Bull. Environ. Contam. Toxicol. (1989) 42:119–125

Bulletin of Environmental Contamination and Toxicology

Influence of Variations in Culture Medium on the Survival and Reproduction of *Daphnia magna*

A. E. Girling and B. M. Garforth

Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent, ME9 8AG, United Kingdom

Daphnia magna (Straus) has become widely accepted as a standard test organism in aquatic toxicology and is recommended as such in current national and international guidelines for the testing of new chemicals (e.g. EEC, 1984; OECD, 1984). However, it has become increasingly apparent that some laboratories, including ourselves, experience difficulties in culturing D. magna. In our laboratory cycles in productivity have been encountered. Other laboratories have experienced difficulties in consistently obtaining the high rates of reproduction and survival demanded in chronic toxicity test guidelines. (Blok, 1981; Gersich et al, 1986; Nebeker, 1982).

From a review of the literature it appears that several factors might contribute to the variability in survival and rates of reproduction of D. magna in the laboratory. Factors that have been investigated include light intensity, photoperiod, temperature, food and feeding regime (Lee et al 1986); cadmium stress, selenium deficiency and water temperature (Winner and Whitford, 1987); diet (Cowgill et al, 1986); water hardness and diet (Lewis and Maki, 1981) and temperature and diet (Stephenson and Watts, 1984). Researchers have, however, not been able to identify the causes of this variability.

We have now extended the list of factors investigated to include the culture medium on the basis that current guidelines permit the use of any natural or synthetic medium provided that D. magna can survive and reproduce in it without exhibiting signs of stress.

MATERIALS AND METHODS

Six experiments, were carried out in which the survival and reproductive performance of D. magna was compared in different types of culture media. Briefly, the comparisons drawn and the experiment designs were as follows:
Survival and reproduction in:
1. A reconstituted water versus a filtered river water versus an autoclaved river water. (28-day test, 10 D. magna/treatment individually held in 100 mL of medium changed twice weekly).

Send reprint requests to A.E. Girling at the above address.

2. A reconstituted water versus a filtered river water versus blends of the reconstituted (50-99%) and filtered river (50-1%) waters. (21-day test, 5 D. magna/treatment individually held in 100 mL of medium changed twice weekly).
3. A reconstituted water versus a soil extract versus blends of the reconstituted water (50-99%) and soil (50-1%) extract. (21-day test, 5 D. magna/treatment individually held in 100 mL of medium changed twice weekly).
4. A medium changed daily versus a medium changed three times per week. (14-day test, 4 groups of 10 D. magna/treatment in 500 mL of 98% reconstituted water and 2% soil extract).
5. A medium aged for 24 hours in the presence of D. magna, Chlorella and yeast versus a medium aged for 24 hours in the presence of Chlorella and yeast only. (14-day test, 4 groups of 10 D. magna/treatment in 500 mL of medium changed daily).
6. A reconstituted water containing 2% soil extract versus a dechlorinated tap water containing 2% soil extract. (14-day test, 4 groups of 10 D. magna per treatment in 500 mL of medium changed thrice weekly.

D. magna used in the experiments were taken from a clonal stock culture derived initially from individuals obtained from the Institut National Recherche Chimique Applique, France. Approximately 100 stock D. magna of the same age were held in glass vessels containing approximately 2 L of dechlorinated tap water at 18-22°C with a 16 L/8 D, light regime. The water was continuously aerated and renewed weekly. Neonates were removed periodically from the stock vessels to set up new cultures or for use in experiments. The stock culture was fed a daily diet of the unicellular green alga, Chlorella vulgaris (10^6 cells/mL) which had been grown in 5-10 L of sterile culture medium. Prior to use the algal cells were concentrated by filtration and resuspended to approximately 100-200 x 10^6 cells/mL in reconstituted hard water. Active dried bakers yeast (Allinsons, Castleford, UK) was also added to the culture medium at the rate of 5 mg/L.

For each experiment, the D. magna initially introduced into the test vessels - subsequently referred to as the 'parent daphnids' - were taken from the same stock culture vessel and at the start of the experiment were all less than 24 h old.

During the experiments the D. magna were fed daily on Chlorella vulgaris and yeast as follows:-

Chlorella vulgaris	Days 0-3	1 x 10^5 cells/mL
	Days 4-7	2 x 10^5 cells/mL
	Day 8-end of test	5 x 10^5 cells/mL.
Yeast (active dried bakers yeast)	-	5 mg/L of medium.

All waters used in the experiments were saturated with air before use. The reconstituted hard water used in the experiments was prepared in accordance with a recipe recommended by the US Environmental Protection Agency (EPA 1975). The river water used in two of the experiments was collected from the River Sherway at Headcorn in Kent. The dechlorinated tap water used in one experiment was obtained from bore holes in the chalk of the Kent North Downs.

The only treatment prior to its arrival in the laboratory was chlorination to 0.1 mg/L. In the laboratory it was filtered (Balston EDC-200-35-C, Balston Ltd. Maidstone, UK) to remove particles larger than 10 µm and passed through activated carbon filters (Cuno model CT, Flowtech Ltd., Reading, UK) to remove chlorine and organic contaminants.

Soil extract used in two of the experiments was prepared by autoclaving 1 kg of a 'general soil' in 10 L of reconstituted hard water for 15 min at 120°C and 1.05 g/cm^2 (one atmosphere). The soil used consisted primarily of sterilised loam (60%), peat (15%) and durite sand (25%). However it also contained normal super phosphate (1 g/L), sulphate of potash (0.6 g/L), nitroform (0.4 g/L), fritted trace elements (0.2 g/L) and chalk (1.4 g/L). After autoclaving, the soil extract was allowed to stand for 24 h before being filtered through a Whatman GF/C filter (Whatman Ltd., Maidstone, UK).

All the experiments were conducted in a temperature controlled room at 18-22°C with a 16 L/8 D, light regime.

Survival and reproduction of the parent daphnids were recorded daily for the duration of each experiment. Any young produced were removed on the day of production. Reproduction data was analyzed either by one-way analysis of variance of untransformed data followed by separation of the means using the Student - Newman-Keuls procedure or using a Students 't' test on untransformed data. Both procedures were in accordance with Zar (1974).

RESULTS AND DISCUSSION

Significant differences in reproductive response to the various test media are indicated by letters in parentheses in Tables 1 to 6. Within each table, different letters denote responses that are significantly different ($p < 0.05$).

In the first experiment (Table 1) D. magna cultured in reconstituted water reproduced at a lower rate (101 young/parent in 28 days) than those cultured in autoclaved, filtered river water (176 young/parent) or filtered river water (181 young/parent). Autoclaving the river water did not alter its suitability for the culture of D. magna. These results are supported by the data from

Table 1 - Daphnia magna reproduction and survival data at 28 days in various kinds of water (Experiment No. 1).

Test medium	Mean No. of young per parent	Parent survival (%)
Reconstituted water	101 (a)	100
Filtered river water	181 (b)	80
Autoclaved, filtered river water	176 (b)	100

the second experiment (Table 2) in which the effects of various proportions of reconstituted and river water were tested. An

Table 2 - Reproduction and survival of Daphnia magna after 21 days in reconstituted hard water, filtered river water and four mixtures (Experiment No. 2).

Test medium (% filtered) river water)	Mean no. of young per parent	Parent survival (%)
0	83 (a)	80
1	102 (a,b)	100
5	128 (b)	100
10	135 (b)	100
50	125 (b)	100
100	170 (c)	100

increase in the quantity of river water in the medium relative to the amount of reconstituted water was associated with an increase in reproduction from 83 young/parent in 21 days in 100% reconstituted water to 128-135 young/parent in 5-50% river water and 170 young/parent in 100% river water. Similar improvements in reproduction were associated with the addition of soil extract to the culture medium in the third experiment (Table 3). The addition of 5 and 10% soil extract to reconstituted water resulted in higher rates of reproduction (212 young/parent in 21 days) than in 100% reconstituted water (87 young/parent) or reconstituted water containing higher or lower concentrations of soil extract (76-150 young/parent). Survival of the parents was generally high

Table 3 - Reproduction and survival of Daphnia magna after 21 days in soil extract, reconstituted hard water and four mixtures (Experiment No. 3).

Test medium (% filtered soil extract)	Mean no. of young per parent	Parent survival (%)
100	128 (a,b)	60
50	76 (a)	40
10	212 (b)	100
5	212 (b)	80
1	150 (a,b)	100
0	87 (a)	100

(>80%) in all three experiments. Low values of 40% and 60% were however recorded in the third experiment at soil extract concentrations of 50 and 100% respectively.

The results of the first three experiments indicate that some factors associated with the river water and soil extract are beneficial to D. magna reproduction. The mode of action of the substances is unclear. However, they may function as nutrients or buffers to potential toxins in the media.

The effect of the frequency with which the medium was changed was examined in the fourth experiment (Table 4). Changing the medium

Table 4 - Effect of frequency of change of medium on reproduction and survival of Daphnia magna over 14 days (Experiment No. 4).

Frequency of medium change	Mean no. of young per parent	Parent survival (%)
Daily change	53 (a)	100
3 x per week change	109 (b)	100

less frequently was associated with an improvement in reproduction from 53 young/parent in 14 days under a regime of daily change to 109 young/parent under a regime of thrice weekly change. This result suggests that ageing the medium has beneficial effects on its properties for the culture of D. magna. A similar conclusion can be drawn from the results of the fifth experiment (Table 5) in which the effects of ageing the medium prior to use were examined.

Table 5 - Daphnia magna reproduction and survival data at 14 days in freshly prepared medium and two aged media (Experiment No. 5).

Test medium	Mean no. of young per parent	Parent survival (%)
(a) Freshly prepared medium	53 (a)	100
(b) Medium left standing with D. magna Chlorella and yeast for previous 24 h	112 (b)	100
(c) Medium left standing with Chlorella and yeast only for previous 24 h	114 (b)	100

Reproduction rates improved from 53 young/parent in 14 days in fresh medium to 112-114 young/parent in the aged media. The presence or absence of D. magna in the media during the ageing process did not influence its subsequent suitability for D. magna culture.

The improvement in the properties of the culture media following less frequent changing or deliberate ageing would seem to indicate either a detoxificiation process or the production of nutritionally or otherwise beneficial substances. If the latter is the case the experiments show that these substances are not a product of the D. magna themselves.

Culture in reconstituted water compared to culture in dechlorinated tap water yielded higher rates of reproduction (46 versus 13 young/parent in 14 days) in the sixth experiment (Table 6). This feature has been noted on previous occasions in our laboratory and suggests that the tap water either contains an excess of substances which are chronically toxic to D. magna or is deficient in substances which are essential to good reproduction.

Table 6 - D. magna reproduction and survival data at 14 days in reconstituted hard water and dechlorinated mains water (Experiment No. 6).

Test medium	Mean no. of young per parent	Parent survival (%)
Reconstituted hard water	46 (a)	75
Dechlorinated tap water	13 (b)	100

The findings of a recent workshop which addressed similar problems in the culture of Ceriodaphnia to those we have described with D. magna support the former hypothesis (DeGraeve and Cooney, 1987). Evidence presented at the workshop suggested that treatment processes do not necessarily remove all trace contaminants from tap water. Common elements to the most successful treatment systems were distillation, activated carbon filtration and the use of ion-exchange resin columns.

Survival of the parents in all of the experiments was not so dependent as reproduction on the quality of the culture medium. High mortality (>40%) of the parents was only obvious in the third experiment when soil extract was added to the medium at concentrations of >50%. At these high rates of addition it is possible that substances present in the soil extract were being introduced to the culture medium at concentrations toxic to D. magna.

We can conclude from the results of these experiments that variations in culture medium, permitted under current international guidelines, can influence the survival and in particular reproductive performance of D. magna under the conditions of a chronic toxicity test. Addition of soil extract, river water or other similar substances to the culture medium is permitted under current guidelines and has been shown to enhance culture performance. This practice is however undesirable since extraneous contaminants may be introduced to the test medium. Further work is therefore required to assess the significance of these permitted variations and to identify the optimum culture medium.

Acknowledgements

The technical assistance of S. Mattock and Y. Dutnall and the comments of other colleagues at Shell Research Ltd. is acknowledged.

REFERENCES

Blok H (1981) Measuring the reproduction rate of Daphnia magna. Proceedings of "Colloque International d'Ecotoxicologie", Institut National de la Sante et de la Recherche Medicale, Villeneuve, France, November 1981

Cowgill UM, Emmel HW, Hopkins DL, Takahasi IT and Parker WM. Variations in chemical composition, reproductive success and body weight of Daphnia magna in relation to diet. Int Revue ges Hydrobiol 71 (1): 79-99

EEC (1984) Council directive 79/831/EEC Annex 5 Part C. Methods for the determination of ecotoxicity: C 2 Acute toxicity for Daphnia. Official Journal of the European Communities No. L251/3: 155-159

EPA (1975) Methods for acute toxicity testing with fish, macro-invertebrates and amphibians. US Environmental Protection Agency EPA/660/3-75/009

Gersich FM, Blanchard FA, Applegath and Park CN (1986). The precision of Daphnid (Daphnia magna Straus, 1820) static acute toxicity tests. Arch Environ Contam Toxicol 15: 741-749

De Graeve GM and Cooney JD (1987) Ceriodaphnia: An update on effluent toxicity testing and research needs. Environ Toxicol Chem 6: 331-333

Lee CM, Turner CA and Huntingdon E (1986) Factors affecting the culture of Daphnia magna. Aquatic Toxicology and Environmental Fate: Ninth Volume, ASTM STP921, T.M. Poston and R. Purdy, Eds., American Society for Testing and Materials, Philadelphia: 357-368

Lewis MA and Maki AW (1981) Effects of water hardness and diet on productivity of Daphnia magna Straus in laboratory culture. Hydrobiologia 85: 175-179

Nebeker AV (1982) Evaluation of a Daphnia magna renewal life-cycle test method with silver and endosulphan. Water Res 16: 739-744

OECD (1984) Guidelines for testing of chemicals. Section 2: Effects on biotic systems. ISBN 92-64-12221-4

Stephenson RR and Watts SA (1984) Chronic toxicity tests with Daphnia magna: The effects of different food and temperature regimes on survival, reproduction and growth. Environ Pollut (Ser. A) 36: 95-107

Winner RW and Whitford TC (1987). The interactive effects of a cadmium stress, a selenium deficiency and water temperature on the survival and reproduction of Daphnia magna Straus. Aquatic Toxicol 10: 217-224

Zar JH (1974) Biostatistical analysis. Prentice Hall, Englewood Cliffs, New Jersey, USA

Received January 19, 1988; accepted June 24, 1988.