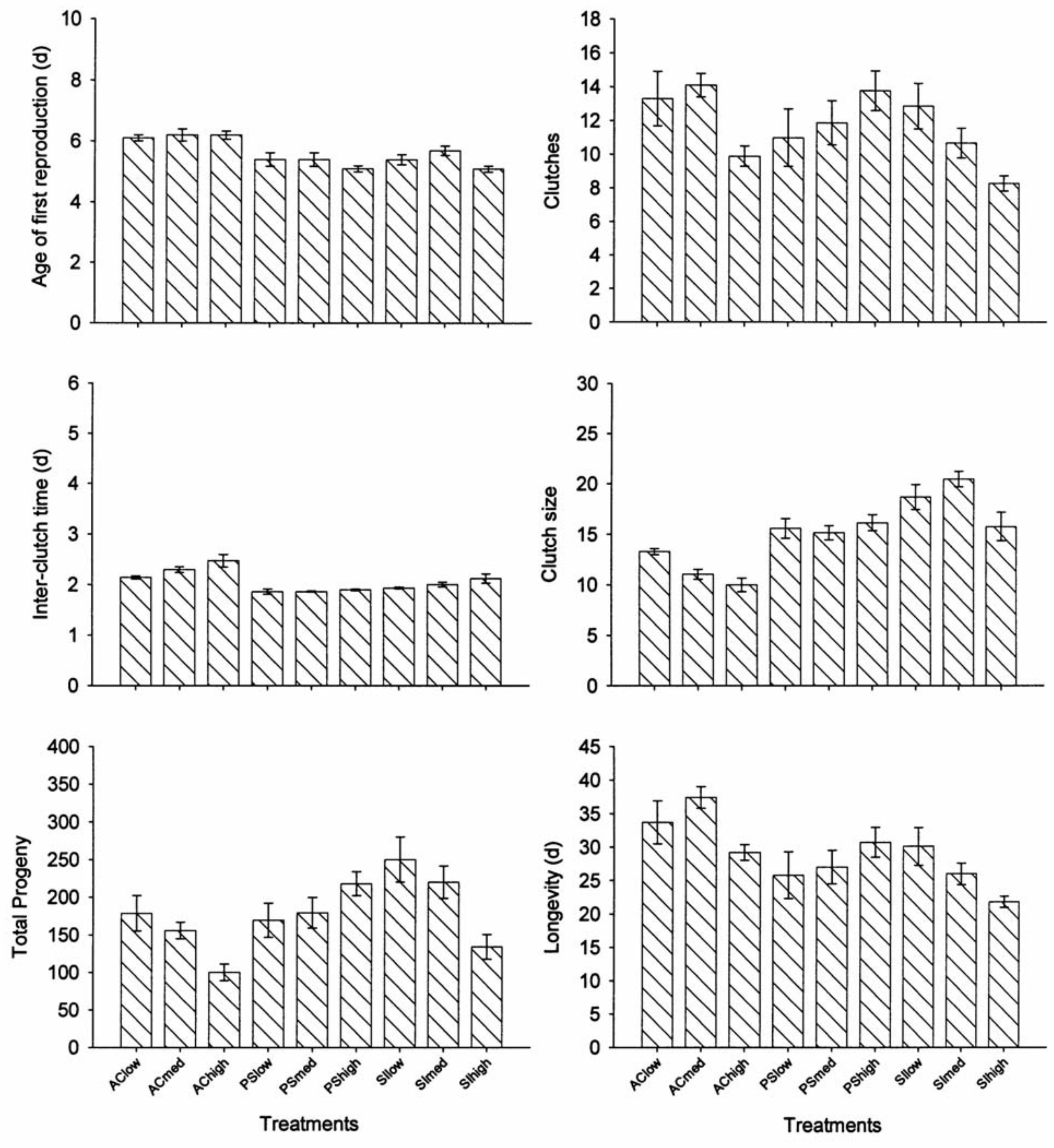

Fig. 2. Reproductive responses and longevity of *Daphnia pulex* fed with three different concentrations (low, med, and high) of the green microalgae *Ankistrodesmus convolutus* (AC) *Pseudokirchneriella subcapitata* (PS), and *Scenedesmus incrassatulus* (SI). Average values and standard error limits.

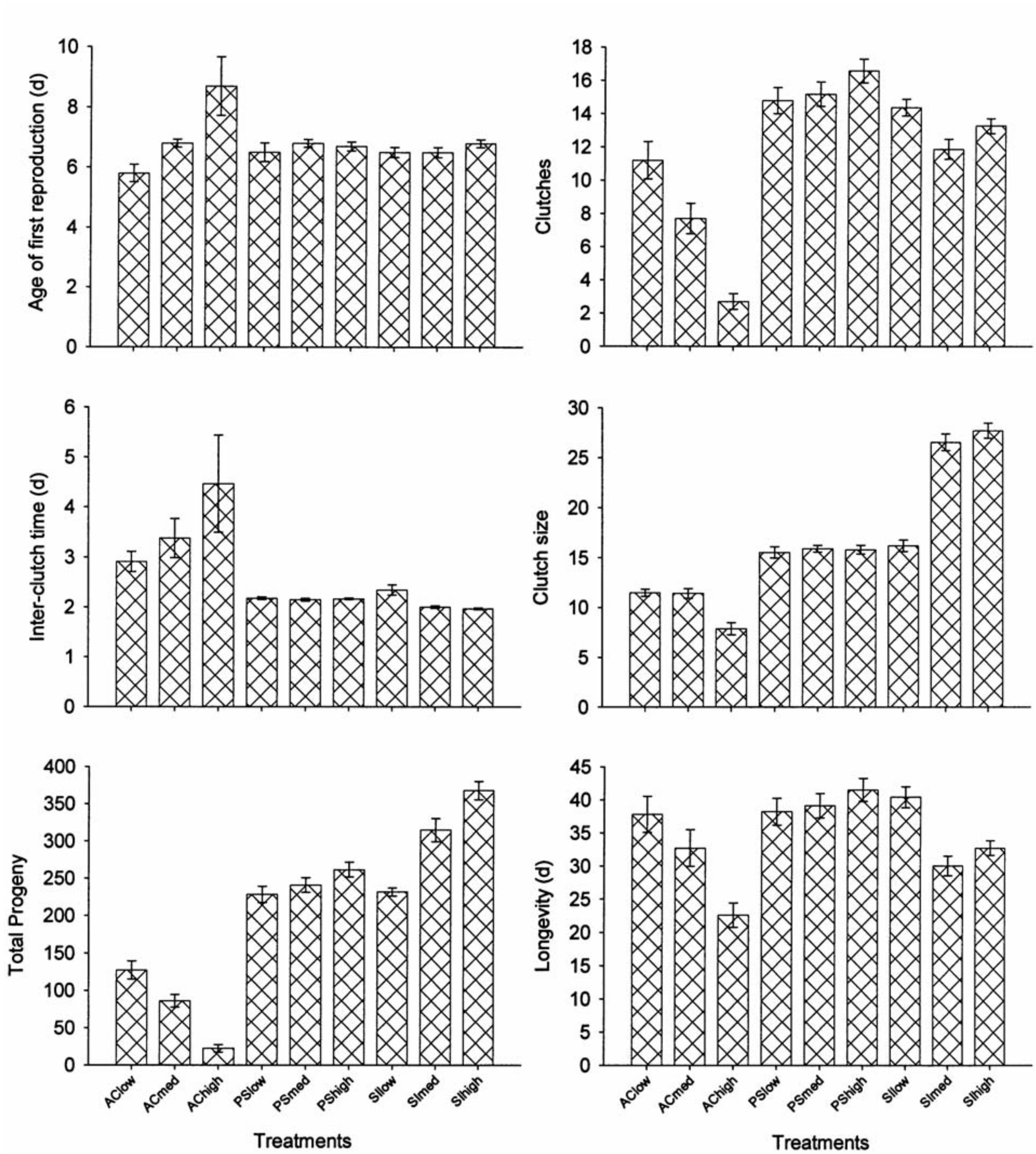

Fig. 3. Reproductive responses and longevity of *Simocephalus mixtus* fed with three different concentrations (low, med, and high) of the green microalgae *Ankistrodesmus convolutus* (AC) *Pseudokirchneriella subcapitata* (PS), and *Scenedesmus incrassatulus* (SI). Average values and standard error limits.

limits growth, size and weight of *Daphnia*, delays the age of first reproduction, reduces fecundity and the reproductive rate, and shortens life-span (Sterner et al. 1993), as observed with the AC treatments. Sterner et al. (1993) concluded that total protein, carbohydrates, lipids, and phosphorus vary among microalgal species, despite culture conditions, as observed in this study; this seems to be a trait related with species-specific characteristics more than a feature modified by growth conditions. This fact is important because it could be thought that cladocerans, as a non selective filter feeding species (as frequently considered), are able to ingest and assimilate with similar results any food item within the size range of their filtering capacity.

Margalef (1983) reported that, under natural conditions, phosphorus content per cell in microalgae is between 0.3 and 2.0 pg cell^{-1}; the lowest value is similar to that determined in *A. convolutus*, whereas the other two algae had values above the maximum. Cowgill et al. (1985) and Cooney et al. (1992a) fed *A. convolutus* and *S. capricornutum* microalgae to *C. dubia* and obtained low fecundity (<5 neonates), but this was probably a consequence of using cell densities that were below nutritional requirements, rather than inherent to the nutritional content of the algae. This condition was not an issue in our treatments because the lowest algal densities were well above the highest levels reported by these authors.

To achieve high fecundity in *D. magna*, Martínez-Jerónimo (1995) used *S. incrassatulus* at three concentrations (0.65, 1.3, and 1.95 x10^{6} cells mL^{-1}) and found the best results at the intermediate concentration. In our study, the highest R_o values were obtained with the highest concentrations of food supplied to *C. dubia* and *D. pulex*, while *S. mixtus* depicted the highest fecundity with the smallest concentration of the used microalgae. Nutritional requirements and variations in feeding efficiencies among cladoceran were evident.

At 14 days, average fecundity of *C. dubia* fed PS diets (8.64 neonates with PS_{med}) was above that obtained by Cooney et al. (1992a) with the same diet (2.1 neonates), a finding that could be explained because these authors used higher test volumes, as well as a higher female density per container. The PS diet and the YCT mixture (yeast, Cerophyll®, and food for trout) have been proposed as food for *C. dubia* culture for toxicological assays in 30-mL vessels with 15 mL test volume (Cooney et al. 1992a, Environment Canada 1990, Patterson et al. 1992, DeGraeve et al. 1992, U.S. EPA 2002). In our study, it was feasible to grow *C. dubia* using *P. subcapitata* as food, in 7.5-mL assay tubes (as test containers), with 5 mL of test volume per culture, reducing markedly expenses and space requirements.

Cogwill et al. (1990) found an average longevity of 9.5 and 45.5 days for *C. dubia* specimens kept in 15- and 100-mL volumes, respectively. In our study, this cladoceran was kept in far lower volume containers, and the specimens lived between 25.3 and 26.8 days, values that are acceptable for test organisms' production cultures.

When *P. subcapitata* was supplied as food to *D. pulex*, longevity, fecundity, and inter-brood time did not show significant differences among the three concentrations, possibly a consequence of the relatively constant amount of cells filtered and consumed by *D. pulex* in a given time period, independently from the concentration of feed. Price et al. (1990) fed *D. pulex* with *P. subcapitata* at a concentration 13-times lower than our lowest concentration, yet they recorded the highest fecundity in reconstituted hard water at 24 ºC. However, the reproductive values they recorded were 2.6 times lower than the ones we recorded. One consideration is that they tested eight specimens in 50 mL water, and density of specimens is a major variable because *D. pulex* shows high algae ingestion when cultivated at low densities and the opposite when cultivated at high densities (Helgen 1987). Since high ingestion can produce better growth and fecundity rates (Sanders et al. 1996), differences in results between both reports may have resulted from differences in the density of specimens per container, as shown by Cox et al. (1992) and Guisande (1993) using the related *D. magna* species. Additionally, it is likely that competition among *D. pulex* for space decreases fecundity, as occurs with *D. magna* (Martínez-Jerónimo et al. 2000). It is also possible that productivity differences are due to the amount of food provided in these experiments.

Groeger et al. (1991) fed *S. obliquus* to *D. pulex*, achieving excellent reproductive results when algae were cultured in a nitrogen-rich medium; results are similar to those obtained by us with the SI_{low} and SI_{med} treatments. This indicates that the microalgae culture medium in our study possessed suitable nutritional quality, at least in the case of *S. incrassatulus* and *P. subcapitata*. Quality and quantity of mineral nutrients for algal growth have an effect on the size and biochemical composition of microalgae (Kilham et al. 1997), which subsequently influence growth and development of microalga consumers. All the essential macronutrients in the culture medium used for algae production were in no-restraining concentrations, which leads to unrestricted conditions that could have contributed to an increase in the main macromolecules consumed by the filtering organisms.

For *S. mixtus* consuming PS diets, fecundity was slightly lower than that reported by other researchers for the similar species *S. vetulus* (Corigliano 1978, de Bernardi et al. 1978, Sharma & Pant 1982). Although these reports were based on different test conditions, which hinder making more detailed comparisons, they all agree in that fecundity has a direct relation with available food concentrations.

Garcia & Pereira (2000) studied the life cycle of two *Simocephalus* species (*S. acutirostratus* and *S. latirostris*) fed *P. subcapitata* at 1×10^6 cells mL^{-1} and obtained shorter life spans (25 and 30 days, respectively) and lower population growth rates (0.19 and 0.22, respectively) than those here recorded. Although the subject species were different, for *S. mixtus*, grown in equivalent feeding condition (PS_{med}), we recorded much longer life-spans and higher population growth rates than those recorded by them.

Although we did not test the effects of population densities, it is interesting to mention that Lee & Ban (1999) observed that high population density negatively affects *S. vetulus* growth and reproduction, even when the amount of food is not limited; they recorded an age of 11 days at first reproduction, longer than that recorded in this study with all tested feeding conditions. These authors recorded, at the lowest female density, a maximum value of 58.3 neonates per female, which is remarkably lower than the brood size recorded in our study. The same negative effect of increased density on reproduction was observed in *C. cf dubia* by Rose et al. (2002).

Despite that the green microalgae *A. convoutus* was supplied at a dry weight equivalent to the amount of supplied *P. subcapitata* and *S. incrassatulus*, it yielded the lowest reproductive response in the three cladoceran species used in this study. Results also show that reproduction and survival of filter-feeding organisms are specific; we determined close to optimal feeding conditions for each of them (microalga species and concentrations) and, based on these results, the best combination for increased fecundity and longevity can be inferred. Our results were obtained with laboratory cultures of very small volumes and under practical and low cost conditions, providing information for the controlled production of cladoceran neonates with valuable applications for biological research and commercial demands.

This study provides detailed biological information for people interested in the culture of cladocerans as a means of getting biological material for several applications. It also shows that the response of cladocerans to feeding conditions is species-specific and that there are food items that can produce better outcomes (see Figs. 1-3). From the present study, it can also be pointed out that, in general, *A. convolutus* is not an adequate feed for the cladocerans here tested, and that food concentration is a determinant factor for reproduction and survival.

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