Belg. J. Zool. — Volume 124 (1994) — issue 1 — pages 3-9 — Brussels 1994

Manuscript received on 30 August 1993

GENOTYPE-DEPENDENT DAYTIME VERTICAL DISTRIBUTION OF *DAPHNIA MAGNA* IN A SHALLOW POND

by

Luc De MEESTER [1], Johan VANDENBERGHE [1], Konjev DESENDER [2] and Henri J. DUMONT [1]

[1] Laboratory of Animal Ecology, University of Ghent, K.L.Ledeganckstraat 35, 9000 Gent, Belgium

[2] Royal Belgian Institute of Natural Sciences, Department Entomology, Vautierstraat 29, 1040 Brussel

SUMMARY

The daytime vertical distribution of *Daphnia magna* Straus genotypes, characterized by differences at two enzyme loci (*Got* and *Pgi*), was analysed in a shallow pond. Though all genotypes tended to occur predominantly in the lower half of the water column, we observed significant differences in the vertical distribution of genotypes marked by differences at the *Got* locus. Our results indicate that, in shallow water bodies too, the vertical distribution of zooplankton is not merely random, and that vertical distribution and migration patterns may be genotype-dependent in such small-scale systems.

Keywords : Daphnia, vertical distribution, genotype, habitat selection.

INTRODUCTION

A substantial amount of the information on diurnal vertical migration of zooplankton has been derived from laboratory experiments on *Daphnia magna* (Clarke, 1931 ; Harris and Wolfe, 1955 ; Ringelberg, 1964 ; Calaban and Makarewicz, 1982 ; Dumont *et al.*, 1985 ; De Meester, 1991a). *D. magna* is indeed easy to culture and, due to its size, easy to manipulate. Moreover, there is an abundance of background information available on the physiology, ecology and genetics of this species (Peters and De Bernardi, 1987), including a great deal of data on phototactic behaviour under laboratory conditions (reviewed by Ringelberg, 1987). However, *D. magna* being a pond species, there is little information available on its vertical migration behaviour in the field. Ringelberg (1964) reports on a nocturnal vertical migration (evening ascent) of a *D. magna* population in a shallow canal. Ranta and Nuutinen (1985) also observed nocturnal migration of *D. magna* in shallow rock-pools.

In previous work, we have shown that there is a significant genetic component to the variability in phototactic behaviour in *D. magna* (DE MEESTER, 1989, 1991a, 1993b; DE MEESTER and DUMONT, 1989; VAN UYTVANCK and DE MEESTER, 1990). The vertical distribution of different genotypes in outdoor containers is in concordance with expectations drawn from laboratory observations on phototactic behaviour (DE MEESTER, 1993a). These results suggest that the vertical daytime distribution of *D. magna* in natural populations may be genotype-dependent. Studies using electrophoretic markers have shown such genotype-dependent vertical distributions for typical lake *Daphnia* species (MÜLLER and SEITZ, 1993) as well as for *D. pulex* in a large deep pond (6.5 m depth; WEIDER, 1984). In the present study, we analyse the vertical distribution of *D. magna* genotypes in a shallow pond. Experiments on *D. magna* clones isolated from this pond have provided evidence for intrapopulational variability in phototactic behaviour (DE MEESTER, 1991b).

MATERIAL AND METHODS

The study site is Driehoekvijver (Heusden, Eastern Flanders, Belgium), a small (1/2 ha surface area) and shallow (< 1 m depth) eutrophic pond. *D. magna* and *D. pulex* are the most abundant cladocerans, but their densities are subject to rapid and extensive changes due to periodical flushing, and partial drying out of the pond in summer. The pond contains fish (*Rutilus rutilus* LINNAEUS and *Gasterosteus aculeatus* LINNAEUS), but they are never abundant, probably due to the unstable nature of the pond system.

Samples were taken on 5 February 1992, 14.00 h, by towing a series of rectangular plankton nets simultaneously through the water over a distance of 1.2 m, after which all nets were closed simultaneously. Each net was 10 cm high, with a sampling surface of 50 cm^2 (total volume sampled per net : 6 liter). The nets were mounted on a wooden bar in such a way that the complete water column was sampled with a depth interval of 10 cm. The sampling site was 60 cm deep, with an artificial (concrete) bottom, facilitating efficient sampling of the near-bottom water layer. From each depth sample, 30 adult *D. magna* females were randomly picked out for electrophoretic analysis and preserved in liquid nitrogen. Only 15 adult females were analysed from the upper waterlayer (0-10 cm), because of the low number of animals caught. The remainder of the sample was fixed with formaldehyde (4 %) for subsequent counting.

Cellulose acetate electrophoretic analysis was done according to the methods described in HEBERT and BEATON (1989). Small animals (< 2.5 mm) were homogenized in 50 μl Tris-glycine buffer solution (pH = 8.5), larger animals in 70 μl. Two enzymes were screened : glutamate-oxaloacetate transferase (*Got*, EC 2.6.1.1.) and phosphoglucose isomerase (*Pgi*, EC 5.3.1.9.).

We tested the null hypothesis that there is no genotype-dependent vertical distribution by means of G-tests of Independence (SOKAL and ROHLF, 1981) grouping individuals according to their genotype at the two loci (*Got* and *Pgi*). Two-locus as well as pooled one-locus genotype frequencies were tested. Though analyzing the loci separately (pooled data) results in a potential loss of information, the statistical reliability is increased considerably due to higher cell frequencies.

RESULTS

The number of adult *Daphnia magna* caught in the different nets is given in Table 1. The animals are not uniformly distributed, but show a tendency to reside close to the bottom. Almost 50 % of the adults were found in the lower 10 cm, whereas less than 3 % occurred in the upper 10 cm.

TABLE 1

Depth distribution of adult *Daphnia magna* in Driehoekvijver, 5 February 1992, 14.00 h. N : number of adults caught ; $N\,l^{-1}$: density per liter ; % : percentage of total population caught at given depth ; avg.: average.

Depth	N	$N\,l^{-1}$	%
0-10 cm	17	2.8	2.4
10-20 cm	32	5.3	4.6
20-30 cm	42	7.0	6.0
30-40 cm	130	21.7	18.5
40-50 cm	150	25.0	21.4
50-60 cm	330	55.0	47.1
avg.	116.8	19.5	

All possible two-locus genotypes were represented in the population (Table 2). An overall RxC G-Test of Independence indicates a genotype-dependent vertical distribution when the data are pooled according to genotype at *Got* (G-statistic = 25.98, df = 10, $p < 0.005$). When the data are pooled for genotype at *Pgi*, the null hypothesis is not rejected (G-statistic = 8.11, df = 10, $p > 0.5$). Pooling depth strata two by two (0-20 cm, 20-40 cm, 40-60 cm) yields similar results (*Got* : G-statistic = 9.77, df = 4, $p < 0.05$; *Pgi* : G-statistic = 1.90, df = 4, $p > 0.5$). The vertical distribution of genotypes heterozygous for *Got* is found to be significantly different from that of both homozygous genotypes by G-tests ($p < 0.001$). No significant differences were observed in the vertical distribution of *Pgi* genotypes (G-tests, all $p > 0.1$).

Analysing the frequency data of all two-locus genotypes that represent > 5 % of the population yields some additional differences (see Table 2), but caution should be taken in interpreting these results, as sample sizes are small. Figure 1 shows that, though the genotypes marked by differences at the *Got* locus were all

TABLE 2

Genotype of 165 adult *Daphnia magna* sampled at different depths in the Driehoekvijver. Number of animals sampled at depth 0-10 cm is 15, and 30 at all other depths. n° : number designated to genotypes ; % : relative abundance of genotype in pooled sample (corrected for reduced sample size at depth 0-10 cm) ; G : pairwise comparisons of distribution over depth of genotypes (in case of two-locus genotypes, only those representing > 5 % of the sampled individuals analysed) by G-tests, numbers refer to genotypes for which the vertical distribution is significantly different at p = 0.05 ; ns : no significant differences were observed for any of the comparisons ; / : data not analysed due to low sample size (< 5 % of the population).

A : Two-locus (*Pgi* — *Got*) genotypes.

Depth n°	SS,SS 1	SF,SS 2	FF,SS 3	SS,SF 4	SF,SF 5	FF,SF 6	SS,FF 7	SF,FF 8	FF,FF 9
0-10 cm	1	2	3	1	5	3	0	0	0
10-20 cm	3	7	7	1	3	4	0	1	4
20-30 cm	4	5	6	1	4	6	1	2	1
30-40 cm	0	6	5	1	7	9	0	1	1
40-50 cm	2	9	6	1	2	4	1	3	2
50-60 cm	5	9	5	1	3	2	1	1	3
Total	15	38	32	6	24	28	3	8	11
%	8.9	22.2	19.4	3.9	16.1	17.2	1.7	4.4	6.1
G	2, 3, 5, 6	1,5,6	1	/	1, 2, 9	1, 2, 9	/	/	5, 6

B : One-locus genotypes.

Depth n°	*Pgi*			*Got*		
	SS 1	SF 2	FF 3	SS 1	SF 2	FF 3
0-10 cm	2	7	6	6	9	0
10-20 cm	4	11	15	17	8	5
20-30 cm	6	11	13	15	10	5
30-40 cm	1	14	15	11	17	2
40-50 cm	4	14	12	17	7	6
50-60 cm	7	13	10	19	6	5
Total	24	70	71	85	57	23
%	14.5	42.5	43.0	51.5	34.6	13,9
G	ns	ns	ns	2	1, 3	2

more abundant in the lower 30 cm of the water column than in the upper 30 cm, the extent to which the animals aggregate in the near-bottom 10 cm was genotype-dependent.

DISCUSSION

Our results clearly indicate a genotype-dependent daytime vertical distribution of *Daphnia magna* in a shallow pond. Genetic differentiation in vertical distribution and migration patterns in *Daphnia* have been reported before (WEIDER, 1984; STIRLING *et al.*, 1990; MÜLLER and SEITZ, 1993). Our observations show that such habitat partitioning may occur in a very shallow system, on a scale of less than one meter. Previous work on clones isolated from the same pond had already indicated significant interclonal differences in phototactic behaviour (DE MEESTER, 1991a, 1991b). In concordance with these previous results, the present observations indicate that genotypes with a preference for a shallow day-depth are absent or rare in this population. Indeed, all genotypes are predominantly found in relatively deep water (see Table 2, Figure 1). This may be explained by the fact that this population co-occurs with fish (mainly *Rutilus rutilus* and *Gasterosteus aculeatus*). So far, we have not been able to hatch positively phototactic clones out of ephippia collected from the pond (DE MEESTER, pers. obs.). However, positively phototactic clones have been obtained in the laboratory through selfing clones isolated from the Driehoekvijver (DE MEESTER, 1991a), indicating that this population has not lost

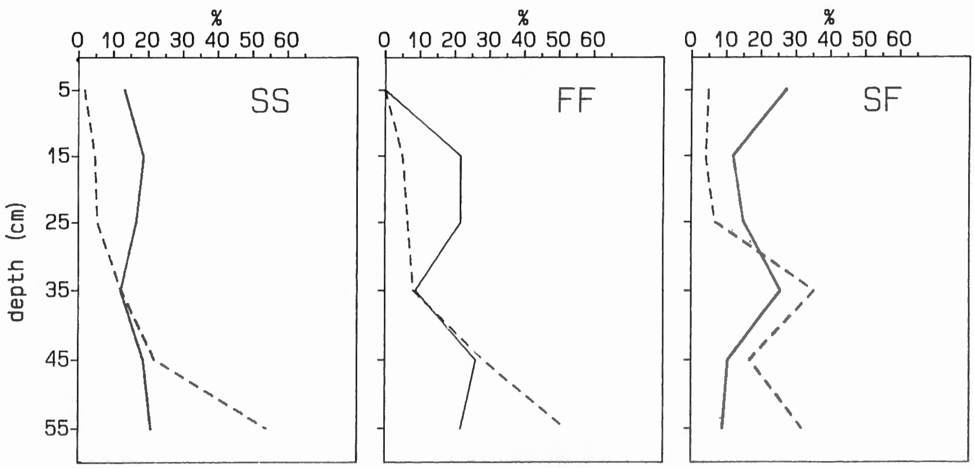

Fig. 1. — The vertical daytime distribution of *Daphnia magna Got* genotypes in Driehoekvijver, 5 February 1992 : Solid line : % adults of given genotype observed at different depths, when the same number of animals is analyzed at all depths (relative distribution). Broken line: estimates adjusted for differences in density of the *Daphnia* population at different depths (estimate of absolute distribution).

the capacity for an evolutionary change of its daytime distribution, *e.g.* by becoming partly positively phototactic, if the environmental conditions would allow so.

Our results are based on one vertical profile and a resolution of only two loci. The differences in vertical distribution that were observed for genotypes marked by their alleles at the *Got* locus most probably are the result of a linkage disequilibrium effect, though a direct effect of *Got* on phototactic behaviour or a physical linkage of the *Got* locus with loci influencing phototactic behaviour, cannot be excluded. Resolution of more loci might reveal more clear-cut vertical distribution patterns, as the multi-locus genotypes thus obtained would more closely correspond to true single genotypes.

Irrespective of these limitations, our results indicate that the vertical distribution of zooplankton in shallow ponds is not merely random, and that vertical distribution and migration patterns may be genotype-dependent even in such small-scale systems. This adds to the confidence that differences in phototactic behaviour between *D. magna* clones isolated from this population, as observed in the laboratory (*e.g.* De Meester, 1991a, 1991b), are not merely laboratory artefacts. In order to evaluate the full extent of genetic differences in vertical migration patterns of *Daphnia* and other zooplankton in shallow ponds, a more comprehensive study is needed, analysing the vertical distribution of genotypes over several day-night cycles.

ACKNOWLEDGEMENTS

We thank E. Ranta, K. Schwenk, P. Spaak, L. Weider and two anonymous reviewers for their remarks on an earlier version of the manuscript, though not all comments were incorporated in the present paper. LDM is a postdoctoral researcher with the National Fund for Scientific Research, Belgium.

REFERENCES

Calaban, M.J. and J.C. Makarewicz (1982) — The effect of temperature and density on the amplitude of vertical migration of *Daphnia magna*. *Limnol. Oceanogr.*, **27** : 262-271.

Clarke, G.L. (1931) — Quantitative aspects of the change of phototropic sign in *Daphnia*. *J. exp. Biol.*, **9** : 180-211.

De Meester, L. (1989) — An estimation of the heritability of phototaxis in *Daphnia magna* Straus. *Oecologia*, **78** : 142-144.

De Meester, L. (1991a) — An analysis of the phototactic behaviour of *Daphnia magna* clones and their sexual descendants. *Hydrobiologia*, **225** : 217-227.

De Meester, L. (1991b) — Evidence for intra-population genetic variability for phototactic behaviour in *Daphnia magna* Straus, 1820. *Biol. Jb. Dodonaea*, **58** : 84-93.

De Meester, L. (1993a) — The vertical distribution of *Daphnia magna* genotypes selected for different phototactic behaviour : Outdoor experiments. *Arch. Hydrobiol., Beih. Ergebn. Limnol.*, **39** : 137-155.

De Meester, L. (1993b) — On the genetical ecology of phototactic behaviour in *Daphnia magna* (Crustacea : Cladocera). *Academiae Analecta*, **55** : 145-187.

De Meester, L. and H.J. Dumont (1989) — Phototaxis in *Daphnia*: Interaction of hunger and genotype. *Limnol. Oceanogr.*, **34** : 1322-1325.

Dumont, H.J., Y. Guisez, I. Carels and H.M. Verheye (1985) — Experimental isolation of positively and negatively phototactic phenotypes from a natural population of *Daphnia magna* Strauss : A contribution to the genetics of vertical migration. *Hydrobiologia*, **126** : 121-127.

Harris, J.E. and U.K. Wolfe (1955) — A laboratory study of vertical migration. *Proc. R. Soc. B.*, **144** : 329-354.

Hebert, P.D.N. and M.J. Beaton (1989) - *Methodologies for allozyme analysis using cellulose acetate electrophoresis*. Helena Laboratories, Beaumont, Texas.

Müller, J. and A. Seitz (1993) — Habitat partitioning and differential vertical migration of some *Daphnia* genotypes in a lake. *Arch. Hydrobiol., Beih. Ergebn. Limnol.*, **39** : 167-174.

Peters, R.H. and R. De Bernardi (1987) - *Daphnia*. Ist. ital. Idrobiol., Pallanza.

Ranta, E. and V. Nuutinen (1985) — *Daphnia* exhibit diurnal vertical migration in shallow rock-pools. *Hydrobiologia*, **127** : 253-256.

Ringelberg, J. (1964) — The positively phototactic reaction of *Daphnia magna* Straus — a contribution to the understanding of diurnal vertical migration. *Neth. J. Sea Res.*, **2** : 319-406.

Ringelberg, J. (1987) — Light induced behaviour in *Daphnia*. In R.H. Peters and R. De Bernardi (eds.), *Daphnia*, Ist. ital. Idrobiol., Pallanza : 285-323.

Sokal, R.R. and F.J. Rohlf (1981) — *Biometry*. Freeman W. H. & Co., San Francisco.

Stirling, D.G., D.J. McQueen and M.R.S. Johannes (1990) — Vertical migration in *Daphnia galeata mendotae* (Brooks) : Demographic responses to changes in planktivore abundance. *Can. J. Fish. aquat. Sci.*, **47** : 395-400.

Van Uytvanck, J. and L. De Meester (1990) — Phototaxis in *Daphnia magna* : The influence of temperature and acidity on the phototactic behaviour of *Daphnia* genotypes. *J. Plankton Res.*, **12** : 1089-1097.

Weider, L.J. (1984) — Spatial heterogeneity of *Daphnia* genotypes : Vertical migration and habitat partitioning. *Limnol. Oceanogr.*, **29** : 225-235.