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Suitability of Commercially Available Spring Waters as Standard Medium for Culturing *Daphnia magna*

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The maintenance of healthy cultures of daphnid is an essential prerequisite to perform toxicity tests with these organisms. Food and medium can be considered as the key components of the culture method; nevertheless, they are often very different among laboratories. If regarding diet, there is an increasing consensus that living algal cells are the best food source, the choice of the culture medium is often determined by the availability of a source of water of good quality, so that different laboratories culture their daphnids in waters from many different sources.

Reconstituted water, well water, tap water and lake water are the most common media. A totally defined reconstitued water, that toward a standardization would be the most desirable, often gives contradictory results in different laboratories: some of them have never experienced any problem with synthetic media (Lee et al. 1986) while others verified its complete inadequacy to maintain a healthy culture of daphnid (Cowgill 1987). In the latter case, deficiency of some trace elements is advocated; however, also the addition of 1 µg Se/L, that is suggested to be included to synthetic media, ensures an healthy culture for no longer than 4 mon (Vigano', unpublished results). Therefore, selenium can improve the performance of a reconstituted water but some other trace nutrients are necessary to ensure the undefined maintainance of the organisms (Elendt and Bias 1990).

The complete pool of minor elements is provided by natural freshwaters that generally encourage culture performance. However, the quality of surface water is usually variable over time; spring and well waters are more suitable to rearing pourpose, although, in industrial and agricultural areas, wells are often

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exposed to the risk of chemical contamination.

This study examined the suitability of commercially available spring waters for culturing *Daphnia magna*. To this aim, five Italian mineral waters were compared and the effects of the material of their containers were also examined.

MATERIALS AND METHODS

In this study, five mineral waters were examined. Their springs are located in five different Italian regions and will be referred herein as: PA (Toscana), LE (Lombardia), SB (Piemonte), FI (Lazio) and VE (Veneto). In Table 1, their chemical compositions are partially reported as determined by university and government laboratories of the respective regions. To compare these waters, reagent-grade salts were added to achieve the same hardness, alkalinity, and Ca/Mg and Na/K ratios. Final values of these parameters were approximately 150 mg/L as $CaCO_3$, 120 mg/L as $CaCO_3$, 4 and 10, respectively.

In a preliminary test only three spring waters (PA, LE, SB), packed in PVC bottles, were examined and compared to tap water medium. This last one has been routinely used to culture *D. magna* in this laboratory, so that it was considered as a reference medium. Comparisons were based on the results of a 23-d (six broods) exposure test. According to this preliminary test, one mineral water (SB) was chosen to start a massive culture of *D. magna*. From the preliminary test, however, a negative effect of the PVC containers seemed also evident; therefore, the massive culture was maintained in the selected brand of water (SB) preferring that available in glass bottles. After 6 mon of successful maintenance, a second 23-d (six broods) exposure test was carried out. In this test, beyond tap water, seven potentially different waters were tested, i.e., the same three brands (PA, LE, SB) of spring waters examined in the first test but with different storage periods in their PVC bottles, two glass bottled waters (LE, SB), one of which (SB) was that used for the massive culture of *D. magna* and finally, two new waters (VE, FI), of which the former is commercially available in PET container and the latter in glass bottle only.

D. magna were routinely maintained in dechlorinated tap water diluted with Milli-Q water. Final tap water medium had a hardness of 150 mg/L as $CaCO_3$ and an alkalinity of approximately 130 mg/L as $CaCO_3$. Both culture and 23-d exposure tests were conducted in a climate chamber at 20 ± 2°C with a 16 hr light - 8 hr dark photoperiod. Light intensity was approximately 1000 lux. Each test was initiated with ten neonates

≤24-hr old that were individually maintained in 50 mL of medium in glass beackers. Daphnids were daily fed a diet composed of 3 x 10^5 cells/mL of Selenastrum capricornutum and 3 x 10^5 cells/mL of Saccharomyces cerevisiae. This same diet was used for the culture of daphnids. Organisms were daily transferred to fresh medium and newborn daphnids were counted. At the end of the second test, the mean weight of neonates of each exposure group was determined pooling in sets of two the sixth broods of the organisms. Each pool of broods was washed three times with Milli Q water and placed in a oven at 60°C for 24 hr, after which it was allowed to cool in a dessicator before being weighed with a Sartorious microbalance. After the experimental animals laid the sixth brood, their length was measured from the top of the head to the base of the spine with an ocular micrometer.

Comparisons between tap water and spring waters were performed on the following endpoints: cumulative number of neonates per female, body length and neonate dry weight. Differences between mean values of measured parameters were tested for significance using a one-tailed Dunnett's test (P=0.05).

RESULTS AND DISCUSSION

In Table 2 the results obtained from the preliminary 23-d (six broods) test are reported. The cumulative number of neonates per female was significantly higher for daphnid in SB medium than for those exposed to LE and PA media as well as to tap water. According to these preliminary results, SB spring water was chosen to start a massive culture of D. magna. However, an inverse relationship between reproduction rate and storage time of water in PVC bottles seemed evident; therefore, the massive culture was maintained with the selected SB water preferring that available in glass bottles.

After 6 mon of successful maintenance in this medium, a second 23-d (six broods) exposure test was carried out. Table 3 presents the results obtained from this test for tap water and seven media, differing either for water identity or for bottle material. Also in this second test, exposure waters differed in their effect on the demographic performances of D. magna.
The mean number of neonates per female was significantly higher for glass bottled waters (FI, LE, SB) than for animals exposed to tap water, again assumed as a reference medium. On the contrary, PVC bottled waters (SB, PA, LE) as well as the PET bottled one (VE), did not cause any significant improvement in offspring production. Referring to the mean number of neonates obtained with PVC bottled waters in both tests, a large part of the observed variation can be

explained by the length of the time of storage in plastic bottle. In fact, plotting the mean cumulative number of neonates against the logarithm of the time of storage, the slope of the regression line was significantly negative ($P<0.05$) and the explained variability was about 70% ($R^2 = 0.704$). This effect can be related to the leaching of contaminants into mineral water from PVC bottles, such as plasticizers and impurity from polymerization process (Junk et al. 1974; Carmignani and Bannet 1976). Accordingly, the inclusion of reproduction rates obtained with glass bottled waters and their times of storage, did not determine a significant regression between the above mentioned parameters. The influence of the container material can also be appreciated comparing the average reproduction rate of PVC bottled waters to that of glass bottled ones, considered as two exposure groups. From the data of the second exposure test, the comparison also being valid if extended to the preliminary test, it can be observed that the average reproduction values are 138.0 (n=30) and 150.9 (n=29) respectively, which are different at a highly significant level ($P<0.001$).

Less defined is the effect of the different media on body length and neonate dry weight (Table 3). For the former parameter, LE and SB glass bottled media determined a significant increase when compared to tap water, as well as when compared to the same kinds of water (LE, SB) bottled in PVC containers. On the other hand, also PA, a PVC medium, promoted a significant increase in carapace length, while organisms reared in FI water, a glass bottled medium, did not differ from those of tap water. Referring to carapace length, PVC and glass bottled waters cannot be distinguished as two groups, in fact the average length of adult female is 4.35 mm for both groups (n=28 and n=29, respectively). Apparently, body length seems to be less responsive to contaminants desorbed from plastic bottle. Anyway, it has already been observed that growth and reproduction can be differently affected, in such a way that the former or the latter can result as the most sensitive parameter to detect a sublethal effect (Gersich and Milazzo 1990).

Mean neonate dry weights, that are reported in Table 3, show that daphnids reared in PA water produced the lightest neonates (9.6 µg) followed by those reared in SB, a glass bottled water (10.2 µg). For the remaining media, no significant effect could be detected. On the other hand, comparing glass and PVC bottled waters, again as two exposure groups, neonate dry weight is on average significantly higher ($P<0.05$) for the former (11.2 µg; n=15) than for the latter (10.2 µg; n=14). Therefore, organisms reared in glass bottled spring waters produced, on average, large broods with heavy neonates.

Table 1. Original chemical composition of tested spring waters (mg/L)

	SB[c]	LE	FI	PA	VE
Ca	12.3	17.4	16.0	31.2	34.1
Mg	0.6	1.9	4.9	6.1	12.5
Na	0.6	1.3	6.5	6.5	2.2
K	0.3	1.6	4.6	0.9	0.4
Cl	0.7	0.6	12.6	8.9	1.2
SO_4	2.0	10.9	5.1	18.6	15.8
Hardness[a]	33.1	51.5	60.0	103.0	136.0
Alkalinity[a]	30.8	44.5	50.0	85.0	119.6
Conductivity[b]	63.5	96.8	166.5	225.1	253.0

[a] As mg/L $CaCO_3$
[b] As µS/cm at 20°C
[c] See methods for definitions of these abbreviations

Table 2. Results from preliminary 23-d exposure of *D. magna* to tap water and spring waters.

Test water	Bottle material	Storage time days	Neonates/ female[a] mean (cv %)	Mean brood size	Adult mortality %
Tap	–	–	126.8 (14.6)	21.1	0
SB	PVC	12	*152.5 (17.7)	25.4	0
PA	PVC	21	138.2 (17.3)	23.0	0
LE	PVC	56	128.6 (14.3)	21.4	0

[a] Values are mean cumulative number for surviving female up to the sixth brood
* Significantly different from tap water (P=0.05)

It has been noted that unstressed well fed daphnids produce large broods consisting of small young (Cowgill et al. 1985a; Enserink et al. 1990). However, it has been observed that sublethal concentrations of a toxicant can have a similar effect, i.e., enhances reproduction rate while decreasing neonate weight (Bodar et al. 1988). On the other hand, both no effect on reproduction with a significant reduction of newborn body length and also the opposite were observed (Flickinger et al. 1982; Cowgill et al. 1985b). According to these Authors, it is not easy to evaluate the observed responses of *D. magna* to the different media. In an attempt to compare the etherogeneous reproduction strategies, for each exposure medium, the measured endpoints, i.e., reproduction rate, carapace length and neonate dry weight, were integrated in a single value that was expressed in terms of biomass. To this aim,

Table 3. Results from 23-d exposure of D. magna to tap water and spring waters.

Test water	Bottle material	Storage time days	Neonates/ female[a] mean (cv %)	Mean brood size
Tap	–	–	132.0 (5.2)	22.0
VE	PET	27	140.0 (10.8)	23.3
LE	PVC	33	142.0 (3.7)	23.7
PA	PVC	34	140.6 (8.8)	23.4
SB	PVC	114	131.3 (8.6)	21.9
FI	Glass	39	*144.8 (8.6)	24.1
LE	Glass	26	*147.9 (9.0)	24.6
SB	Glass	34	*159.4 (5.4)	26.6

Table 3. Continued.

Test water	Mean Body length mm (cv %)	Mean neonate dry weight µg (cv %)	Adult mortality %	Biomass output[b] µg
Tap	4.26 (2.5)	11.6 (2.9)	10	1934.5
VE	4.19 (4.1)	11.8 (4.4)	0	2042.1
LE	4.35 (1.9)	10.5 (5.7)	0	1916.2
PA	*4.41 (2.3)	*9.6 (7.8)	0	1800.6
SB	4.29 (2.1)	10.7 (6.8)	0	1817.4
FI	4.22 (2.2)	11.8 (12.2)	10	2112.5
LE	*4.41 (3.1)	11.5 (5.3)	0	2143.9
SB	*4.42 (3.5)	*10.2 (14.9)	0	2072.6

[a] Values are mean cumulative number per surviving female up to the sixth brood
[b] Biomass output is derived by calculation from reproduction rate, body length and neonate dry weight. See text
* Significantly different from tap water (P=0.05)

adult female carapace lengths were converted into dry weights by means of the relationship $W = 0.009\ L^{2.63}$ (Burns 1969). The calculated values were added to the reproduction output, expressed in terms of biomass and resulted by multiplying the mean weight of neonate by the corresponding mean number of young per female (Table 3). Since toxicants and general stressors can reduce daphnid filtration rate (Gliwicz and Seniawska 1986; Flickinger et al. 1982; Cooley 1977), it is expected that under stressful conditions when the same amount of food is made available, it is converted into less biomass either as growth and/or reproductive output. Examining biomass final values that were calculated as above and are reported in Table 3, it is evident that daphnids reared in the three glass bottled waters and in the only PET bottled water that was tested (VE) performed very similar food conversion into biomass, which exhibited the highest values among the exposure groups. On the contrary, organisms exposed to two PVC bottled waters (PA, SB), evidenced the lowest food conversion, while tap water and the third PVC medium (LE) can be placed at an intermediate efficiency position.

Based on these results, a commercially available glass bottled spring water can represent a suitable base of trace elements from which a partially synthetic medium with defined hardness, alkalinity and Ca/Mg, Na/K ratios, can be easily obtained by addition of no more than four reagent-grade salts. Six months of successful maintenance of D. magna in the SB medium (glass bottled) provide evidence that there are no problems both of trace nutrient deficiency and of toxicity, which are the most common problems of synthetic media (Winner 1976; Elendt and Bias 1990). Young daphnids (≤24-hr) obtained from the SB massive culture were acutely tested for sensitivity to chromium (as $K_2Cr_2O_7$). From four tests that were conducted over the six months of maintenance, there resulted an average 24-hr EC50 of 381.3 µg Cr/L with a coefficient of variation of 8.2%. Finally, it can be mentioned that the variability of spring water quality seems low. In fact, hardness and alkalinity measured for SB partially synthetic medium over a period that, at the time of writing, amounts to 11 mon, have a coefficient of variation of 1.4% and 4.1%, respectively.

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REFERENCES

Bodar CW, Van Leeuwen CJ, Voogt PA, Zandee DI (1988) Effect of cadmium on the reproduction strategy of Daphnia magna. Aquat Toxicol 12:301-310

Burns CW (1969) Relation between filtering rate, temperature and body size in four species of Daphnia. Limnol Oceanog 14:693-700

Carmignani GM, Bennet JP (1976) Leaching of plastics used in closed aquaculture systems. Aquaculture 7:89-91

Cooley JM (1977) Filtering rate performance of Daphnia retrocurva in pulp mill effluent. J Fish Res Board Can 34:863-868

Cowgill UM, Hopkins DL, Applegath SL, Takahashi IT, Brooks SD, Milazzo DP (1985a) Brood size and neonate weight of Daphnia magna produced by nine diets. In Bahner RC, Hansen DJ (eds) Aquatic Toxicology and Hazard Assessment: Eighth Symposium, ASTM STP 891, American Society for Testing and Materials,

Philadelphia, pp 233-244
Cowgill UM, Emmel HW, Hopkins DL, Applegath SL, Takahashi IT (1985b) The influence of water on the reproductive success and chemical composition of laboratory reared populations of Daphnia magna. Wat Res 20:317-323
Cowgill UM (1987) Critical analysis of factors affecting the sensitivity of zooplankton and the reproducibility of toxicity test results. Wat Res 21:1453-1462
Enserink L, Luttmer W, Maas-Diepeveen H (1990) Reproductive strategy of Daphnia magna affects the sensitivity of its progeny in acute toxicity tests. Aquat Toxicol 17:15-26
Elendt BP, Bias WR (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 D. magna. Wat Res 24:1157-1167
Flickinger AL, Bruins JF, Winner RW, Skillings JH (1982) Filtration and phototactic behavior as indices of chronic copper stress in Daphnia magna Straus. Arch Environ Contam Toxicol 11:457-463
Gersich FM, Milazzo DP (1990) Evaluation of a 14-day static renewal toxicity test with Daphnia magna Straus. Arch Environ Contam Toxicol 19:72-76
Gliwicz M, Sieniawska A (1986) Filtering activity of Daphnia in low concentrations of a pesticide. Limnol Oceanog 31:1132-1138
Junk GA, Svec HJ, Vick RD, Avery MJ (1974) Contamination of water by synthetic polymer tubes. Environ Sci Tech 8:1100-1106
Lee CM, Turner CA, Huntington E (1986) Factors affecting the culture of Daphnia magna. In Poston TM, Purdy R (eds) Aquatic Toxicology and Environmental Fate: Ninth Volume, ASTM STP 921, American Society for Testing and Materials, Philadelphia, pp 357-368
Winner RW (1976) Toxicity of copper to daphnids in reconstituted and natural waters. Ecol Res Ser EPA 600/3-76-051, US Environmental Protection Agency, Washington DC

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