



The influence of fish kairomones on the induction and vertical distribution of sexual individuals of the *Daphnia galeata* species complex

Piet Spaak^{1,2} & Maarten Boersma²

¹Department of Limnology, EAWAG, Überlandstrasse 133, Postfach 611, 8600 Dübendorf, Switzerland

²Max-Planck-Institut für Limnologie, Postfach 165, D-24302 Plön, Germany

Tel: +41-1-823-5617. Fax: +41-1-823-5315. E-mail: spaak@eawag.ch

Abstract

To assess the potential production of hybrids and backcrosses in a semi-natural environment, we studied the combined effect of fish kairomone, and food level on the production of males and ephippial females in different clones of five *Daphnia* taxa from the *D. galeata* species complex. We also studied the diel vertical migration (DVM) of these sexual daphnids under the same varying conditions. This was done to test the hypothesis that males and ephippial females have different migrating strategies, which would increase their mating probability. The study was carried out in two large-scale indoor mesocosms, the so-called 'plankton towers' in the Max-Planck Institute in Plön, Germany.

Although all of the *Daphnia* taxa produced ephippial females in the course of the experiment, only *D. galeata* produced a significant number of males. Fish kairomones had a significant negative influence on the production of ephippial females. We found no DVM in the *D. galeata* males. They stayed at a depth between 5 and 6 m both day and night, 1 or 2 m above the thermocline. The ephippial females of *D. cucullata x hyalina* migrated, whereas ephippial females of the other taxa showed no DVM but came significantly closer to the surface in the presence of fish kairomones. We conclude that males and sexual females co-occur in this species complex both in time and space. Therefore, a regular production of hybrids and backcrosses in this species complex seems likely. Fish kairomones do not seem to significantly influence this process.

Introduction

Hybridization is a significant phenomenon within the *Daphnia galeata* complex (Schwenk & Spaak, 1995). The parental species *D. galeata*, *D. cucullata* and *D. hyalina* co-occur in many European lakes with one or more of their hybrids *D. galeata x hyalina*, *D. cucullata x galeata*, or *D. cucullata x hyalina* (Schwenk & Spaak, 1995; Spaak, 1997). However, hybridization in *Daphnia* is not restricted to this species complex. Colbourne & Hebert (1996) described several hybrid taxa of North America, and Hebert & Wilson (1994) did the same for Australia. Several genetic studies have shown that hybridization among *Daphnia* species is an ongoing process and that as a result, introgression takes place (Taylor & Hebert, 1992; Spaak, 1996). The fact that both hybrids and parental species are still genetically distinct shows, however, that pre- and/or post-mating barriers must exist that prevent the complete mixing of different species.

Daphnids are cyclic parthenogens; they alternate asexual with sexual reproduction. Whether a *Daphnia* individual reproduces sexually depends on external (inducing) factors as well as internal (genetic) factors. Sexual reproduction is primarily limited to restricted periods of the year and induced by factors that precede harsh conditions. Parthenogenetic females produce males and sexual females. When a sexual female is fertilised a so-called ephippium is produced. This ephippium contains one or two embryos that might hatch at favourable times. By going into diapause *Daphnia* can survive harsh conditions such as drought, food limitation or strong predation pressure. Therefore, it is important for a *Daphnia* clone or taxon to change over to sexual reproduction before these catastrophes happen. Hence, it is critical to react to the right environmental conditions. Obviously, interspecific hybridization can only occur during these periods of sexual reproduction. Hybrid *Daphnia* taxa have no problems reproducing parthenogenetically. In some lakes, they can even survive the winter period as

parthenogenetic females. Therefore, recurrent hybridization events are not necessary to maintain a hybrid *Daphnia* population.

Laboratory studies on sex-induction in *Daphnia* have focused mainly on factors necessary to switch from parthenogenetic reproduction to the production of ephippia. In addition to well-known factors such as temperature, photoperiod and density (Carvalho & Hughes, 1983; Korpelainen, 1989; Hobæk & Larsson, 1990), chemicals excreted by fish have recently been found to induce the production of ephippia in *D. magna* (Ślusarczyk, 1995; Pijanowska & Stolpe, 1996; Ślusarczyk, 1999). Differences in cues or in combinations of cues necessary to induce the switch to sexual reproduction between taxa can act as prezygotic reproductive barriers. Indeed, Spaak (1995) showed that different taxa from the *D. galeata* species complex produced different numbers of males and ephippial females when exposed to the same combination of stress factors. Moreover, he also showed that clonal variation existed within taxa for the ability to produce males and ephippial females. These different induction cues could lead to temporal differences in the presence of males and ephippial females for different taxa. Field studies have shown that, within the *D. galeata* complex, *D. cucullata* tended to reproduce sexually in autumn, whereas *D. galeata* showed sexual reproduction predominantly in spring (Spaak, 1995), which supports the hypothesis of reproductive isolation between these taxa.

In addition to temporal segregation of sexual forms of *Daphnia* taxa, spatial segregation may also occur in a lake. *Daphnia* migrate to deeper layers of a lake during the day to escape visual predators (Loose, 1993). At dusk, they move up into the warmer upper layers. This behaviour, known as Diel Vertical Migration (DVM), differs among *Daphnia* taxa. In Lake Constance, for example, *D. hyalina* shows a strong migrating behaviour, whereas *D. galeata* does not migrate (Stich & Lampert, 1981; Stich, 1989). Almost nothing is known about the vertical migration of sexual *Daphnia*. In a laboratory study with *D. pulicaria*, Brewer (1998) found that males stayed just above the thermocline, probably to reduce their predation risk while increasing their chances of mating with the migrating sexual females (see also Gerritsen, 1980). It is unknown whether this is a general pattern in daphnids or whether sexual forms of different taxa show different migration strategies.

The aim of the present study was to investigate the induction of sexual reproduction under semi-natural

conditions within the *D. galeata* species complex. Since fish kairomones seem to induce the production of ephippial females in *D. magna* under laboratory conditions, we investigated if this is a general phenomenon in *Daphnia*. Moreover, we tested if ephippial females and males from different taxa differ in their DVM behaviour. To answer these questions, we used the data from an experiment, which we carried out in the plankton towers at the Max-Planck Institute for Limnology in Plön. Data were collected to study the competition between 5 *Daphnia* taxa co-occurring in the Plußsee (Northern Germany) under different food and predation pressure conditions. In this paper, we describe the induction of males and ephippial females, its phenology and the vertical distribution of sexual daphnids in the towers.

Materials and methods

Experimental set-up

A large-scale competition experiment was conducted in the plankton towers (Lampert & Loose, 1992) using 20 clones (4 clones of each taxon: *D. galeata*, *D. cucullata*, *D. galeata* × *hyalina*, *D. cucullata* × *galeata* and *D. cucullata* × *hyalina*) collected from the Plußsee, Northern Germany in autumn 1995 (for details see Spaak et al., 2000). To ensure replication, both towers received the same treatment, and the effect of different treatments (food level, fish factor) was tested sequentially. The towers were filled with filtered (pore diameter 10 µm) water from the Schöhsee. Both towers had a thermocline (20 °C / 8 °C) at a depth of 8 m. Under the thermocline an anoxic zone was created to mimic the natural Plußsee conditions. The entire experiment was carried out under summer light conditions (16 h light, 8 h dark).

Because the conditions in the towers become difficult to control after roughly 50 days, the experiment consisted of two parts. At day 50, the towers were emptied and cleaned before the second part of the experiment. In the first part of the experiment, we first changed the food levels. They were changed on day 24 from 0.8 mg C l⁻¹ (high) to 0.3 mg C l⁻¹ (low) (see Fig. 1). Food levels were established once a day during the high food treatment and twice a day during the low food treatment. Two algal species (*Scenedesmus obliquus* and *Rhodomonas minuta*) were used as food in a 9:1 mixture. Moreover, after 37 days, a fish factor treatment was established in both towers. Water was

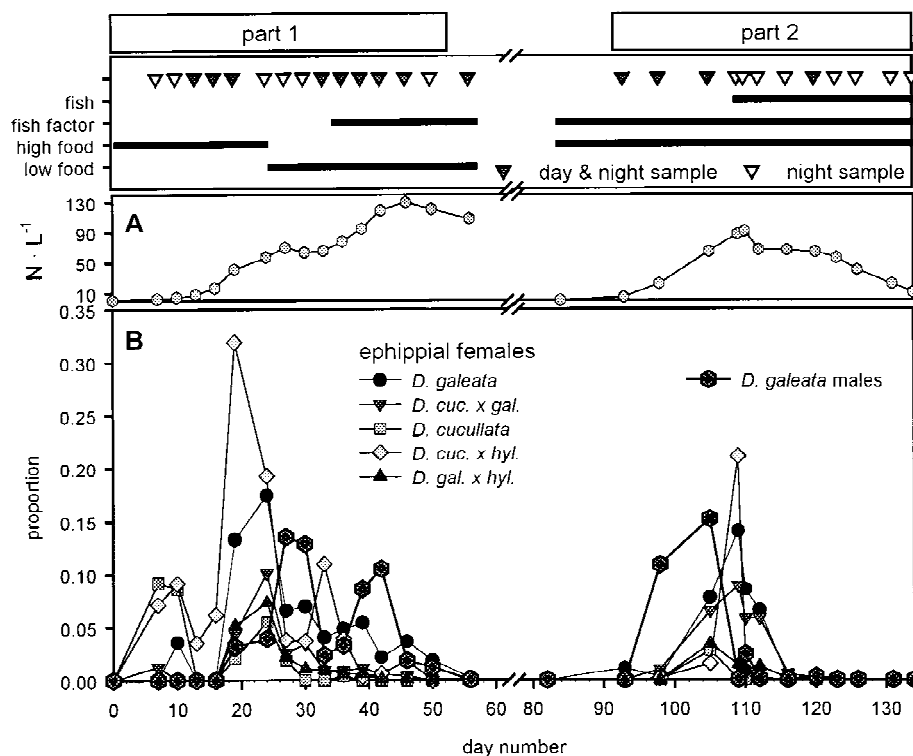


Figure 1. The upper panel (A) shows the experimental design of the tower experiment. The first part of the experiment started on 14 February 1996 (day 1), the second part on 8 May (day 84). The middle panel (B) shows the total density (N per litre) of all five taxa in the towers. The lower panel (C) show the proportion (of the total individuals of a given taxa) of ephippial females for all five taxa, and the proportion of *D. galeata* males.

pumped from the towers at a depth of 6 m into a 60 l container holding 50 individuals of *Leuciscus idus* (mean length 6 cm), and then returned to the towers. In the second part of the experiment, which started on day 82, fish chemicals were added continuously as described above (Food levels were $0.8 \text{ mg C} \cdot \text{l}^{-1}$). On day 109, we introduced two fish into each tower, two more fish were added on day 116 and day 120. We will refer to the several combinations of food level, presence of fish factor and fish as *treatments* in the rest of this paper.

Each tower was stocked with 300 adult *Daphnia* females per clone in the first part of the experiment and 200 per clone in the second. The clones were grown in 12 l buckets and acclimatised to the experimental temperature of $20 \text{ }^\circ\text{C}$, light and food conditions. Samples were usually taken at night, but additional day samples were taken to examine diurnal vertical migration (DVM). Sampling was carried out at 10 depths (0.6, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 m) using pumps and flow through traps attached to sampling ports (Lampert & Loose, 1992).

Over the course of the experiment, we took 27 night samples and 13 day samples. Per port, 60 randomly chosen adult individuals (if available) were measured and frozen individually in microtiter plates at $-80 \text{ }^\circ\text{C}$ for later electrophoretic analysis enabling taxonomic and clonal determination (Spaak et al., 2000). The remaining individuals from each sample were preserved in 4% formaldehyde and counted later to determine densities. When counting these samples we distinguished among adults, juveniles, males and ephippial females. Finally, the taxon distribution per sampling date and depth was combined with the formalin counts to calculate the absolute densities from each taxon.

Data analysis

In this paper, we concentrated on the taxon level, which means that the data of four individual clones is combined. The total numbers of individuals at a certain depths at a specific date was calculated by adding the electrophoretically analysed animals to the formalin counts. These totals (juveniles and adults)

were multiplied by the taxon fractions as determined with the electrophoresis to calculate the number per litre per taxon per depth, per date. These densities per depth were summed to calculate the taxon densities for the whole tower. We used the proportion of ephippial females and males per taxon for our analysis. Interspecific differences in the proportions of male and ephippial broods were analysed using a four-way analysis of variance with taxon, fish-factor and food level as fixed factor and tower as random factor. Tower was used as a factor in order to test if both towers were statistically identical. As these data obviously violate the assumptions of ANOVA (not normally distributed, Sokal & Rohlf, 1995), and could not be solved by transforming the data, we compared the calculated F values to F values produced by 100 randomisations of the data for each test (Innes & Dunbrack, 1993). In our analysis, we used the proportion of sexual females or males on a given sampling date as individual observation, and multiple dates within a 'treatment' as replicates.

The mean depth of the different taxa was determined as the weighted mean of the number of adult females at a certain depth. In this way we obtained 54 night observations (27 samples from two towers) and 26 day observations distributed over the several treatments (Fig. 1). We restricted the analysis of DVM to the part of the experiment without fish in the towers, since almost no sexual daphnids were present when fish were present in the towers. Using three-way ANOVAs, we tested for significant main effects (sampling time: day and night, fish factor: yes and no, and food level: high and low) and interactions.

Results

After 20 days, ephippial females were found for all five taxa simultaneously (*D. galeata*, *D. cucullata*, *D. hyalina*, *D. cucullata* × *galeata*, *D. galeata* × *hyalina* and *D. cucullata* × *hyalina*) in both parts of the experiment (Fig. 1). *D. cucullata* × *hyalina* and *D. galeata* produced the highest frequency of ephippial females in both parts of the experiment. The number of males produced was lower than the number of ephippial females, with essentially only *D. galeata* males found in the towers (Fig. 1), and only sporadically for the other taxa. During the first part of the experiment, most sexual forms were observed at the end of the high food phase. During the second part, the ephippial females and males disappeared soon after the intro-

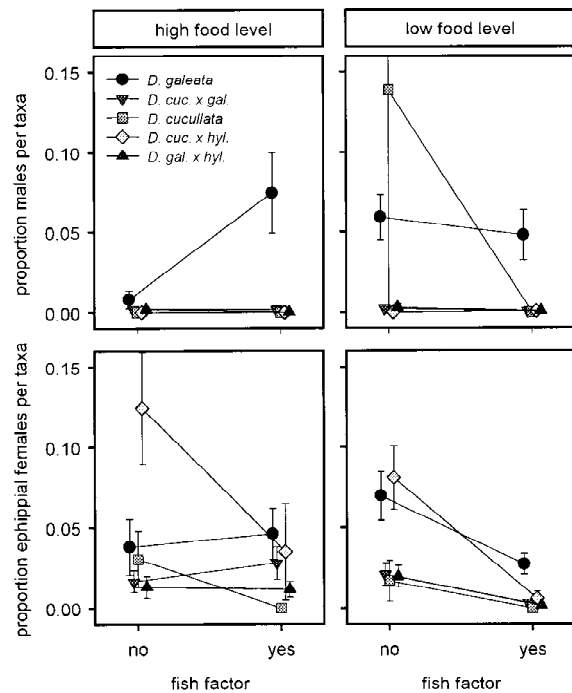


Figure 2. Reaction norms for the five taxa studied for the proportion of males (upper panels) and ephippial females (lower panels) under conditions with and without the presence of fish chemicals. Panels at the left show high food conditions (0.8 mg C l^{-1}), at the left low food conditions (0.3 mg C l^{-1}). Error bars indicate 1 SE.

duction of fish. The disappearance of the ephippial daphnids was faster than the reduction in the total number of *Daphnia* after the introduction of fish (Fig. 1A,C), suggesting a positive selection of ephippial daphnids by fish.

The production of *D. galeata* males was not significantly influenced by the presence of fish factor in the water of the towers (Fig. 2, Table 1). With respect to the proportion of ephippial females found in the towers, we found a significant taxon effect, and also a significant interaction between taxon and food level and between taxon and fish factor (Fig. 2, Table 1). Eight out of 10 reaction norms had a negative slope, indicating a lower proportion of ephippial females under fish factor conditions. *D. cucullata* × *hyalina* produced the largest number of ephippial females in the absence of fish factor on both food levels. *D. galeata* showed the highest proportion of ephippial females under fish factor conditions (Fig. 2).

The mean depth of *D. galeata* males showed no significant differences between day and night for the food and the fish factor treatments (Fig. 3). The mean depth was always around 5 m with one excep-

Table 1. Results of four-way ANOVAs for the characters: proportion male broods (Proportion males) and proportion of ehippial broods (Proportion ehippial females). Tower was treated as a random factor. Significances were determined using the bootstrap method discussed in the text

	df	Proportion males		Proportion ehippial females	
		MS	F	MS	F
Tower	1	0.00176	0.455	0.00948	3.205
Food	1	0.00697	1.795	0.00780	9.700
Fish factor	1	0.00031	0.197	0.04806	100.594
Taxon	4	0.02477	5.701	0.03087	15.537 ^a
T×F	1	0.00388	1.002	0.00080	0.272
T×Ff	1	0.00156	0.403	0.00048	0.162
F×Ff	1	0.01700	4.658	0.00614	510.576 ^a
T×Tax	4	0.00435	1.122	0.00199	0.672
F×Tax	4	0.00346	0.947	0.00396	1.805
Ff×Tax	4	0.00748	1.650	0.01619	11.650 ^a
T×F×Ff	1	0.00365	0.942	0.00001	0.004
T×F×Tax	4	0.00366	0.944	0.00219	0.741
T×Ff×Tax	4	0.00453	1.169	0.00139	0.470
F×Ff×Tax	4	0.00617	1.663	0.00337	7.215
T×F×Ff×Tax	4	0.00371	0.957	0.00047	0.158
Error	270	0.00387		0.00296	

^aSignificant with bootstrap method.

tion (no fish – high food – night) when the animals were found at only 1 m depth. We had enough data for all taxa, except *D. cucullata*, to calculate mean depths of ehippial females. One taxon (*D. cucullata*×*hyalina*) showed a DVM pattern under fish conditions, both with high and low food (Fig. 3, Table 2); without fish factor this pattern was reversed. A significant time×fish factor interaction (Table 2) evinced this. For the other three taxa, no DVM patterns could be observed for the sexual females. However, these three (*D. galeata*, *D. cucullata*×*galeata* and *D. cucullata*×*hyalina*) showed a significantly lower mean depth (higher in the water column) under the fish factor conditions compared to the control both day and night under the high food condition (Fig. 3, Table 2).

Discussion

Our results indicate that interspecific differences exist within the *D. galeata* species complex with respect to the production of males and ehippial females. Under the different conditions offered in our experimental set-up, *D. galeata* was the only taxon that produced males. This was unexpected. In an earlier study, Spaak (1995) showed that under a combined set of stress factors in the laboratory, *D.*

cucullata×*hyalina* clones produced the highest proportion of male broods, whereas *D. galeata* produced no males at all. These results confirm earlier findings that male induction in *Daphnia* is taxon specific, and that the factors that induce the production of ehippial females and males are not identical, although it is generally more difficult to induce the production of ehippial than of male offspring (Hobæk & Larsson, 1990; Kleiven et al., 1992). These differences in induction promote sexual isolation of the species of the *D. galeata* complex, which is corroborated by data collected in the field. Schwenk (1997) mostly found males of *D. galeata* in spring and of *D. cucullata* in autumn in Tjeukemeer (The Netherlands), whereas the hybrid *D. cucullata*×*galeata* produced males in both seasons but to a lower extent. In Plußsee, the lake of origin for our experimental animals, Denk (pers. comm.) only found males and ehippial females in autumn, and not in spring when densities were highest. They also observed that the same taxa that were present as parthenogenetic females produced males and ehippial females.

We found ehippial females for all taxa during our experiment although the proportions differed significantly between the taxa. Our results show that hybrid taxa can produce ehippial females, and this illustrates that sexual hybrids and parental species can co-occur

Table 2. Results of three-way ANOVAs to test for the effect of Time (day–night), Food (high–low), Fishfactor (yes–no) and their interactions on the mean depth of *D. galeata* males and *D. galeata*, *D. cucullata*×*galeata*, *D. cucullata*×*hyalina* and *D. galeata*×*hyalina* ephippial females

	df	<i>D. galeata</i>		<i>D. galeata</i>		<i>D. cuc. × gal.</i>		<i>D. cuc. × hyl.</i>		<i>D. gal. × hyl.</i>	
		Male		Sexual female		Sexual female		Sexual female		Sexual female	
		n=30		n=38		n=23		n=25		n=21	
		MS	F	MS	F	MS	F	MS	F	MS	F
Time	1	7.187	1.969	0.894	0.950	3.649	1.223	0.002	0.001	0.612	0.144
Food	1	3.363	0.921	5.837	6.204*	8.327	2.791	0.522	0.287	9.308	2.185
Fish fac	1	4.682	1.282	4.247	4.515*	16.591	5.560*	2.164	1.189	20.022	4.701*
Ti×Food	1	4.972	1.362	1.523	1.619	2.405	0.806	0.344	0.189	0.351	0.082
Ti×Ff	1	1.703	0.467	0.794	0.844	2.736	0.917	14.320	7.867*	0.706	0.166
Food×Ff	1	10.821	2.964	2.829	3.007	28.531	9.562**	13.513	7.424*	7.535	1.769
To×Food×Ff	1	12.701	3.479	1.095	1.164	1.330	0.446	0.152	0.083	0.006	0.001
Error		3.651		0.941		2.984		1.820		4.259	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

under the same conditions making backcrossing a possibility. This confirms the results of genetic studies showing that introgression occurs within this species complex (Spaak, 1996; Gießler, 1997).

In our study, we found significant interactions between the effect of fish kairomones and taxon and between the presence of fish kairomones and food level on the proportion of ephippial females produced (Table 1). At the low food level, all reaction norms were negative, and at the high food level three out of five were negative (Fig. 2). Based on earlier studies on the effect of fish kairomones on the induction of ephippial females in *D. magna* (Ślusarczyk, 1995; Pijanowska & Stolpe, 1996), we expected an increase in sexual reproduction in the presence of fish kairomones. This is thought to be adaptive, since the resting stages are known to pass through the digestive tract of predators without loss of viability (Proctor, 1964). In this way *Daphnia* could 'survive' fish predation in its resting stage. One explanation for this discrepancy could be the food of the fish used in the experiments. Although several reports exist indicating that the feeding conditions of the fish do not influence the production of kairomones (e.g. Loose et al., 1993), recent evidence suggests that alarm substances of daphnids might be involved in the chemical communication (Pijanowska, 1997; Ślusarczyk, 1999). As we fed the fish with chironomid larvae, we avoided mixing direct fish effects and possible effects of alarm substances, but there is no reason to assume that different food would result in an opposite reaction. Another explanation for our deviant results could be the different genetic ori-

gin. The previous work on ephippia induction under fish conditions was done with one single *D. magna* clone, whereas we used 20 clones from five different taxa. It is possible that the specific *D. magna* clone behaved differently. As Boersma et al. (1998) showed that, within *D. magna*, large clonal variation exists for the reaction to fish kairomones this might well be the case. This demonstrates that it is important that the experiments on predator induced diapause are repeated with additional clones. Alternatively, the differences in reactions to fish kairomones might be caused by species differences. *D. magna* is a pond species, and as a result, relies on sexual reproduction to survive harsh conditions and to re-colonise their ephemeral habitats. Species of the *D. galeata* complex mainly occur in lakes and populations in lakes are much more stable (Berendonk, 1998). More importantly, lake species can also avoid predation by vertical migration. Hence, for lake-species it might be adaptive to produce less ephippia in the presence of fish, since their vulnerability to predation increases when carrying an ephippium, as they are much easier detected by fish (Mellors, 1975). In the present study, we observed that different taxa from the *D. galeata* species complex significantly reduced their ephippia production in the presence of fish factor. This could be adaptive for this species complex. Indeed, we found an almost complete disappearance of ephippial animals in the towers shortly after the introduction of fish. This reduction was larger than the overall reduction in *Daphnia* densities (Fig. 1), which indicates that ephippial females were preferred by the fish in our experiment. However,

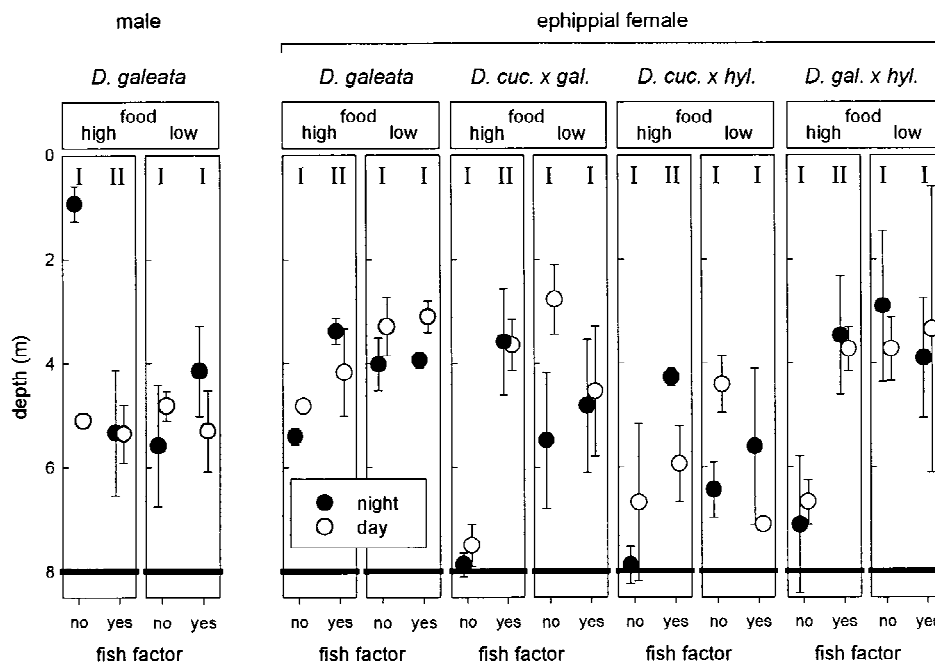


Figure 3. Mean day and night depths (with SE) for *D. galeata* males and ehippial females of *D. galeata*, *D. cucullata* × *galeata*, *D. cucullata* × *hyalina* and *D. galeata* × *hyalina* for several treatments. Depths are plotted for fish factor and no fish factor conditions both for high and low food levels. The thermo- and oxycline at 8 m depth is indicated. Roman I and II indicate phases one and two of the experiment.

our findings should be interpreted with some care. The treatments in the towers were applied after each other and as a result are not independent in a statistical sense, even though the variation between towers was small and not significant. All the same, controlled experiments, with different clones and species have to be done to test the hypothesis that lake and pond species of *Daphnia* react different to fish kairomones with respect to the production of ehippial females.

Our data clearly show that, as was found for parthenogenetic *Daphnia* before (Stich & Lampert, 1981), interspecific variation exists for day and night depths of sexual animals. *D. galeata* males showed no significant variation related to the different factors tested. They almost always had a mean depth between 4 and 6 m. This corresponds with the observation of Brewer (1998) in 1 m long tubes with sexual individuals of *D. pulicaria*, who found males positioned on the thermocline and, like in our study, without any DVM behaviour. Brewer hypothesised that this behaviour, low predation risk and relatively slow developmental rates, increases the likelihood that males meet a sexual female than if they swam up to regions with an increased predation risk and search for sexual females. We found DVM behaviour of ehippial daphnids as a reaction to fish factor only for *D. cucullata* × *hyalina*

both under high and low food conditions (Fig. 3). The other taxa did not show DVM behaviour. These other taxa however, (*D. galeata*, *D. cucullata* × *galeata* and *D. galeata* × *hyalina*) showed a significant reaction to fish factor (Table 2), especially under high food conditions (Fig. 3). The mean depth of ehippial females (during day and night) decreased with several meters when the daphnids in the towers were exposed to fish factor. To our knowledge, no published data exists about the depth distribution of sexual daphnids, although a lot is known about the vertical distribution and migration of parthenogenetic *Daphnia* (Stich & Lampert, 1981; Ringelberg, 1991; Weider & Stich, 1992; Loose et al., 1993). Since ehippial females are better visible by predatory fish one would expect ehippial females to be deeper in the water column during the day compared to the night to reduce their predation risk by visually hunting predators. Furthermore, De Meester et al. (1995) showed that larger *D. galeata* × *hyalina* individuals migrated deeper compared to smaller ones. Therefore, it is difficult to understand why in our experiment the larger taxa (*D. galeata* and *D. galeata* × *hyalina*) do not migrate as one of the smaller taxa (*D. cucullata* × *hyalina*) does.

Although the experimental conditions in the first and second part of the experiment were identical (ex-

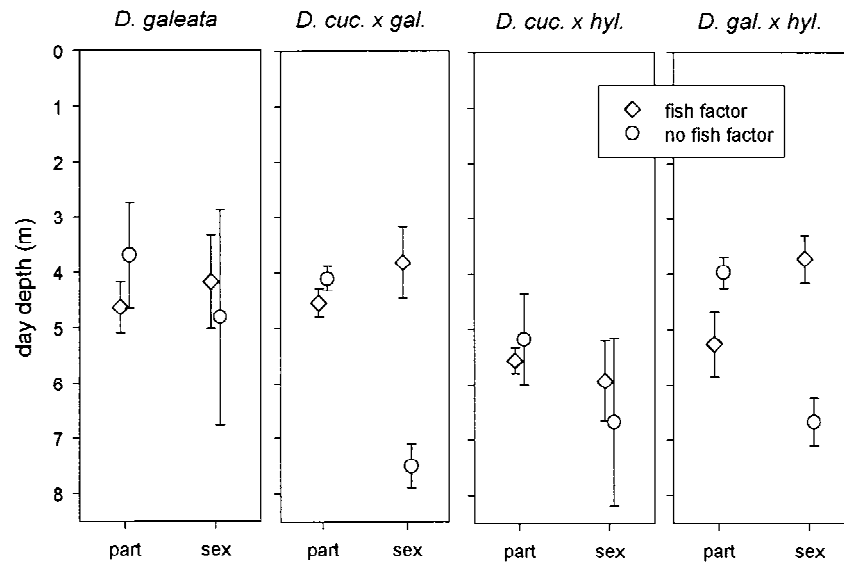


Figure 4. Mean day-time depths for parthenogenetic and ephippial *Daphnia* under high food conditions in the towers. The period with fish predation is excluded from the analyses. Error bars indicate 1 SE.

cept for the 'treatments'), it is still possible that our results are an artefact of our experimental design. The fish kairomone, high-food, treatment comes from the second part of the experiment, and this treatment yields very similar results compared to both low food treatments in the first part. It cannot be excluded that the food condition in the second part of the experiment were lower than we wanted, although this option is less likely since fecundity did not differ between the first weeks of both parts of the experiment.

The mean day depth of ephippial females in the towers was deeper than the mean day depth of the parthenogenetic females in the treatments without fish (Fig. 4). This effect disappears under fish kairomone conditions (Fig. 4). This cannot be an artefact since parthenogenetic and ephippial females were collected simultaneously. Interestingly, also in the field it was observed that ephippial daphnids (*D. hyalina* and *D. galeata* × *hyalina*) tended to be higher in the water column during the day compared to parthenogenetic daphnids (Spaak unpubl. results). To our knowledge no studies have been carried out on the influence of fish kairomones on the behaviour, life history or morphology of ephippial *Daphnia*. It could be the case that ephippial daphnids have to put so many resources in the production of their sexual eggs, that they cannot afford to migrate to deeper layers with less food and lower temperatures.

In conclusion, in the present study we could show that fish kairomones influence the proportion of ephip-

pial females produced in *Daphnia*, but that the production decreased when fish kairomones were present. No influence on the production of males was found. Differences between taxa (species and hybrids) could be shown. Hence, we conclude that hybrids and backcrosses can be produced within the *D. galeata* species complex since males and ephippial females of different taxa co-occur. We could show high variation in the vertical distribution of *D. galeata* males and ephippial females from all other taxa of the *D. galeata* species complex. There was no distinct spatial separation between taxa that could circumvent hybridization events. The different DVM strategies and spatial reactions to fish kairomones will, however, influence the predation risk of the individual taxa.

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