



## COMPARISON OF REPRODUCTIVE SUCCESS OF *Daphnia magna* Straus CULTURED IN DIFFERENT TYPES OF WATER

Neda Mažuran<sup>1</sup>, Goran Kovačević<sup>2\*</sup>

<sup>1</sup> Salopekova 2b, HR-10000 Zagreb, Croatia

<sup>2</sup> Faculty of Science, University of Zagreb, Division of Biology, Horvatovac 102a, HR-10000 Zagreb, Croatia

\*Corresponding Author: goran.kovacevic@biol.pmf.unizg.hr

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### ABSTRACT

In laboratory culture, *D. magna* is very sensitive to environmental conditions such as temperature, water chemical composition, pH value, illumination, food quality and quantity, and population density. Unfavourable conditions affect reproductive performance and survival. In this work, the reproductive success of *D. magna* was compared in different types of water recommended in toxicity test guidelines and in the literature on cultivation. Four types of water were compared, two natural types of water (dechlorinated tap water and unpolluted surface water) and two synthetic types of water (ISO dilution water and MS media) with ten *D. magna* individuals per water type. The best reproductive results in terms of cumulative number of neonates and brood size were obtained for MS media ( $P < 0.005$ ) containing vitamins, macronutrients and trace elements. ISO dilution water and tap water showed similar reproductive results (no significant differences). All three types of water met the validity criteria of the OECD guidelines of a mean  $\geq 60$  surviving offspring per parent and  $\leq 20\%$  mortality in 21 days. The worst results were obtained for surface water, probably due to the unfavourable chemical composition and high conductivity of the water. This study confirmed that the chemical composition of the culture water has a pronounced effect on the reproductive potential of *D. magna*.

#### How to Cite

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## INTRODUCTION

Daphnids have been used in toxicology for decades. Nowadays, toxicity tests with *Daphnia magna* are widely used in laboratories all over the world for ecological risk assessment of commercial chemical substances, evaluation of industrial wastewater, environmental monitoring of various effluents and receiving waters. *Daphnia* sp. are also used in aquaculture for fish nutrition (Castro–Mejía et al., 2016; El-feky and Abo-Taleb, 2020; Monavar et al., 2020). In laboratory culture, *D. magna* is very sensitive to environmental conditions, including temperature, water chemical composition, pH, illumination, food quality and quantity, and population density. Unfavourable conditions affect reproductive performance and survival. Over the years of scientific progress, the testing and culturing methods have developed with respect to reproducibility and reliability of tests and constant good reproductive output of a consistent quality in healthy *Daphnia* stock cultures (Adema, 1978; Baird et al., 1989; Bradley et al., 1993; Buikema et al., 1980; Canton and Adema, 1978; Cowgill et al., 1986; Cox et al., 1992; OECD 1984, 2004, 2012; Naylor et al., 1992; Stephenson and Watts, 1984; Ten Berge, 1978; US EPA 1985, 1988). That has led to the optimization and standardization of culturing methods in terms of food ration quality and quantity, stock density, temperature and light conditions, and maintenance and handling of the stock culture. Current test protocols are highly standardized and validated through interlaboratory comparison studies. Culturing and maintenance methods of *Daphnia magna* stock culture are explained in detail in toxicity tests guidelines and in the relevant literature, and within those different types of culture water the following are recommended: natural water (groundwater and surface water, dechlorinated tap water) and several types of synthetic water such as EPA reconstituted hard water (EPA, 1985), MS media (Keating, 1985), ISO dilution water (ISO, 1996; OECD, 2004), Elendt M4 and M7 media (OECD 2004, 2012). Whatever water is used for the culture of *D. magna*, it must be of the highest quality (Jonczyk and Gilron, 2005). Water with a hardness of over 140 mg/L as CaCO<sub>3</sub> and with a pH in the range of 6-9 is recommended (OECD, 2012). Water used for preparation of dilution or reconstituted media should be ultrapure (Elendt and Bias, 1990) with conductivity of ≤ 10 µS/cm (ISO, 1996; OECD, 2004). In the present study, the reproductive success of *D. magna* was compared in some types of water recommended for cultivation and dilution. Four different media were compared, two types of natural water (dechlorinated tap water and unpolluted surface water) and two types of synthetic water (ISO dilution water and MS media) (Keating, 1985) from which OECD Elendt M4 and M7 were derived.

## MATERIALS AND METHODS

### *Daphnia* culture

Laboratory culture of *Daphnia magna* obtained from the local pond population was reared in the laboratory for a long time in the dechlorinated tap water of drinking quality and hardness of > 80 mg/L Ca<sup>2+</sup> which was passed through activated charcoal. The diet was a daily mixture of a suspension of two green algae, *Chlorella vulgaris* and *Scenedesmus obliquus*, supplemented with suspended dry baker's yeast once a week. The photoperiod was 12/12 (550 lux), pH was 7 and the temperature was 20 ± 2°C. Under these conditions, the stock culture permanently showed no presence of males and ehippia or aborted eggs, and no increased mortality.

### Culture water

Surface water was analyzed and no traces of anthropogenic contaminants such as pesticides, heavy metals, detergents, unionised ammonia, oils and phenols were found. It contained 46 mg/L Ca<sup>2+</sup> and 38 mg/L Mg<sup>2+</sup>, total hardness as CaCO<sub>3</sub> was 273 mg/L, conductivity was 710 µS/cm, particulate matter was 1.84 mg/L and COD was 2.6 mg/L. Dechlorinated tap water was of drinking quality, it had 94 mg/L Ca<sup>2+</sup> and 14.3 mg/L Mg<sup>2+</sup>, total hardness was 242.90 mg/L CaCO<sub>3</sub>. Synthetic media were prepared with deionised water and chemicals of analytical grade produced by Merck (Darmstadt, Germany). ISO dilution water was prepared by mixing four salt solutions (calcium chloride, magnesium sulphate, sodium bicarbonate and potassium chloride) according to the recommendations of the ISO 6341 standard. MS media consist of two separately prepared solutions, M which contains 14 various trace elements, especially trace metals, and S solution which contains 7 major nutrients and vitamins (B12, biotin and thiamine).

### Experimental procedure

The experiment was run for 44 days. Neonates from 5<sup>th</sup> brood ≤ 24-hour old from mothers acclimated to examined media were placed individually in 50 ml of medium in 10 glass beakers for each medium. The media and glass beakers were changed three times a week and the produced neonates were enumerated and removed. Feeding and illumination regimes were the same as culture maintenance conditions described above, except for the temperature which fluctuated between 24 and 26 °C due to technical difficulties. During the experiment, the pH value was from 7 to 8 in all four types of water, and dissolved oxygen was above 5 mg/L. The parameters observed and calculated were the following: neonates produced in 44 days, number of broods and brood size, time of the production of the first brood, viable neonates per female per day, maximum number of neonates per brood and mortality.

The significance of differences between treatments was assessed by the Student t-test.

## RESULTS

The demographic data obtained after comparing the reproduction and survival of *Daphnia magna* cultured in four different types of water in a 44-day experiment and in 21 days are shown in Table 1. The greatest number of neonates and brood sizes were produced in MS media, and the smallest in surface water. Neonate production and brood sizes in ISO solution water and tap water were almost the same and did not significantly differ from each other, but they were significantly lower compared to MS media ( $P < 0.005$ ). In MS, ISO and tap water brood production was irregular; the females frequently released broods every two days or each day in contrast to the usual synchronous pattern of releasing a brood every third day. That resulted in more broods of smaller sizes. In all three types of water, the highest number of neonates were produced in the fourth and fifth brood between days 18 and 21, when the brood size of all females ranged from 18 to 31, 25 and 22 neonates, respectively. Neonate production, brood size and number of broods in surface water were significantly lower compared to all other three types of water ( $P < 0.0001$ ). Average survival for females in MS, ISO and tap water was almost identical, 37 days. In all three types of water, a few females survived until the end of the experiment, but most stopped breeding on day 35. In surface water, the average survival was only 18 days. During the whole experiment, there were no aborted eggs in any of the four types of water. Results in 21 days, which is the duration of a standard *D. magna* chronic reproduction test, showed the same relation among four

types of water, the number of neonates and brood size was significantly greater in MS media compared to ISO and tap water ( $P > 0.005$ ), and all variables were significantly lower in surface water ( $P < 0.0001$ ) than in the other three types of water. Average days to first brood in MS, ISO and tap water ranged from 10.4 to 11.4, while in surface water it was 8 days for all females but one.

In order to define the maximum life span and maximum fecundity in our *D. magna* laboratory population, we observed four females in ISO dilution water under the same laboratory conditions as in the 44-day experiment, except for the temperature which was  $22 \pm 2$  °C. The results are shown in Table 2.

**Table 2.** Maximum reproduction and longevity of *Daphnia magna* in ISO dilution water

Variable	Females				$\bar{X}$
	1	2	3	4	
Longevity	56	70	67	70	65.75 ± 6.65
No. of neonates	221	219	223	281	236 ± 30.04
Viable neonates/day	3.9	3.1	3.3	4	3.58 ± 0.44
No. of broods	14	16	18	14	15.50 ± 1.91
Brood size	15.8	13.7	12.4	20	15.48 ± 3.33

**Table 1.** Reproduction and survival of *Daphnia magna* in four different types of water at experiment termination (44 days) and in 21 days; values are presented as means ± standard deviation

Demographic variable	MS media	ISO water	Tap water	Surface water
No. of neonates/female	139 ± 11.48*	116.6 ± 12.45	114.2 ± 6.88	38 ± 13.40**
Viable neonates/surviving female/day	3.79 ± 0.44	3.23 ± 0.63	3.39 ± 0.32	2.25 ± 0.94
No. of broods/female	8.9 ± 1.15	8.9 ± 1.13	9 ± 0.67	3.2 ± 0.63**
Brood size	15.78 ± 3.18	13.1 ± 1.89	12.75 ± 1.24	12.92 ± 7.85**
Days to 1 <sup>st</sup> brood	11 ± 0.92	11.4 ± 2.46	10.4 ± 0.97	8.2 ± 0.64
Max. no. of neonates in a brood	31	25	22	60
Survival (days)	36.8 ± 2.89	36.7 ± 4.02	37.3 ± 1.76	18.1 ± 3.03**
No. of neonates in 21 days/female	84.5 ± 6.77*	69.3 ± 6.99	72.5 ± 7.04	38 ± 13.40**
Viable neonates/surviving female/day	4.04 ± 0.28	3.3 ± 0.34	3.45 ± 0.36	2.25 ± 0.94
No. of broods in 21 days	5.7 ± 1.15	5.5 ± 0.52	5 ± 1.08	3.2 ± 0.63
Brood size in 21 days	14.82 ± 2.96	12.64 ± 1.22	13.6 ± 1.07	12.92 ± 7.85
Survival in 21 days (%)	100	100	100	20**

Maximum longevity ranged from 56 to 70 days, maximum fecundity (in 70 days) was 281 neonates, maximum number of broods was 18, and maximum brood size was 20. Average brood sizes and number of viable neonates per female per day were comparable to the results obtained in three types of water (MS, ISO and tap) in the 44-day experiment.

## DISCUSSION

*Daphnia magna* is not only sensitive to various toxic chemical compounds but also to environmental conditions which can affect reproductive performance, sensitivity to toxicants and survival (Baird et al., 1989). Reproduction success of *D. magna* in laboratory culture, besides genetic factors, depends on the environmental conditions under which the daphnids are reared. Investigations have shown that reproductive performance depends on food availability and temperature (Jonczyk and Gilron, 2005). At lower food levels, as a result of increased stocking density, fewer neonates are produced (Cox et al., 1992). Daphnids reproduction increases when they are fed a mixed algal diet, a mixed diet of at least two green algae, supplemented with yeast or trout chow, as recommended (Jonczyk and Gilron, 2005). Cultures fed two or three algae produce more progeny than cultures fed a single alga (Keating, 1985). Stock density also influences the reproduction of *D. magna*; a high population density reduces fertility and delays the day of the first offspring (Olkova, 2021). Bradley et al. (1993) showed that reproductive performance also depends on the different culture media; the best reproductive performances were in the media with the addition of trace elements and vitamins. Research has shown that simple media composed of a few salts like ISO medium are deficient in trace nutrients and that low reproduction and high mortality in ISO medium could be improved by the addition of selenium (Elendt and Bias, 1990). Ten Berge (1978) observed improved reproduction of *D. magna* with the addition of yeast as a source of vitamins to the culture water. The results of the present study confirmed that in media which contain trace elements, including selenium, nutrients and vitamins, the reproductive performance of *D. magna* is statistically significantly better compared to other media. Keating (1985) showed that MS supported a long-term, healthy culture of daphnids with 50+ generations and 300+ progeny per mother. Other investigations found that reproductive success was also different in different natural types of water depending on chemical composition, and different time of the first brood was also related to water chemistry (Cowgill et al., 1985). Poor reproductive performance and short survival in surface water in this study could be a result of some unrevealed contaminant in the water or unfavourable chemical composition such as high conductivity (710  $\mu\text{Scm}^{-1}$ ) or Ca:Mg ratio (1:1). The fact that 90% of the

females in the surface water released their first brood 2–3 days earlier than in other three types of water can be ascribed to water composition effect since all other conditions in four treatments were identical and constant. Survival of 37 days in MS, ISO and tap water was the result of temperature which was 24–26 °C, in contrast to the recommended temperature of 18–22 °C. *Daphnia magna* can live up to 40 days at 25 °C and about 56 days at 20 °C (Jonczyk and Gilron, 2005). The irregular brood release in these three types of water could also be ascribed to high and fluctuating temperatures. Reproductive and survival results in 21 days show that *Daphnia magna* culture in MS, ISO and tap water in this experiment met the OECD guideline validity criteria for the toxicity test; mortality of the parent animals < 20% in controls and the mean number of surviving offspring per parent in 21 days  $\geq 60$ . Also, animals for the toxicity test should be derived from a healthy stock, i.e. no high mortality, presence of males and ephippia, and delay in the production of the first brood (> 12 days). The experiment with the definition of maximum life span and maximum fecundity showed that ISO medium with a diet of two algae and an addition of yeast could sustain individuals of *Daphnia magna* for up to 70 days with a fecundity of up to 281 neonates. Some studies reported the maximum life span of *Daphnia magna* as 72–114 days at 20 °C, depending on stock density (Olkova, 2021), and mortality in 75 days of 80–86.7% (Olkova, 2022).

## CONCLUSIONS

This study confirmed the importance of the chemical composition and temperature of the water on healthy and productive *Daphnia magna* laboratory culture. For reproduction toxicity tests with *D. magna* to be valid, performance criteria for control animals are required, such as  $\geq 60$  surviving offspring produced per parent and a maximum 20% mortality of the parent animals at the end of the 21-day test duration. These performance criteria can not be met if inadequate culture and test medium are used.

## USPOREDBA REPRODUKTIVNOG USPJEHA VRSTE *Daphnia magna* Straus U RAZLIČITIM UZGOJNIM VODAMA

### SAŽETAK

U laboratorijskim kulturama *D. magna* je vrlo osjetljiva na uvjete okoliša poput temperature, kemijskog sastava vode, pH vrijednosti, osvjetljenja, kvalitete i količine hrane i gustoće populacije. Nepovoljni uvjeti djeluju na reproduktivni ishod i preživljavanje. Ovdje je uspoređen reproduktivni uspjeh vrste *D. magna* u nekima od voda preporučenih u smjernicama za testove toksičnosti i

u literaturi za uzgoj. Uspoređene su četiri vode: dvije prirodne vode (deklorirana vodovodna voda i nezagađena površinska voda) i dvije sintetičke vode: ISO voda za razrjeđivanje i MS medij, po 10 jedinki *D. magna* u svakoj vodi. Najbolji reproduktivni rezultati s obzirom na kumulativni broj neonata i veličinu legla dobiveni su u MS vodi ( $P < 0.005$ ) koja je sadržavala vitamine, makronutrijente i elemente u tragovima. ISO voda za razrjeđivanje i vodovodna voda pokazale su slične reproduktivne rezultate (bez značajne razlike). Sve tri vode zadovoljavale su kriterije valjanosti OECD smjernica od  $\geq 60$  živih potomaka po roditelju i  $\leq 20\%$  smrtnosti u 21 dan. Najlošiji rezultati dobiveni su u površinskoj vodi vjerojatno zbog nepovoljnog kemijskog sastava i visoke vodljivosti. Ova studija pokazuje da kemijski sastav uzgojne vode ima izrazit učinak na reproduktivni potencijal u vrste *D. magna*.

**Ključne riječi:** Laboratorijska kultura dafnije, reprodukcija, okolišni uvjeti

## REFERENCES

- Adema, D. M. M. (1978): *Daphnia magna* as a test animal in acute and chronic toxicity tests. *Hydrobiologia*, 59, 2, 125-134.
- Baird, D. J., Soares, A. M. V. M., Girling, A., Barber, I., Bradley, M. C., Calow, P. (1989): The long-term maintenance of *Daphnia magna* Straus for use in ecotoxicity tests: problems and prospects. In Proceedings of the 1st European Conference on Ecotoxicology, SECOTOX. Lokke, H., Tyle, H., Bro-Rasmussen, F. (eds.), Technical University, Lyngby, Denmark, pp. 144-148.
- Bradley, M. C., Naylor, C., Calow, P., Baird, D. J., Barber, I., Soares, M. V. M. (1993): Reducing Variability in *Daphnia* Toxicity Tests – A Case for Further Standardization. In Progress in standardization of aquatic toxicity tests, Chapter 4, Lewis Publishers, pp. 57-70.
- Buikema, A. L., Jr., Geiger J. G., Lee D. R. (1980): *Daphnia* toxicity tests. In Buikema, A. L., Jr., Cairns, J., Jr. (eds.) Aquatic Invertebrate Bioassay, STP 715, ASTM International, pp. 48-69.
- Canton, J. H., Adema, D. M. M. (1978): Reproducibility of short-term and reproduction toxicity experiments with *Daphnia magna* and comparison of the sensitivity of *Daphnia magna* with *Daphnia pulex* and *Daphnia cucullata* in short-term experiments. *Hydrobiologia*, 59, 2, 135-140.
- Castro-Mejía, J., Ocampo-Cervantes, J. A., Castro-Mejía, G., Cruz-Cruz, I., Monroy-Dosta, M. C., Becerril-Cortes, D. (2016): Laboratory production of *Daphnia magna* (Straus 1820) fed with microalgae and active dry yeast. *Journal of Entomology and Zoology Studies*, 4, 2, 548-553.
- Cowgill, U. M., Emmel H. W., Hopkins D. L., Applegath S. L., Takahashi I. T. (1986): The influence of water on reproductive success and chemical composition of laboratory reared populations of *Daphnia magna*, *Water Research*, 20, 3, 317-323
- Cox, E. J., Naylor, C., Bradley, M. C., Calow, P. (1992): Effect of differing maternal ration on adult fecundity and offspring size in laboratory culture of *Daphnia magna* Straus for ecotoxicological testing. *Aquatic Toxicology*, 24, 1-2, 63-74.
- Elendt, B. P., Bias, W. R. (1990): Trace nutrient deficiency in *Daphnia magna* cultured in standard medium for toxicity testing. Effects of the optimization of culture conditions on life history parameters of *Daphnia magna*. *Water Research*, 24, 9, 1157-1167.
- El-feky, M. M. M., Abo-Taleb H. (2020): Effect of feeding with different types of nutrients on intensive culture of the water flea, *Daphnia magna* Straus, 1820, 24, 1, 655-666.
- International Organization for Standardization (ISO 6341) (1996): Water Quality – Determination of the Inhibition of Mobility of *Daphnia magna* Straus (Cladocera, Crustacea), Third Edition, Geneva Switzerland, Report No. ISO 6341:1996.
- Keating, K. I. (1985): A system of defined (*sensu stricto*) media for *Daphnid* (Cladocera) culture. *Water Research*, 19, 1, 73-78.
- Khan, M. A., Hasan, M. M., Sumon, K. A., Rashid, H. (2020): Culture of freshwater zooplankton *Daphnia magna* fed with different feed combination, *Bangladesh Journal of Fisheries*, 32, 1, 55-59.
- Naylor, C., Bradley, M. C., Calow, P. (1992): Effect of algal ration – quality and method of quantification – on growth and reproduction of *Daphnia magna*. *Arch. Hydrobiological*, 125, 3, 311-321.
- OECD (Organization for Economic Cooperation and Development) (1984): OECD Guideline for Testing of Chemicals. *Daphnia* sp., Acute Immobilisation Test and Reproduction Test, TG 202, adopted 13. April 1984.
- OECD (Organization for Economic Cooperation and Development) (2004): OECD Guideline for Testing of Chemicals. *Daphnia* sp., Acute Immobilisation Test, TG 202, adopted 13. April 2004.
- OECD (Organization for Economic Cooperation and Development) (2012): OECD Guideline for Testing of Chemicals. *Daphnia magna* Reproduction Test, TG 211, adopted 02. October 2012.
- Olkova, A. (2021): Control of Suitability of the Culture *Daphnia magna* Straus for Bioassays of Aquatic Environments, Taking into Account Demographic Indicators of Model Populations. *Water*, 13, 1, 47.
- Olkova, A. (2022): Chronic Toxicity Testing with *Daphnia magna* in Three Generations. *Environmental Research, Engineering and Management*, 78, 1, 31-37.

Stephenson, R. R., Watts, S. A. (1984): Chronic Toxicity Tests with *Daphnia magna*: the Effects of Different Food and Temperature Regimes on Survival, Reproduction and Growth. *Environmental Pollution, Series A*, 36, 2, 95-107

Ten Berge, W. F. (1978): Breeding *Daphnia magna*. *Hydrobiologia*, 59, 2, 121-123.

USEPA (United States Environmental Protection Agency) (1985): Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms (Third edition). Environmental Monitoring and Support Laboratory, Cincinnati OH45268, Document EPA/600/4-85/013.

USEPA (United States Environmental Protection Agency) (1988): Protocols for Short Term Toxicity Screening of Hazardous Waste Sites, Document EPA/600/3-88/029.