THE ROLE OF DENSITY IN REPRODUCTION STRATEGY OF *DAPHNIA MAGNA* – IMPLICATIONS FOR CHRONIC ECOTOXICITY TESTS

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Abstract:

We examined the possible influence of the parental culturing pattern on offspring reproductive strategy, as an implication for chronic toxicity tests. Clutch sizes of F_0 daphnids living in groups were smaller than those of living singly. Clutch sizes of F_1 daphnids living singly were bigger then those of living in groups. However, the clutch size of F_2 daphnids living alone were not significantly different from the clutch sizes of F_1 daphnids living singly. Neonates intended for chronic toxicity tests should be F_1 deriving from the daphnids (F_0) taken out of the batch cultures as neonates, living singly.

Key words

Daphnia magna, toxicity tests, maternal effect, intraspecific interaction

1. INTRODUCTION

Ecotoxicologists use a suite of acute and chronic toxicity tests to predict the effect of chemicals in the environment. Those results have been and will be used for setting EQS (environmental quality standards) under the current EU legislation [3]. Additionally, whole effluent toxicity testing is a common method for effluent quality monitoring [14]. *Daphnia magna* is, by far, the most often used test species for either purposes [1,6,7,11,12,14,15,16], while reduction in fecundity, as measured by the production of juveniles by parental Daphnia is the most commonly used end-point to estimate the chronic toxicity of substances and mixtures. All standardised testing methods set the minimum number of neonates in control treatments to declare a test as a valid one, and one of the important issues of intercalibration process of chronic tests with daphnids had been standardisation of control reproduction [11,12,13,]. It has been, so far, well documented that reproduction of *D. magna* is obviously related to food rations [8,10], since at low food levels small broods of large neonates were produced, whereas at high food levels many tiny

young were born. Yet, no unequivocal relationship was found between maternal food level and the sensitivity of the young [4]. However, the aim of this paper was to examine the possible influence of the parental culturing pattern on offspring reproductive strategy as an implication for chronic toxicity tests.

2. MATERIALS AND METHODS

The daphnids (*Daphnia magna*, laboratory clone NSV) were bred and experiments were run according to standard method [14], in static-renewal conditions. Animals used for experiments were kept in 50 ml beakers, in 30 ml culture medium and fed with 0.1 ml of YCT mixture and 0.1 ml of *Selenastrum capricornutum* concentrate per animal three times a week, 2 h before the renewal of culture medium.

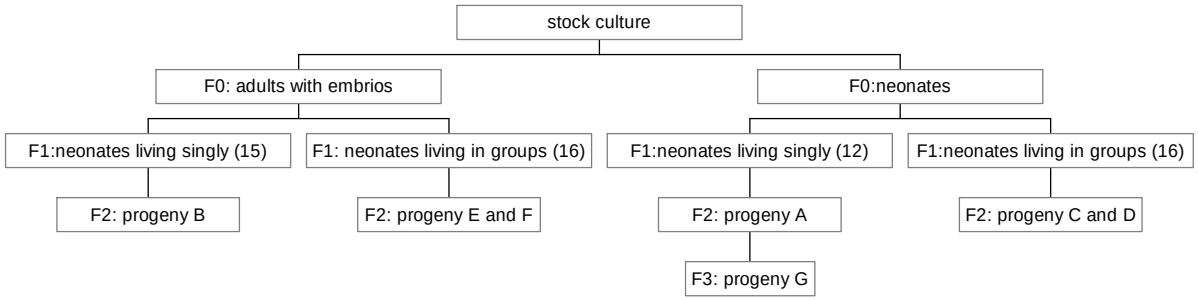

Fig. 1. *Schema of experiments on the role of density in reproduction strategy of Daphnia magna*

Progeny A represents the offspring (F2) of neonates living singly, born form parental Daphnids living also singly (F1), as they were taken from stock culture (F0) as neonates. Progenies C and D are neonates (F2) of daphnids (F1) living in groups (5), although they have been taken out of stock culture as neonates. Progeny B represents the neonates (F2) of singly living daphnids (F1) born from the parents living in groups. Progeny E and F are the neonates (F2) of daphnids (F1) living in groups (5) in two successive generations. Additionally, progeny G (F3) represents the neonates born from daphnids living singly in three successive generations. Differences between progeny E and F as well as between C and D originates from expression of the average number of neonates: C and E, are expressed as average number of neonates of all animals in the test chamber (5), regardless of their survival until the end of test, while D and F are expressed as average number of neonates born from parents surviving to the end of test, till day 21 (fig.1).

3. RESULTS AND DISCUSSION

The highest mean number of neonates (30.58 ±8.72, range 18-44) after 14 days was recorded in progeny G (offspring of progeny A) – singly living animals during 3 successive generations, while the lowest number

(8.78 ±4.3, range 1.8-16.2) occurred in progeny C – animals living in groups, born from singly living parents, but the mean number expressed as average number from the test chamber (5), regardless of the survival of the animal (fig. 2).

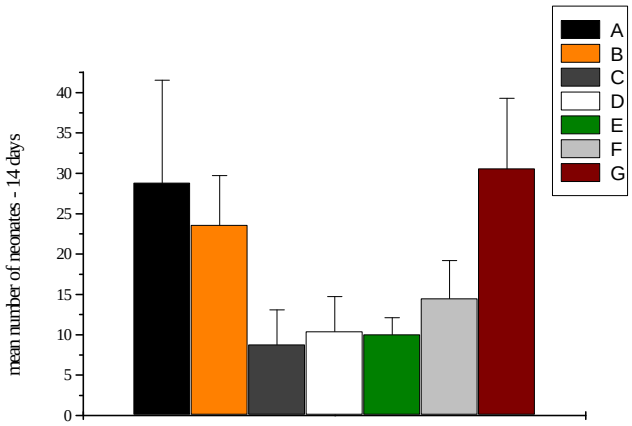

***Fig 2**. The role of density in reproduction strategy of D. magna –14 days*

However, detail analysis (table 1) indicates that the mean number of neonates in progeny G, although higher, does not significantly differ from progeny A. Also, the average number of neonates from progeny C (although the lowest of all) does not significantly differ from progenies D and E. The mean numbers of neonates from progeny A and B also do not significantly differ each other, in spite of the unlike origin of the animals used in experiment (fig. 1).

The highest mean number of neonates (84.45±23.71, range 49-119) after 21 days was again recorded in progeny G (offspring of progeny A) – singly living animals during 3 successive generations, while the lowest number (18.05 ±8.73, range 4-32-16.2) occurred in progeny C – animals living in groups, born from singly living parents, but the mean number expressed as average number from the test chamber (5), regardless of the survival of the animal (fig. 3), as it was the case after 14 days.

***Table 2**. Detailed analysis of reproduction patterns – 14 days*

	A	B	C	D	E	F
B	**p=0.17**	-				
C	p=0.00002*	p=9x10^{-8}*	-			
D	p=0.00005*	p=7x10^{-7}*	**p=0.34**	-		
E	p=3x10^{-6} *	p=3x10^{-9}*	**p=0.32**	**p=0.74**	-	
F	p=0.0003*	p=0.00007*	p=0.002*	p=0.02*	p=0.001*	-
G	**p=0.7**	p=0.002*	p=4x10^{-8}*	p=1.2x10^{-7}*	p=3x10^{-9}*	p=1.1x10^{-6} *

** statistically significant (one - way ANOVA, $p \leq 0.05$)*

Also, detail analysis (table 2) indicates that the mean number of neonates in progeny G, although higher, does not significantly differ from progeny A, while the average number of neonates from progeny C (although the lowest of all) does not significantly differ from progeny E. However, after 21 days, neither mean number of neonates from progenies

B and F nor A and F significantly differed. Yet, average offspring of E and F, as well as A and B, differed significantly.

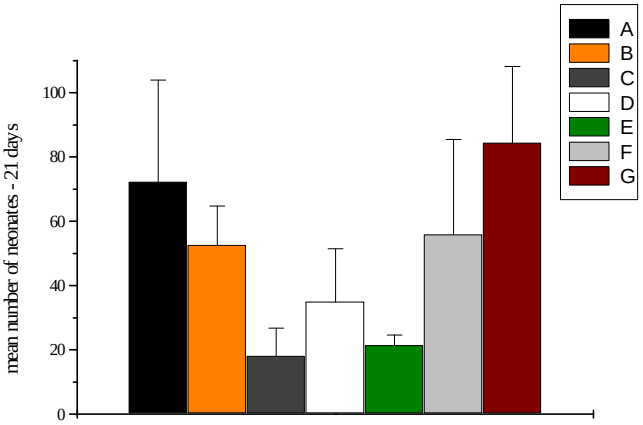

***Fig 3**. The role of density in reproduction strategy of D. magna – 21 days*

In *D. magna,* high animal density, even when sufficient and equal food levels are available to all test animals, unquestionably causes a mutual intraspecific influence called life-strategy shift by intraspecific interaction, as suggested earlier [2,9]. The mean number of neonates in progenies A, B and C (singly living animals) was significantly higher than the average offspring of animals living in groups (C, E, D and E), in spite of the equal food levels available to all test animals. Summarising, but without the intention of going deeply into complex biology and ecology of species, at present there is no evidence to counter the chemical hypothesis [5] that chemical substances, which release might be stimulated by mechanical interaction, are responsible for eliciting life-strategy shift by intraspecific interaction.

Table 2. Detailed analysis of reproduction patterns – 21 days

	A	B	C	D	E	F
B	p=0.04*	-				
C	$p=4x10^{-6}$*	$p=5x10^{-9}$ *	-			
D	p=0.001*	p=0.003 *	p=0.003*	-		
E	$p=8x10^{-7}$ *	$p=8x10^{-11}$ *	**p=0.17**	p=0.03*	-	
F	**p=0.17**	**p=0.69**	p=0.0001 *	p=0.03 *	p=0.00006 *	-
G	**p=0.31**	p=0.0001 *	$p=3x10^{-9}$ *	$p=5x10^{-6}$ *	$p=1x10^{-10}$ *	p=0.01 *

* statistically significant (one - way ANOVA, $p \leq 0.05$)

However, our concern were only the implications such reproduction pattern might have on chronic toxicity testing. To avoid any possible influence of culturing patterns on offspring, the animals intended for chronic toxicity testing should be derived from singly-living parents, cultured and kept during the experiments in separate test vessels. The reason is very simple – in mass cultures it is virtually impossible to

distinguish between over-crowding and test substance's impact on reproduction pattern, while the purpose of laboratory tests basically is to provide such conditions that environmental factors need not be taken as co-variables when evaluating the possible effect of test substances or mixtures.

Therefore, the old test design - to keep test animals in groups, 10 per tests vessel, in 4 replicates per each test concentration (total of 40 animals) was changed [11]: the new method uses 10 animals housed individually. Apart from the fact that the results of the tests conducted according to old method are under direct influence of over-crowding, it was extremely difficult to accurately evaluate reproduction status. Although the animals used in tests derive from the same clone, it virtually never happens that all animals in tests release clutches simultaneously, the offspring usually appear in 2-3 days intervals and overlap, so it is impossible to tell between the offspring of test animals housed in groups. Therefore, the whole set of valuable information on clutch size and timing are lost. Besides, the new experimental design uses 10 real replicates instead of pseudoreplicates used in old method, and therefore, provides more information from the test: the exact size of the clutch, the timing of the distinctive clutches, the exact number of possibly aborted embryos or dead neonates per parent animal, the time intervals between two successive clutches etc. Contrary to expectations, 1 x 10 practice, although requires less animals per treatment, results in same statistical power of the test as 4 x 10, as the experimental unit is the test vessel, not the animal, and there are more vessels in each treatment in new design than in the old one (10 as opposed to 4), which will tend to increase the power [13].

In chronic toxicity tests, particularly those based on reproduction strategy of test animals, the influence of status, reproduction potential, test design and parental culturing method (especially crowding) could not be easily excluded. Mean number of neonates in offspring of individually housed animals (series A and B) differs significantly after 21 day, being 72 and 52, respectively. The situation in offspring derived form parental animals cultured in groups is rather vague, so the direct connection between parental crowding and offspring reproduction potential couldn't be found. One of the possible explanations of higher number of neonates in individually housed animals definitely is the higher rate of adult and pre-mature death in animals housed in groups.

However, the mean number of neonates in series A and G do not differ significantly, although the average number of offspring in series G was higher that any other, including A, after 14 as well as after 21 days. Basically, it could be concluded that it is not necessary to keep animals intended for chronic tests individually till F3 generation, but it is not advisable to take adult animals (with embryos) out of mass cultures and use their offspring in reproduction tests, either. Optimal design would be to use animals as series A, in other word, it is ideal to take neonates from

mass cultures, house them individually, and to use their offspring (third clutch) in chronic toxicity tests.

4. REFERENCES:

1. APHA, AWWA, WPCF. *Standard Methods for Examination of Water and Wastewater*, 19th ed., Washington, DC, 1995.
2. CLEUVERS, M., GOSER, B., RATTE, H. T. Life-strategy shift by intraspecific interaction in *Daphnia magna*: change in reproduction from quantity to quality. *Oecologia*, 110, 337-345, 1997.
3. Council Directive 2000/60/EC of 23 October 2000 establishing a framework for Community action in the field of water policy Official Journal L 327/1, P. 0001 – 0072, 22.12.2000.
4. ENSERINK, L., DE LA HAYE, M., MAAS, H. Reproductive strategy of *Daphnia magna*: implications for chronic toxicity tests. *Aquatic Toxicology*, 25, 111-124, 1993.
5. GOSER, B. AND RATTE, H. T. Experimental evidence of negative interference in *Daphnia magna* Strauss. *Oecologia* 98: 354-361, 1994.
6. ISO. Determination of the inhibition of mobility of *Daphnia magna* Straus (Cladocera, Crustacea)–Acute toxicity test. ISO 6341-1996.
7. ISO. Determination of long term toxicity of substances to *Daphnia magna* Straus (Cladocera, Crustacea). ISO 10706:2000.
8. KLUTTGEN, B., RATTE, H. T. Effect of different food doses on cadmium toxicity to Daphnia magna. *Environ. Toxicol. Chem.,* 13 (10), 1619-1627, 1994.
9. MATVEEV, V. An investigation of allelopathic effects of *Daphnia*. *Freshwat. Biol.* 29, 99-105, 1993.
10. NORBERG – KING, T., J., Schmidt, S. Comparison of effluent toxicity results using *Ceriodaphnia dubia* cultured on several diets. *Environ. Toxicol. Chem.,* 12, 1945-1955, 1993.
11. OECD. Draft Guideline 202, part II on *Daphnia magna* Reproduction Test. Organisation for Economic Co-operation and Development, Paris, 1994.
12. OECD. Report of the Final Ring Test of the *Daphnia magna* Reproduction Test, OECD, Series on Testing and Assessment, No. 6, Paris, 1997.
13. SIMS, I., VAN DIJK, P. The statistical power and biological information of two *Daphnia* juvenile production test design. *Water Research*, 30, (4), 1030-1035, 1996.
14. US EPA. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th ed., EPA/600/4-90/027F, 1993.
15. US EPA. Aquatic invertebrate acute toxicity test, freshwater daphnids. EPA 712-C-96-120, 1996.
16. US EPA. Daphnid chronic toxicity test. EPA 712-C-96-121, 1996.