Effect of fish kairomone and bacterial flora of fish in two differently sized *Daphnia* species

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Introduction

The effect of kairomones on zooplankton behaviour (Gliwicz, 1990), morphology (Boersma et al., 1994) and life history traits () has been extensively studied for more than 20 years. However, regardless of numerous studies, the nature of fish kairomones remains unknown. Recently, it has been suggested that since chemical signalisation of the predator presence cannot be an advantage, bacteria associated with fish skin or fecal pellets are 'to be blamed' for the kairomones (Ringelberg and Van Gool, 1998). Here we tested if the life history and phenotypic changes in *Daphnia* can be partially explained by the additional food source provided by the bacteria related to fish exudates and fecal pellets.

The ability to exploit bacterial food depends on the intra-setular distances specific for the species and developmental stage of *Daphnia* (cit). Therefore we used Daphnia species of different sizes: smaller but efficiently grazing on bacteria, *Daphnia cucullata,* and bigger but not retaining bacteria, *Daphnia hyalina*. Our results indicate that efficient usage of bacterial food associated to fish modifies the response of *Daphnia* to kairomones.

*Daphnia hyalina* (**a**) and *D. cucullata* (b) at maturity

Materials and methods

In each of the 3 life history experiments, freshly born neonates (<8h old) of two *Daphnia* species, larger *D. hyalina* (clone HG011) and smaller *D. cucullata* (clone C006 NIOO), were randomly distributed in 320 beakers (1 animal per beaker, 160 per sp.) filled with filtered lake water. Different filtration procedures (EXP.1: 1.6 µm, EXP.2: 0.45 µm and EXP.3: 0.2 µm) were used to modify the initial biomass and size spectrum of available bacterial food. Prior to each filtration, half of the aged, filtrated (0.45) lake water was conditioned with small (<10cm) roach (*Rutilus rutilus* fed once per day with *Chironomidae*) for 24h at density of 1 fish per 4 liters (kairomone-treatment), the other half remain unconditioned (no-kairmone treatment). The water was then filtered according to the 3 procedures above and mixed with fresh algal suspensions (*Scenedesmus obliguus*, 1 mg algal C L^{-1}).

Daphia were checked for molt every 8h in first 4-6 pre-reproductive instars, after molting each animal was measured (from the top of the eye to the base of the tail spine) and the total volume of lipid reserves in the body was calculated as a total amount of ellipsoidal droplets, each time 2 or 3 random individuals were used for body mass. When Daphnia deposited the first and second clutch of eggs into the brood chamber, life history parameters were followed: age and size at reproduction (AFR; SFR), number and size of eggs, and volume of each egg in the brood cavity.

Triplicate volumes of each medium were analyzed for total organic carbon content (Shimadzu TOC 5050). Bacterial and algal cells in each medium were counted (Olympus HSB) after standard DAPI staining (Porter and Feig, 1980).
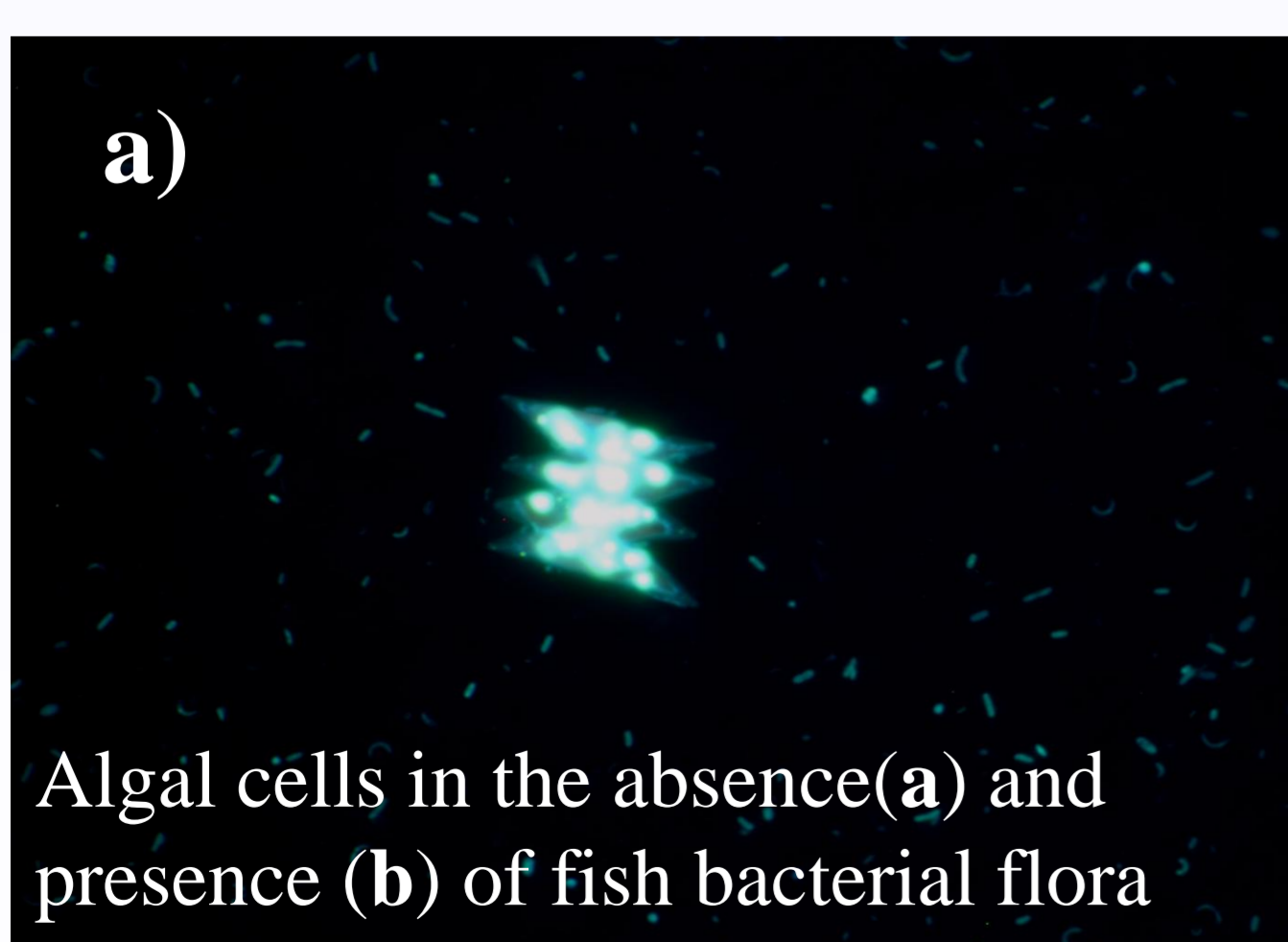
Algal cells in the absence(**a**) and presence (**b**) of fish bacterial flora
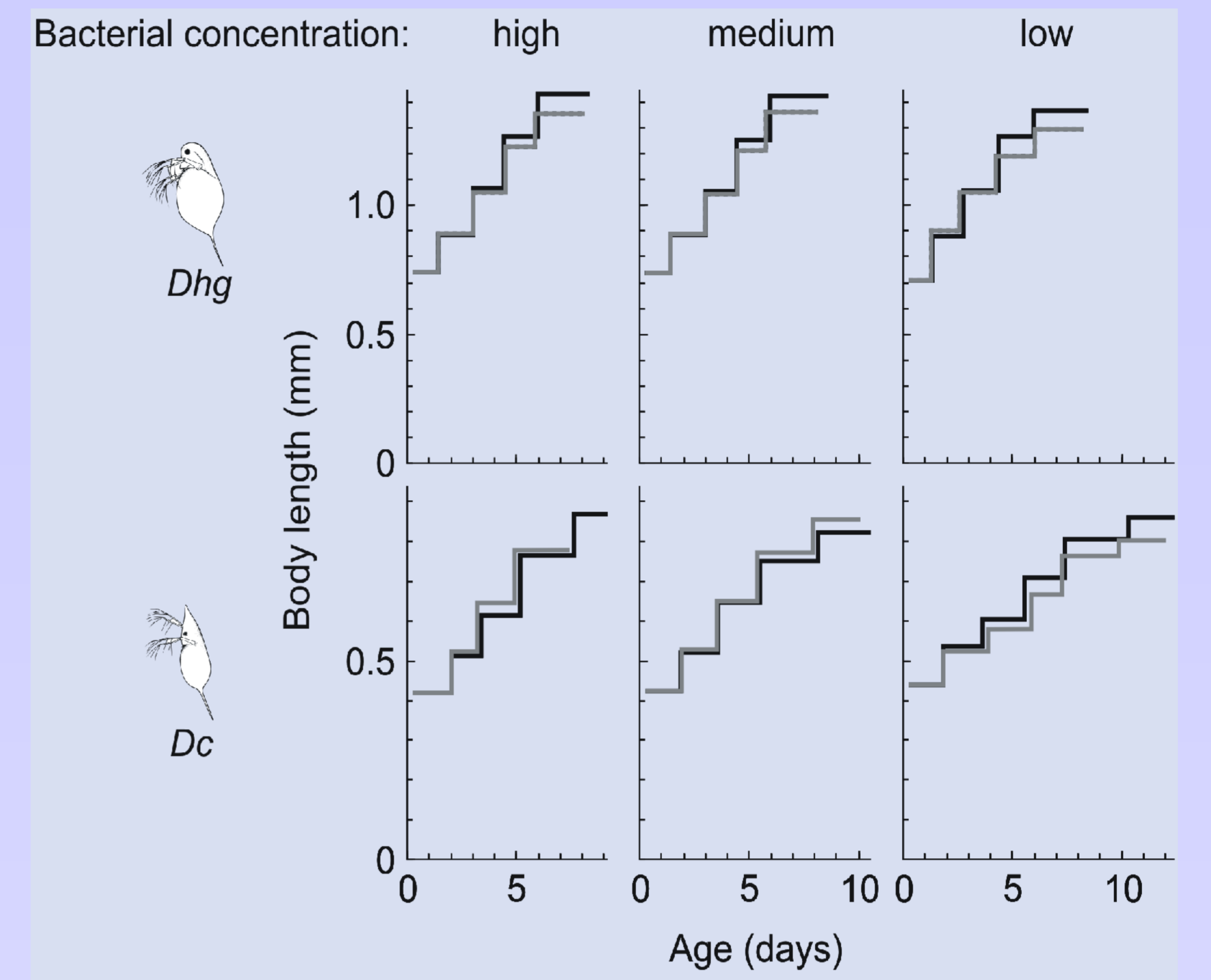
Results

Fig. 1. Instar-to-instar body length increase in *D. hyalina* (upper) and D. *cucullata* (lower panel) in the presence (grey line) and absence (black line) of fish kairomones at high (1 mg C l^{-1}) algal food concentration and at different densities of bacteria as an additional food: high (0.10), medium (0.05), and low (0.03 mg C l^{-1}).
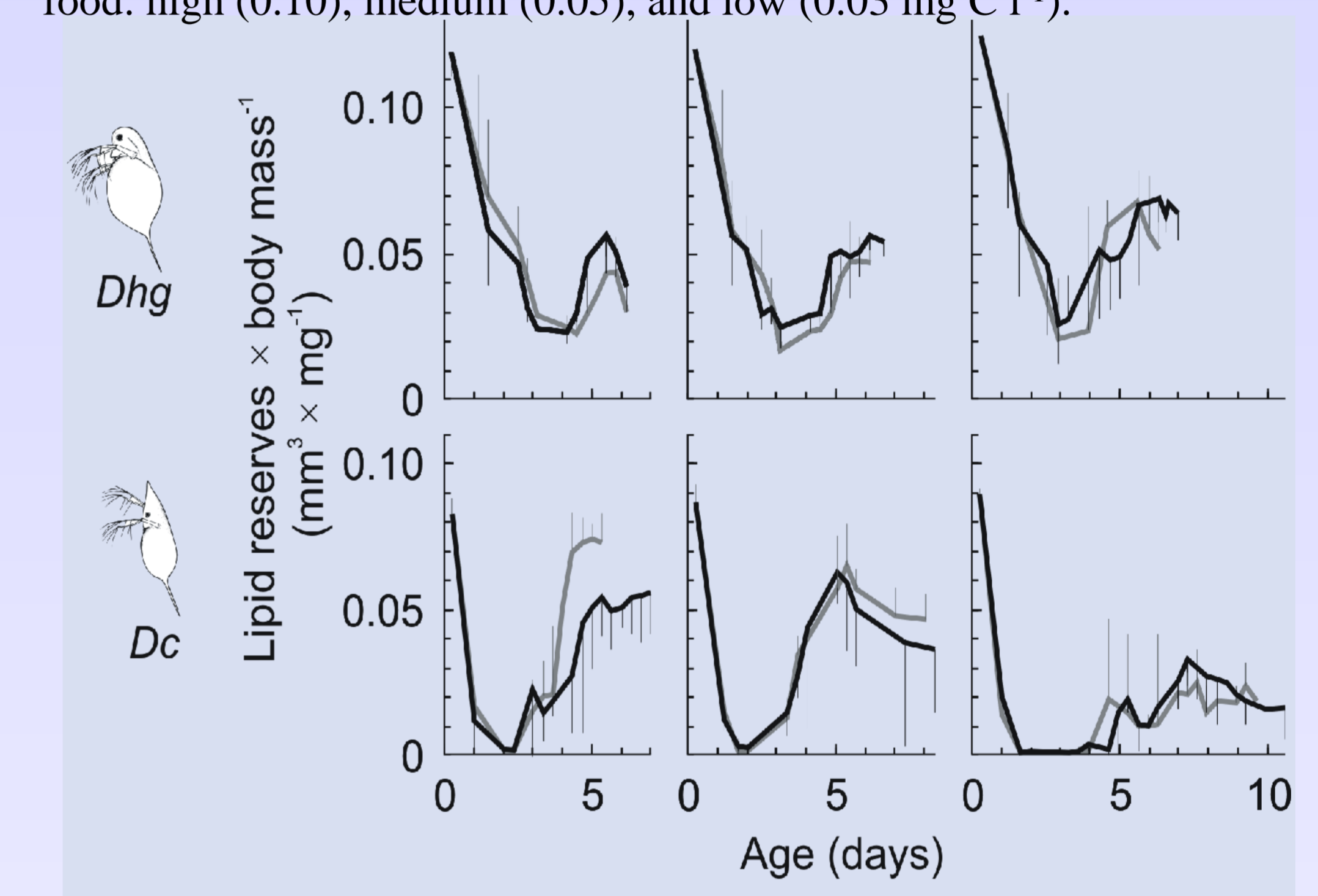

Fig. 2. The evolution of lipid reserves in *D.hyalina* (upper) and *D. cucullata* (lower panel) in the presence (grey line) and absence (black line) of fish kairomones at high (1 mg C l^{-1}) algal food concentration and at different densities of bacteria as an additional food: high (0.1), medium (0.05), and low (0.03 mg C l^{-1}). The lipid reserves shown as the ratio (+ or - SD) of integrated volume of lipid droplets (mm^3) to body mass (mg).

Table 1. Results of three-way ANOVA (F, p and df) test for fish kairomones (F), algal food (Sc), additional bacterial food (B) and interactions between factors on the life history traits in *D. hyalina* (*Dhg*) and *D. cucullata* (*Dc*). Statistical significance is accepted at * $p< 0.05$, ** $p< 0.005$, *** $p< 0.0005$, NS - not significant.

| | Trait | AFR | | SFR | | Instars | | Fat volume | | | | Egg volume | | |
| | | | | | | | | Juvenile | | Adult | | | | |
	Factors; interactions	F	p	F	p	F	p	F	p	F	p	F	p	df
Dhg	F	32	***	118	***	16	***	0	ns	0	ns	64	***	1
	Sc	64	***	167	***	0	ns	16	***	1	ns	8	**	1
	B	20	***	16	***	0	ns	0	ns	61	***	2	ns	2
	$F \times Sc$	5	*	9	**	0	ns	9	**	1	ns	1	ns	1
	$F \times B$	0	ns	3	ns	0	ns	1	ns	3	ns	1	ns	2
	$Sc \times B$	9	***	4	*	0	ns	16	***	2	ns	8	***	2
	$F \times Sc \times B$	0	ns	1	ns	0	ns	0	ns	2	ns	0	ns	2
	Error (*df*)	344		344		344		304		304		319		
Dc	F	79	***	18	***	66	***	3	ns	28	***	46	***	1
	Sc	126	***	1	ns	91	***	2	ns	44	***	1	ns	1
	B	221	***	32	***	163	***	41	***	110	***	11	***	2
	$F \times Sc$	52	***	2	ns	50	***	1	ns	12	**	11	**	1
	$F \times B$	0	ns	3	*	5	*	0	ns	1	ns	5	**	2
	$Sc \times B$	39	***	7	**	28	***	4	*	43	***	1	ns	2
	$F \times Sc \times B$	7	**	18	***	7	***	0	ns	4	*	1	ns	2
	Error (*df*)	304		304		304		303		303		305		

We provide evidence for the effect of fish bacterial flora on life history changes resulting from the presence of fish kairomones. Although Geller and Müller (1981) show that mesh size of Cladocera have implications on the bacterial food exploitation, for the next 30 years fish was considered only as a source of thread to zooplankton. Today we can assume that it can be as well a source of food.