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Life-history responses of *Daphnia pulicaria* to diets containing freshwater diatoms: Effects of nutritional quality versus polyunsaturated aldehydes

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Abstract

Like marine diatoms, some freshwater diatoms produce $\alpha,\beta,\gamma,\delta$-polyunsaturated aldehydes (PUA) when damaged. Some of these oxylipins are suspected of impairing egg viability in marine copepods. To determine whether these compounds also play a role in influencing the trophic interactions in freshwater environments, we measured growth and reproduction of the cladoceran *Daphnia pulicaria* in response to diets composed of seven diatoms differing in PUA production. The juvenile growth rate of *Daphnia* varied with the diatom species, but was not related to oxylipin production. Egg hatching success was nearly 100% in all clutches for all diets, except with a diet of the decadienal producing *Fragilaria* sp., where it decreased dramatically in clutches 5–7. In vitro tests of egg hatching in the presence of PUA showed a dose-dependent inhibition for decadienal. Population parameters (i.e., life-time fecundity and instantaneous rate of population growth) were not affected by PUA, as the contribution of late clutches to them was negligible. Consequently, the wound-activated production of PUA by diatoms cannot be regarded as a defensive mechanism against *Daphnia* population recruitment.

Marine diatoms have been considered a main path of the energy flow from primary producers to copepods (Ryther 1969), but their central role is now under debate. Many copepods, in fact, are omnivorous and prefer protists (Kleppel 1993), and various diatoms have been found to have deleterious effects on copepod reproduction in the laboratory and in the field (Ban et al. 1997; Miralto et al. 1999). As freshwater diatoms can represent a considerable portion of phytoplankton biomass in lakes during seasonal succession (Sommer et al. 1986), they may be frequently included in the *Daphnia* diet. There is evidence that small single-celled diatom species like *Stephanodiscus hantzschii* are high-quality food for daphnids and provide high somatic and population growth rates and fecundity (Infante and Litt 1985; Lundstedt and Brett 1991), while the nutritional value of larger colonial species like *Asterionella* and the genus *Fragilaria* is lower (Infante and Litt 1985).

In recent years, copepods fed widespread bloom-forming diatoms (e.g., *Thalassiosira rotula* and *Skeletonema costatum*) were found to have low egg-hatching success and erratic embryonic development (for a review, see Ianora et al. 2003). These effects were correlated with the occurrence of a group of structurally diverse $\alpha,\beta,\gamma,\delta$-polyunsaturated aldehydes (PUA) produced by mechanically damaged diatom cells (Miralto et al. 1999; Pohnert 2000). PUA belong to the group of oxylipins, compounds derived from the oxidative transformation of polyunsaturated fatty acids (Pohnert 2000), and their production has been interpreted as a diatom defense affecting copepod recruitment into the next grazer generation (Ianora et al. 2004). Among the PUA, 2,4-decadienal as well as other shorter chain-length volatile aldehydes and oxo-acids, all bearing the same structural $\alpha,\beta,\gamma,\delta$-polyunsaturated aldehyde motive, have been found to impair copepod hatching success and cleavage of sea urchin eggs in vitro (d'Ippolito et al. 2002*a*,*b*; Pohnert et al. 2002; Adolph et al. 2003).

It is interesting that the same reactive oxylipins have been detected in freshwater diatoms. They were reported from epilithic diatom biofilms (Jüttner and Durst 1997) and in lake water during the senescent phase of phytoplankton blooms (Watson et al. 2001). In particular, the freshwater diatoms *Fragilaria* sp. and *Melosira varians* produced the volatile aldehydes 2,4-heptadienal, 2,4-octadienal, and 2,4-decadien-

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al (Wendel and Jüttner 1996). *Asterionella formosa* and *Gomphonema parvulum* produce the closely related polyunsaturated oxo-acids, 12-oxo-dodeca-5,8,10-trienoic acid (12-ODTE) and 9-oxo-nona-5,7-dienoic acid (9-ONDE), respectively. Similar lipoxygenase/hydroperoxide lyase reactions as described for marine diatoms are involved in the biosynthesis of these metabolites in freshwater diatoms (for a review, see Pohnert and Boland 2002).

Despite the known production of PUA in freshwater diatoms, their effect on freshwater grazers has rarely been studied. Short-term feeding experiments (Carotenuto and Lampert 2004) rendered the role of PUA as feeding deterrents for *Daphnia* unlikely, but there is still the possibility of long-term effects on growth and reproduction, as in marine copepods. For example, arrested egg development and egg abortion have been observed in field populations of *Daphnia* (Threlkeld 1979; Boersma and Vijverberg 1995) and have been linked to food composition but not to particular species of diatoms. We tested the hypothesis that *Daphnia* react like marine copepods in their responses to diatom diets. We compared the effects of seven diatom species varying in colonial shape and PUA production on the growth and reproduction of *Daphnia.* Concentrating on parameters that determine the fitness of *Daphnia,* we studied the question of whether the wound-activated production of particular oyxlipins by diatoms can be regarded as a defense mechanism against *Daphnia* grazing pressure.

Materials and Methods

Algal culture and preparation of food suspensions—Seven nonaxenic diatom species (*S. hantzschii, Stepanodiscus minutulus, Cyclotella meneghiniana, A. formosa, Fragilaria* sp., *Fragilaria capucina, G. parvulum*) were cultivated in 1-liter conical flasks in modified WC medium (Guillard and Lorenzen 1972) in a growth chamber at 16°C under a 16 : 8 light : dark (LD) cycle. The nonaxenic green alga *Scenedesmus obliquus* was grown in a chemostat in modified CHU12 medium (Müller 1972) at 20°C and continuous light. Properties of six of the strains are presented in Carotenuto and Lampert (2004). *G. parvulum* has similar cell size as the other diatoms and tends to form aggregates.

For use in life-history experiments, stock algal cultures were diluted with membrane-filtered water from a nearby mesotrophic lake (Schöhsee) to a final concentration of 1 mg carbon L^{-1}, which is above the incipient limiting concentration for *Daphnia* (Lampert 1987). The density of the stock algae was estimated by measuring the light extinction (800 nm) in a photometer. The final algal concentration was then adjusted using a separate calibration curve (extinction vs. particulate carbon) for each algal species.

Chemical analysis of diatoms—Screening of exponential growth-phase cultures of the seven diatom species for volatile aldehyde production was performed with modifications based on an established procedure (Pohnert et al. 2002). The algal cultures were gently filtered onto GF/C filters (Whatman). The filters were placed in 5-ml glass vials and the pellet was resuspended in 0.5 ml medium to a final concentration of 10^6–10^7 cells ml^{-1}. Saturated NaCl solution (0.5 ml) was added to damage cells by osmotic pressure. The samples were then sonicated and extracted with solid-phase microextraction as described by Pohnert et al. (2002). Identification of PUA was performed by comparison with synthetic (Pohnert 2000; Adolph et al. 2003) or commercially available reference compounds. For quantification of volatile PUA, samples were prepared as described above, derivatized with O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride, and extracted with hexane according to Ianora et al. (2004). The nonvolatile acidic aldehydes 12-ODTE and 9-ONDE were detected and quantified by reversed phase high-performance liquid chromatography-mass spectroscopy according to Pohnert (2000). Sample preparation included filtration and damaging of the cells by sonication, methanol-precipitation of proteins, and centrifugation.

Life-history trait experiments—Stocks of *D. pulicaria* Forbes were maintained in a temperature-controlled room at 19°C ± 1°C and continuous dim light. They were kept in 1.5-liter jars with filtered Schöhsee lake water and fed *S. obliquus* three times a week. Juveniles of different ages ($n = 90$) were collected from the stocks and raised individually in 200-ml jars with lake water and 1 mg C L^{-1} of *Scenedesmus* until they produced their third clutch of eggs. Offspring from the third brood were used to initiate the growth experiments in the different food conditions. We performed two series of experiments: one to determine growth rate of individuals during the juvenile phase, a good proxy of fitness, the second to calculate effects on population parameters by measuring survival, fecundity, and hatching success. To start experiment 1, a random subsample of ten 24-h-old neonates was dried overnight at 60°C, cooled in a desiccator, and weighed to the nearest 0.1 μg to determine their initial dry mass (W_0). The remaining individuals were randomly distributed among the different experimental treatments, consisting either of monoalgal diatom food and *S. obliquus* or of mixtures (1 : 1 in terms of carbon) of the individual diatoms and *S. obliquus* at always 1 mg C L^{-1}. Six neonates per treatment were raised individually in 100-ml jars, fed daily, and transferred to clean jars with new medium every second day. When they reached maturity and deposited the first clutch of egg into the brood chamber, they were sacrificed, dried, and weighed (W_1). The juvenile growth rate g_j (d^{-1}) was calculated according to the equation $g_j = (\ln W_1 - \ln W_0)/t$ (Lampert and Trubetskova 1996), where t is the duration of the experiment (days). W_1 always included the first clutch of eggs in the brood chamber, hence g_j represents the sum of somatic growth and reproduction of the juveniles. The number of eggs produced by each individual in the first brood was also recorded.

In the second series of experiments, a group of third-clutch neonates of *D. pulicaria* was raised on a *Scenedesmus* diet (1 mg C L^{-1}) until the daphniids deposited the first clutch of eggs into the brood pouch. These young adults were then subjected to the same temperature and food conditions as in the first series, and their egg production and hatching success was monitored over the next seven broods. For each food treatment, 16 females were incubated individually in 200-ml jars filled with the appropriate food suspension (1 mg C L^{-1} in filtered lake water), fed daily, and trans-

Table 1. Means (±1 SD, $n=6$) of the juvenile growth rate (g_j) of *D. pulicaria* fed seven diatom species and *Scenedesmus* either as pure diet or in mixtures of one diatom and *Scenedesmus* (1 : 1 in terms of carbon).

	Diet type	
	Monoalgal	Mixed
Food species	g_j (d^{-1})	g_j (d^{-1})
S. obliquus	0.578 (0.151)	—
S. minutulus	0.661 (0.117)	0.709 (0.031)
A. formosa	0.520 (0.095)	0.651 (0.036)
S. hantzschii	0.476 (0.078)	0.673 (0.038)
C. meneghiniana	0.442 (0.085)	0.659 (0.024)
G. parvulum	0.433 (0.038)	0.684 (0.044)
F. capucina	0.357 (0.028)	0.649 (0.022)
Fragilaria sp.	0.298 (0.083)	0.652 (0.023)

ferred to fresh medium every other day. The number of eggs in the brood pouch of each female was counted under a stereomicroscope each time the medium was changed and the number of live neonates and nonviable eggs was recorded after pouring the contents of the jars through a 100-μm mesh. In addition to the number of viable offspring, the reproduction experiment yielded the numbers of survivors of the original cohort over time and the times of neonate release for the successive broods. These data were used to calculate age-specific survival probability (l_x) and age-specific fecundity (m_x). The instantaneous rate of increase (r) was then calculated for every individual using the Euler equation (Stearns 1992).

In vitro assay with Daphnia embryos—D. pulicaria juveniles from the stock cultures were individually reared in 200-ml jars with 1 mg C L^{-1} *Scenedesmus.* As soon as they released their second clutch of neonates, they were checked every 30 min to detect the deposition of another clutch into the brood pouch, which was considered the zero time of egg development. Only eggs from the third to the fifth broods were used to initiate an in vitro experiment. Six hours after deposition, the eggs were removed from the brood pouch under a dissecting microscope and washed twice with 0.2-μm filtered lake water. To determine the in vitro influence of PUA on the development of embryos, one representative of the oxo-acids (12-ODTE) and one from the volatile aldehydes (2,4-decadienal) were tested. Eggs isolated from 5–6 females were pooled and a batch of 10 was randomly transferred into each depression of a six-well tissue culture plate that contained 5 ml of freshwater with 0.5–3.0 μg ml^{-1} of 2,4-decadienal (SIGMA, Aldrich) in 1.2–7.5 μl ml^{-1} methanol or 1–4 μg ml^{-1} of synthetic 12-ODTE (Pohnert 2000) in water. The appropriate amount of methanol was used as control in the decadienal treatment, whereas freshly filtered water was used in the 12-ODTE treatment. Embryos were maintained in a temperature-controlled chamber at 20°C and 16 : 8 LD cycle. Five replicates were performed for each treatment. Egg development was monitored until the embryos shed the external membrane (less than 24 h after their incubation), which was considered successful hatching.

Table 2. Results of GLM ANOVAs on life history variables of *D. pulicaria* fed seven species of diatoms or mixtures of one diatom and *Scenedesmus* (1 : 1 in terms of carbon) with estimation of the main effects of diatom species (spec) and presence of *Scenedesmus* (mix) and interactions between these. Life-history variables juvenile growth rate (g_j) and number of eggs in the first clutch (eggs) were measured in the first experimental series. The population average cumulative number of eggs in seven clutches (R_o) and the instantaneous rate of increase (r) result from the second experimental series.

Variable	Factor	Degrees of freedom	Mean square	F	p
g_j	Spec	6, 70	5.33	14.1	<0.001
	Mix	1, 70	0.94	251.4	<0.001
	Spec × mix	6, 70	3.05	8.1	<0.001
eggs	Spec	6, 70	8.01	2.0	0.078
	Mix	1, 70	146.67	36.6	<0.001
	Spec × mix	6, 70	1.90	0.5	0.826
R_o	Spec	6, 196	3032	2.5	0.025
	Mix	1, 196	33555	27.3	<0.001
	Spec × mix	6, 196	395.9	0.3	0.925
r	Spec	6, 196	0.014	8.0	<0.001
	Mix	1, 196	0.123	70.2	<0.001
	Spec × mix	6, 196	0.008	4.5	<0.001

Results and discussion

Among the diatoms tested, only three species produced detectable amounts of PUA. *A. formosa* and *G. parvulum* produced 12-ODTE and 9-ONDE, respectively, whereas *Fragilaria* sp. produced 2,4-heptadienal, 2,4-octadienal, and 2,4-decadienal. The other algae tested did not give any detectable PUA even if highly concentrated samples (10^7 cells ml^{-1}) were analyzed. The detection limit for the volatile aldehydes was around 0.1 fmol per cell, depending on the cell size.

All *D. pulicaria* neonates reached maturity both with monoalgal and mixed diets. Algal species had a significant effect on juvenile growth rate (g_j) with monoalgal food (Table 1), but not on the number of eggs in the first clutch (mean = 6.60, SD = 2.29, n = 48). The highest g_j was found for *S. minutulus* and *A. formosa,* not significantly different from the *Scenedesmus* control. The two *Fragilaria* species resulted in the lowest g_j, but their effects on this parameter did not differ from each other. In general, part of the differences in growth rates with monoalgal food was caused by later egg laying of daphnids.

The diatom species effect on g_j was only marginally significant if they were mixed with *S. obliquus.* All mixed diets resulted in higher g_j than pure *Scenedesmus.* For comparison of the effects of pure and mixed diets, we excluded *Scenedesmus* from the pure algal data set and analyzed the data by two-way ANOVA (Table 2) with diatom species and presence or absence of *Scenedesmus* as main effects. Addition of *Scenedesmus* had significant effects on g_j and eggs in the first clutch. There were also significant interactions between species and *Scenedesmus* addition for g_j, indicating a different response to the diatom constituents of the diet. This interaction was not found for the eggs; hence, egg num-

Table 3. Life history parameters of *D. pulicaria* fed seven diatom species and *Scenedesmus* either as pure diet or in mixtures of one diatom and *Scenedesmus* (1 : 1 in terms of carbon). Data include seven broods. Survival denotes the fraction of females that finished the seventh brood.

	Diet type							
	Monoalgal				Mixed			
Species	*n*	Survival (%)	R_o	r (d^{-1})	*n*	Survival (%)	R_o	r (d^{-1})
S. obliquus	16	18.8	24.1 (32.9)	0.197 (0.050)	—	—	—	—
S. hantzschii	13	46.2	47.0 (39.0)	0.246 (0.035)	9	77.8	80.9 (51.5)	0.281 (0.034)
S. minutulus	16	37.5	36.9 (31.7)	0.245 (0.045)	12	58.3	70.6 (54.2)	0.282 (0.034)
A. formosa	16	37.5	27.4 (25.3)	0.236 (0.037)	13	46.2	58.2 (48.4)	0.272 (0.048)
C. meneghiniana	16	31.3	35.1 (27.4)	0.228 (0.035)	16	43.8	52.3 (47.9)	0.267 (0.032)
G. parvulum	16	43.8	26.2 (20.0)	0.211 (0.039)	16	43.8	41.9 (37.0)	0.225 (0.058)
Fragilaria sp.	26	50.0	21.8 (13.0)	0.173 (0.024)	12	41.7	46.0 (34.2)	0.277 (0.074)
F. capucina	16	43.8	26.1 (22.7)	0.168 (0.039)	13	53.9	52.4 (40.1)	0.251 (0.043)

bers were improved by adding *Scenedesmus* to all diatom species (mean = 9.17, SD = 1.78, $n = 42$).

Not all *D. pulicaria* females survived until they released their seventh clutch of eggs. Surprisingly, *Daphnia* fed pure *Scenedesmus* had the lowest survival of all treatments (Table 3). Although overall the pure diatom diets seemed to result in somewhat lower survival (41.4 ± 6.3%) compared with the mixed diets (52.2% ± 12.8%), neither a two-sample *t*-test ($t = 2.0$, $p = 0.078$) nor a paired *t*-test ($t = 2.18$, $p = 0.072$) resulted in a significant difference between the groups.

Survival and age-specific fecundity determined the total number of eggs per female (R_o) produced during seven broods. Due to the varying lifetime of the individuals, R_o showed a large variability (Table 3). A two-way ANOVA after exclusion of the pure *Scenedesmus* treatment resulted in a marginally significant effect of diatom species, whereas the addition of *Scenedesmus* led to significantly higher lifetime reproduction in all treatments (Table 2).

The instantaneous population growth rate (r) was much less variable than R_o, showing a similar contrast between diatom diets as for g_j, with the lowest r-values for the two *Fragilaria* species and the highest for the two *Stephanodiscus* species (Table 3). Differences between species were much smaller for the mixed diets. The two-way ANOVA (Table 2) revealed significant effects of species, diet composition, and the interaction between them. Supplementation of the diatoms with *Scenedesmus* led to an increase of r, but the response varied for different combinations.

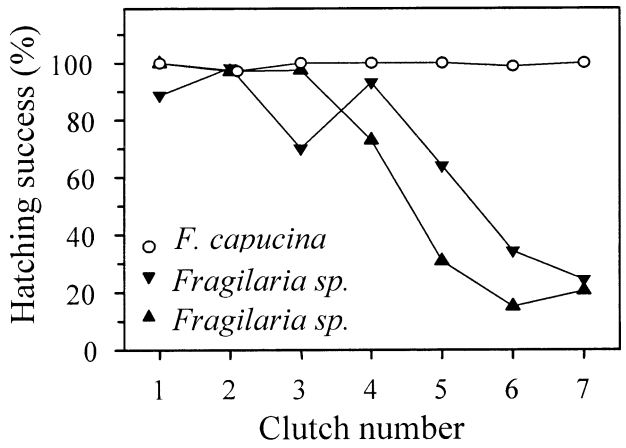

Fig. 1. Percentage of viable eggs in seven broods of *D. pulicaria* fed pure diets of *Fragilaria* sp. (two experiments) or *F. capucina*.

The mean hatching success in all treatments except pure *Fragilaria* sp. was always above 90%. There was no time trend and a typical example is presented as for the *F. capucina* diet in Fig. 1. With pure *Fragilaria* sp. as diet, egg viability dropped dramatically to values as low as 20% after the fourth clutch. This experiment was successfully repeated with very similar results (Fig. 1). Addition of *Scenedesmus* eliminated the effect completely.

D. pulicaria embryos incubated in water containing synthetic 12-ODTE at increasing concentrations (1–4 μg ml^{-1}) did not alter the normal pattern of development, and 100% of the embryos moulted successfully into the second embryonic stage, like the controls incubated in filtered lake water (Fig. 2). The egg membrane was cast off 14–16 h after the incubation, i.e., 20–22 h after deposition into the brood pouch of the female. In contrast, embryos incubated in 2,4-decadienal at concentrations from 0.5 to 3.0 μg ml^{-1} showed a dose-dependent impairment of development. Up to 1.5 μg ml^{-1}, about 80% developed through the first instar, which was consistent with the pure-methanol controls. Only 40% shed the first membrane at 2.5 μg ml^{-1}, and none of the embryos developed at 3.0 μg ml^{-1} (Fig. 2).

Our experiments covered two phases of the *Daphnia* life cycle, juvenile growth and the reproductive phase, both integrating metabolic processes like feeding, assimilation, and respiration. Both phases can be affected by nutritional inadequacy and toxic compounds of the diet. While an earlier study (Carotenuto and Lampert 2004) tested the short-term effects on *Daphnia* carbon uptake caused by algal morphology, digestibility, assimilation efficiency, and oxylipin production, this study looks at long-term effects on individuals and the population.

Besides the morphological differences of cells and colonies, the diatoms selected differ in the ability to produce PUA upon cell damage. Several species are nonproducers, whereas others are similar to well-investigated marine PUA

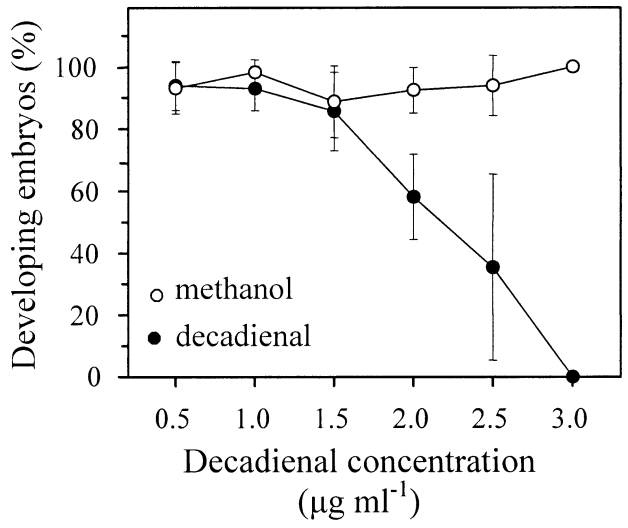

Fig. 2. Effect of methanol (1.2–7.5 μl ml^{-1}) and decadienal in methanol on the development of embryos of *D. pulicaria* in vitro. Development success is defined by shedding of the first egg membrane.

producers. Compared with the marine *T. rotula*, *Fragilaria* sp. produces similar patterns of PUA in comparable quantities, if the smaller cell size of *Fragilaria* sp. is taken into account. While the larger *T. rotula* can produce approximately 4 fmol cell^{-1} (Pohnert et al. 2002), *Fragilaria* sp. used in this study releases 0.1 fmol cell^{-1} $\pm$ 0.01 (n = 3) of PUA upon wounding. Thus, one might expect similar effects on both copepods and *Daphnia*. *A. formosa* can be indirectly compared with the marine diatoms. In sea urchin egg-cleavage tests, the main PUA of this alga (12-ODTE) is 10 times less active than decadienal but acts presumably via the same inhibitory mechanism (Adolph et al. 2003). Because the production of 12-ODTE by *A. formosa* is 10-fold higher (49 fmol cell^{-1}) (Pohnert 2000) than the production of decadienal by *T. rotula*, the toxic potential of these species should again be comparable. 9-ODTE found in *G. parvulum* proved to be inactive in sea urchin egg cleavage assays, presumably due to intramolecular inactivation (Adolph et al. 2003). We found no evidence for any direct toxic effect of the PUA on g_j. *A. formosa* provides good growth and the two *Fragilaria* species are poor food, but the resulting growth rates do not differ significantly despite the presence or absence of aldehydes. Carotenuto and Lampert (2004) used the same argument to exclude the possibility that the aldehydes acted as feeding deterrents, as the ingestion rates did not differ either.

The differing response of *Daphnia* to diatom food in the long-term experiments may have been caused by differences in nutritional quality not detected in the short-term feeding measurements (Carotenuto and Lampert 2004). These differences can comprise a lack of polyunsaturated fatty acids (PUFAs) (Brett and Müller-Navarra 1997) or stoichiometric imbalance (DeMott and Tessier 2002). Evidence for effects of nutritional quality comes from the experiment with mixed diets. In every single case, g_j was higher when *Scenedesmus* replaced half of the diatoms. DeMott (1998) has demonstrated that different algae can complement each other with respect to nutritional quality. A combination of a diatom with *Scenedesmus* may, thus, enhance the overall nutritional balance of the diatoms and the growth rate of *D. pulicaria*.

There is no evidence for an effect of PUA on the survival of adults, the lifetime fecundity (R_0), and the instantaneous rate of population growth (r). The latter is a good measure of fitness (Stearns 1992), and besides age-specific survival and fecundity, it incorporates the viability of the eggs.

We observed reduced hatching of *Daphnia* eggs only for a single diet, pure *Fragilaria* sp., and only for late clutches (after the fourth brood). Although we can again not exclude a nutritional effect with increasing age of the female (all eggs hatched in the mixed diet), the fact that hatching is not affected by *F. capucina* provides evidence for the role of aldehydes produced by *Fragilaria* sp. On the contrary, no effect of the structurally related oxo-acids produced by *A. formosa* and *G. parvulum* was detected. This evidence is supported by the in vitro incubation of *Daphnia* eggs with different oxylipins. While 2,4-decadienal (produced by *Fragilaria* sp.) caused a concentration-dependent cessation of egg development, no such effect was found for 12-ODTE from *A. formosa*. The in vitro effect of 2,4-decadienal is similar as demonstrated for copepod egg hatching and cleavage of sea urchin eggs (Miralto et al. 1999). The lack of an effect with 12-ODTE may be explained by the much lower biological activity of this compound in the sea urchin test (Adolph et al. 2003). Although we detected one possible effect of aldehydes on *Daphnia* (reduced egg viability in late clutches), there is no evidence yet that the production of PUA has an ecological meaning for *Daphnia*–diatom interactions. The contribution of successive clutches to individual fitness is very different in copepods and *Daphnia* due to their different life histories. Copepods spawn in a relatively short time period, have longer larval development times, and there is no overlap in reproduction between females and their offspring. On the contrary, daphniids have a short juvenile phase and overlapping generations. Therefore, all clutches of copepods contribute equally to the next generation, but in *Daphnia*, the first clutches are more important than later clutches (Stearns 1992). Eggs of clutches 4 and later contribute very little to r, consequently, we found no difference in r for diets of *Fragilaria* sp., *F. capucina*, and *Scenedesmus*, despite egg mortality in late clutches with *Fragilaria* sp. diet. Inhibition of egg viability in late clutches cannot be a selective force for the evolution of a grazing defense against *Daphnia*, contrary to copepods. However, we cannot fully exclude the possibility that PUA-producing diatoms can reduce *Daphnia* fitness. Thus, e.g., females fed diatoms from birth on could produce nonviable eggs in earlier clutches or the survival of juveniles could be reduced by a maternal effect as recently demonstrated for copepods (Ianora et al. 2004).

Maintaining the enzymatic tools for the wound-activated production of oxylipins in diatoms may be costly. Thus, it remains an interesting research topic to identify the function of these compounds in providing an advantage to oxylipin-producing diatoms. Although freshwater copepods are not as important grazers as *Daphnia* and diatoms are not a domi-

nant component of their diet, they may eventually be affected like their marine relatives.

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