

Biochemical food quality effects on a *Daphnia* hybrid complex

Tomasz Brzeziński¹

Department of Hydrobiology, Warsaw University, Banacha 2, 02-097 Warsaw, Poland

Eric von Elert²

Limnological Institute, University of Konstanz, 78457 Konstanz, Germany

Abstract

Correlative field studies have shown that the food quality of natural seston for *Daphnia* is highly correlated with the content of eicosapentaenoic acid (EPA), indicating that the growth of *Daphnia* in nature is at least seasonally limited by a low availability of this polyunsaturated fatty acid. Here we test the hypothesis that a putative limitation due to limited availability of EPA in natural seston should lead to genetic adaptation in physiological traits reflected in genotype-dependent differences in the ability of sympatric clones of *Daphnia* to cope with these shortages. We further test if this adaptation to the low availability of EPA provides another explanation for the coexistence of hybrids with their parental taxa. Standardized growth experiments were performed with and without dietary EPA using clones of three taxa from the *Daphnia longispina* complex: *Daphnia galeata*, *Daphnia hyalina*, and their interspecific hybrids. Fitness was estimated from juvenile somatic growth rates, g , and from intrinsic population growth rates, r . There was marked interclonal variability in the susceptibilities of g and r to the absence of EPA from the food, with considerable intraspecific variability. At the taxon level, we noted differences in susceptibility to EPA limitation with regard to the intrinsic rate of population increase r , which demonstrates that the availability of EPA affects the relative fitness of hybrids, but not of the parental species. On average, hybrids seemed less susceptible to the absence of EPA than do parental species. Environmental heterogeneity driven by EPA-mediated changes in the quality of food may in consequence contribute to the maintenance of genetic diversity in *Daphnia* and may be a reason for the temporal dominance of hybrids.

Determining the efficiency with which biomass and energy are transferred from primary producers to higher trophic levels is a major issue in understanding the functioning of aquatic food webs. In freshwater lakes, the key components determining the quality of natural seston as food for the major herbivore *Daphnia* include not only differences in digestibility but also differences in elemental stoichiometry and biochemical composition (Sterner and Schulz 1998).

Among the biochemicals essential for zooplankton, the most important are polyunsaturated fatty acids (PUFAs; i.e., fatty acids with multiple double bonds in the carbon chain) (Müller-Navarra 1995a). Evidence for PUFA limitation of *Daphnia* derives from laboratory experiments with food artificially supplemented with a single PUFA or a mixture thereof (von Elert 2002; Becker and Boersma 2005). Several independent studies have found strong correlations between somatic growth rates in *Daphnia* raised on natural lake seston and the content of one

particular PUFA, eicosapentaenoic acid (EPA), in the seston (Müller-Navarra et al. 2000; Wacker and von Elert 2001)—these correlations indicate limitation of the growth of *Daphnia* where the availability of EPA is low. The quantity of EPA in natural seston is in turn shown to depend on the taxonomic composition of the phytoplankton (Ahlgren et al. 1990) and, to a lesser extent, also on environmental conditions (Müller-Navarra 1995b). These two factors contribute to the between-lake and within-lake fluctuations in the EPA content of seston, indicating that there is seasonal variation to the degree of EPA limitation of *Daphnia* (Wacker and von Elert 2001).

EPA plays an important role in invertebrate physiology in that it regulates the structure and function of membranes and serves as a hormone precursor (Stanley-Samuelson 1994). However, some *Daphnia* genotypes may circumvent limitations on amounts of EPA by using other PUFAs of the (n-3) series to synthesize it, as has been shown for the α -linolenic acid (18:3n-3; von Elert 2002). It is not clear whether and to what extent the ability to synthesize EPA is species- or clone-specific. Similarly, De Mott and Müller-Navarra (1997) hypothesized that species of *Daphnia* may differ in their abilities to cope with PUFA limitation, and this was corroborated experimentally by von Elert (2004), who showed that EPA supplementation of food increased growth rates of a single clone each of *Daphnia hyalina* and *Daphnia galeata*, but not of a clone of the interspecific hybrid clone. However, as the three clones used in that study originated from lakes with seston differing in EPA content, the possibility that the differences in susceptibility result from local adaptations of clones to the availability of

¹ Corresponding author (t.brzezinski@uw.edu.pl).

² Present address: Zoological Institute, University of Cologne, Weyertal 119, 50923 Köln, Germany.

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EPA in the seston cannot be excluded. Based on the seasonal variation in EPA limitation of *Daphnia* in natural systems (Müller-Navarra 1995a; Wacker and von Elert 2001) as well as on the finding that clones within the *Daphnia longispina* hybrid complex differed in their sensitivity to the absence of EPA from their algal diet (von Elert 2004), one may suspect that changes in EPA availability lead to shifts in the relative fitness of different *Daphnia* genotypes within a lake, which allow coexistence of different genotypes. There is a growing body of literature attaching ecological significance to the clonal variation existing in natural populations of *Daphnia*, clones being shown to differ with respect to their tolerances to abiotic factors (e.g., oxygen levels; Sell 1998), predator avoidance (De Meester et al. 1995), and vulnerability to predation (Boersma et al. 1998). However, the influence food quality may have in maintaining clonal diversity in *Daphnia* has only been examined to a limited extent. Only recently has it emerged that there is interclonal variation in *Daphnia* populations with regard to resistance to toxic cyanobacteria (Hairston et al. 2001) and the nutrient content of seston (Weider et al. 2005).

One of the sources of adaptive genetic variation is hybridization—natural crossbreeding between individuals from populations distinguishable on the basis of one or more heritable characters (Grant and Grant 1994; Rieseberg 1997). Hybridization is widespread in a variety of both plant and animal species (Burke and Arnold 2001). While hybrid individuals may suffer from genetic incompatibilities, segregation of additive genetic factors may give certain hybrid genotypes a fitness that may exceed that of the parental species. Thus, thanks to the introgression of genes between species, hybridization is seen as a potentially creative evolutionary force (Grant and Grant 1994; Rieseberg 1997). In the genus *Daphnia*, hybridization among closely related sympatric species is a widely reported phenomenon (Wolf and Mort 1986; Schwenk and Spaak 1997). Grouped together with the parental species in the *D. longispina* complex, the hybrids formed by *D. galeata* Sars, *Daphnia cucullata* Sars, and *D. hyalina* Leydig are very common in lowland European lakes, tending to co-occur with parental species, temporarily achieving numerical dominance over them (Schwenk and Spaak 1997).

Heterogeneity of environmental conditions is considered to be a factor that explains the maintenance of genetic variation in ecosystems, not only at the level of the species, but also within given species (Sommer 1984; Chesson and Huntly 1997). The idea that spatio-temporal heterogeneity maintains coexistences among clones (Vrijenhoek 1978) has also been applied to explain the temporal dominance of *Daphnia* hybrids and their coexistence with parental species (Spaak and Hoekstra 1997). There are several environmental factors influencing the fitness of *Daphnia*. Temperature (Weider and Wolf 1991), food level (Weider 1993), the predation regime (Spaak and Hoekstra 1997), and infections by parasites (Wolińska et al. 2006) have all been shown to alter the relative fitness of hybrids and parental species in the *D. longispina* complex. These factors vary in absolute strength and relative significance during the course of the year, ensuring a selective advantage of the

hybrids under some conditions and of the parentals under other conditions. Also known to markedly affect *Daphnia* populations is the quality of food (De Mott 1989), a factor the role of which has been studied to only a limited extent in the context of hybridization in *Daphnia*. Measurements of the performance of hybrids and parental species fed with different species of algae (Repka 1996) have, however, revealed interspecific differences in responses attributable to differences in this food.

Seasonally fluctuating degrees of EPA limitation in *Daphnia* should lead to genetic adaptation in physiological traits reflected in genotype-dependent differences in the ability to cope with limited EPA availability. The study described here has therefore sought to investigate whether coexisting genotypes within the *D. longispina* complex differ in their ability to cope with the absence of EPA from their algal diet. This was tested by measuring the performance (juvenile growth rate, g , and intrinsic rate of population growth, r) in a relatively large set of clones of the *D. longispina* complex originating from one lake. As both juvenile growth rate and the intrinsic rate of population growth are known to affect overall fitness in *Daphnia* (Lampert and Trubetskova 1996), a depression in somatic growth or the intrinsic rate of population growth in response to the absence of EPA from the algal food can be interpreted to be maladaptive.

Methods

We determined juvenile growth rates and intrinsic population growth rate in eight clones of *D. hyalina*, eight clones of the hybrids *D. hyalina* × *galeata*, and two clones of *D. galeata*. All originated from Lake Roś (53°43'N, 21°52'E; Poland)—a dimictic eutrophic lake of medium size (18.9 km²). The maximum depth of the lake is 31 m and the mean 8.1 m, while the Secchi-disc depth is 2.1 m. An allozyme study revealed that three species of the *D. longispina* complex (*D. cucullata*, *D. galeata*, and *D. hyalina*) coexist with their hybrids in Lake Roś. There are seasonal changes in their occurrence and relative abundance in the lake: *D. cucullata* is the most abundant species and persists throughout the year, while *D. hyalina* and *D. galeata* emerge from ephippia in spring and increase in abundance during summer through to autumn, with *D. hyalina* being more abundant than *D. galeata*. The *D. galeata* × *hyalina* hybrids in Lake Roś are more prevalent in spring and summer, when they constitute up to 11% of all daphnids present, whereas parental species dominate the lake in autumn and in winter (Brzeziński unpubl. data). Alongside the seasonal changes in the taxonomic composition of pelagic *Daphnia* in Lake Roś are substantial changes in clonal composition within taxa, something that points to a seasonal succession of genotypes. In spring the size of egg-bearing *D. galeata* is slightly larger than that of *D. hyalina* (1.20 ± 0.10 mm vs. 1.18 ± 0.09 mm; Rykowska and Gliwicz pers. comm.); during the season the body size of *D. galeata* tends to decrease, while that of *D. hyalina* tends to increase.

The clones used were isolated from pelagic samples taken on 11 August 2003 or else were ex-ephippial clones

Table 1. Clones of the *D. longispina* complex used in the study. Allelic designations reflect relative anodal migration distances: F, fast; M, medium; S, slow. The origin of clones is denoted in the last column. See Methods for enzyme designation.

Clone	Taxon	Allozyme pattern*				Source
		AAT	AO	GPI	PGM	
1	<i>D. hyalina</i>	SS	SS	MM	MF	Ehippial
2		SS	SS	MM	MF	Ehippial
3		SS	SS	MM	MM	Ehippial
4		SS	SS	MM	MM	Ehippial
5		SS	SS	MF	MM	Pelagic
6		SS	SS	MF	MF	Pelagic
7		SS	SS	FF	FF	Pelagic
8		SS	SS	MF	FF	Pelagic
1	<i>D. galeata</i> × <i>hyalina</i>	SF	SF	MM	MM	Ehippial
2		SF	SF	SM	MF	Ehippial
3		SF	SF	MM	MF	Ehippial
4		SF	SF	MM	FF	Ehippial
5		SF	SF	SF	MF	Pelagic
6		SF	SF	SS	MF	Pelagic
7		SF	SF	MF	MF	Pelagic
8		SF	SF	MM	MM	Pelagic
1	<i>D. galeata</i>	FF	FF	FF	MF	Ehippial
2		FF	FF	FF	MM	Pelagic

* AAT, aminoaspartate transferase; AO, aldehyde oxidase; GPI, glucose-6-phosphate isomerase; PGM, phosphoglucosyltransferase.

hatched under laboratory conditions from ehippia collected from the sediments of Lake Roś on 22 January 2003. The clones were screened using allozyme electrophoresis (Hebert and Beaton 1993) in four loci: aminoaspartate transferase (AAT; EC 2.6.1.1), aldehyde oxidase (AO; EC 1.2.3.1), glucose-6-phosphate isomerase (GPI; EC 5.3.1.9), and phosphoglucosyltransferase (PGM; EC 5.4.2.2). AAT and AO were used to identify the taxa (Wolf and Mort 1986; Giessler 1997), GPI and PGM to differentiate the different clones obtained from pelagic samples. We obtained 62 pelagic clones and 96 ex-ehippial ones, from which distinct multilocus genotypes (Table 1) were selected to assess intra- and interspecific variation in the response to depleted EPA in food. Time and material constraints confined us to the analysis of eight clones of both *D. hyalina* and the hybrids. As only two clones of *D. galeata* were found, the analysis was of necessity confined to these two.

The food source was *Scenedesmus obliquus* (strain SAG 276-3a) grown in WC medium (Guillard 1975); this strain has repeatedly been shown to be free of EPA (C20:5n-3) (Wiltshire et al. 2000; von Elert 2004; Becker and Boersma 2005). When *S. obliquus* had reached the stationary phase of growth, it was harvested by centrifugation (3,000 × g) before being resuspended in fresh WC medium. The carbon content of the algal suspension was determined by reference to photometric light extinction at 800 nm, along with carbon-extinction equations determined previously. The animals were fed either pure *S. obliquus* or *S. obliquus* supplemented with EPA. Suspensions were prepared using lake water filtered through membrane filters (pore size, 0.45 μm) and conditioned for 24 h. Experiments were carried out in 0.5 liters of conditioned lake water, with

a food level of 2 mg carbon (C) L⁻¹. Food suspensions were renewed daily. Each food treatment consisted of three replicates with 10–12 animals each, all experiments being run at a constant temperature of 20°C (±0.1°C).

Experimental animals originated from the third broods of mothers raised under nonlimiting concentrations of *S. obliquus* for three generations. Synchronized cohorts of 14–24-h-old neonates were used to start the growth experiments. Initial weight was measured in two subsamples of 20 individuals. Growth experiments were run until the animals released the first clutch to the brood chamber. They were then transferred to preweighed aluminum boats and dried at 60°C before being weighed on a Mettler UMT2 electrobalance to the nearest 0.1 μg. Juvenile growth rates were calculated from dry body mass as $g = (\ln M_t - \ln M_0) \times t^{-1}$, where M_t is the body mass at the end of experiment, M_0 represents the initial body mass of individuals, and t is the experiment's duration. Eggs in the brood chambers were counted under a dissecting microscope. The intrinsic rate of population increase was calculated in accordance with the Euler Lotka equation, $r = \sum l_x m_x e^{-rx}$, where l_x represents the age-specific survivorship, m_x the number of newborns on day x , and x the age in days. Since there was no mortality in the experiment until the first clutch, l_x was set at 1 for this period. l_x was in turn set at 0 for older animals, with the intention of mimicking severe size-selective mortality. Since nonviable eggs were found in neither supplemented nor unsupplemented treatments, the value of fecundity was substituted by m_x under the assumption that all eggs would develop into live animals. For each clone the relative “decrease” (sensitivity S_x) of the juvenile growth rate (g) and of the intrinsic rate of population growth (r) in the absence of EPA from the food source was calculated using the formula $S_x = [(X_{EPA-} - X_{EPA+}) \times X_{EPA+}^{-1}] \times 100\%$, where X_{EPA-} is g or r in the absence of EPA and X_{EPA+} is g or r when food has been supplemented by EPA. A more negative value of S_x thus indicates greater sensitivity to the absence of EPA from the algal diet.

Since it was not possible to run single growth experiments involving all of the experimental clones, four separate ones were instead performed, each featuring the reference clone *D. galeata* (Stich and Lampert 1984) growing on pure *S. obliquus*. For each of the experimental clones, all different food treatments were run within the same experiment.

Supplementation with EPA—EPA supplementation was performed after the method of von Elert (2002). Twenty milligrams of bovine serum albumin was dissolved in 5 mL of ultrapure water, then 0.4 mL of a solution of EPA in ethanol (2.5 mg mL⁻¹) was added. A suspension of *S. obliquus* equivalent to a biomass of 4 mg particular organic carbon was added, and the volume was brought up to 40 mL with WC medium. The suspension was incubated for 4 h on a shaker. Algal cells were then concentrated by centrifugation (3,000 × g); pellets were washed twice with WC medium and resuspended in WC medium. The resultant suspensions were used as the food source in the growth experiments.

Statistical analyses—The dependent variables were \log_e -transformed prior to the analysis, to meet the assumptions of normality (Shapiro–Wilk test) and homogeneity of variances (Levene test). We used a mixed general linear model. The model included food type (a two-level factor: pure alga and supplemented alga), taxon (a three-level factor: two parental species and the hybrid), and clone (the number of levels in this factor being equal to the number of clones screened). The clone factor was nested within the taxon factor. The growth rate (g or r) of the reference clone was used as the covariable (REFERENCE), in order to account for the fact that the experimental clones were utilized in different growth experiments. A significant interaction of food type \times taxon would indicate that taxa differ in their susceptibility to the dietary absence of EPA, while a significant interaction of food type \times clone would indicate intraspecific differences in reaction to the dietary absence of EPA. Values of the relative decrease (the sensitivity) were transformed using the jackknife procedure (Sokal and Rohlf 1995), followed by Kruskal–Wallis analysis of variance (ANOVA) and the Mann–Whitney U -test with Bonferroni adjustment (Rice 1989). A significance level of $\alpha < 0.05$ was applied to all of the statistical analysis. Analyses were performed using Statistica 6.0 software.

Results

Reference clone—Supplementation of *S. obliquus* with EPA enhanced the juvenile growth rate and the intrinsic population growth rate of the reference clone *D. galeata*: the juvenile growth rate in the presence of *S. obliquus* increased from $0.46 \pm 0.03 \text{ d}^{-1}$ to $0.50 \pm 0.01 \text{ d}^{-1}$ upon supplementation with EPA (one-way ANOVA: $F_{1,4} = 7.75$, $p < 0.04$). The values are similar to those obtained by von Elert (2002, 2004). Altogether this indicates that the supplementation worked. The intrinsic population growth rate of individuals of the reference clone fed supplemented *S. obliquus* was higher ($0.32 \pm 0.01 \text{ d}^{-1}$) than that of individuals fed the pure alga ($0.26 \pm 0.01 \text{ d}^{-1}$) (one-way ANOVA: $F_{1,4} = 32$, $p < 0.01$).

Absolute values—We found inter- and intraspecific differences in the susceptibility of sympatric clones of the *D. longispina* complex to manipulation of the availability of a single PUFA (EPA) in the food source. Among the 18 clones tested, 10 responded to EPA limitation with significant reduction of g or r , whereas eight were not affected in terms of fitness expressed using these measures (Fig. 1). Susceptible clones showed differential trait patterns in response to a lack of EPA: while both traits (g and r) were affected in some clones, in others it was r or g only that was lower under conditions of deteriorated food.

Interspecific differences—In both food treatments *D. galeata* revealed juvenile growth rates that were higher than those of *D. hyalina* (Tukey Honestly Significantly Different [HSD] test: $p < 0.0001$ for unsupplemented and for supplemented algae) and exceeded the growth rates of the hybrid (Tukey HSD: $p < 0.0001$ for both types of food).

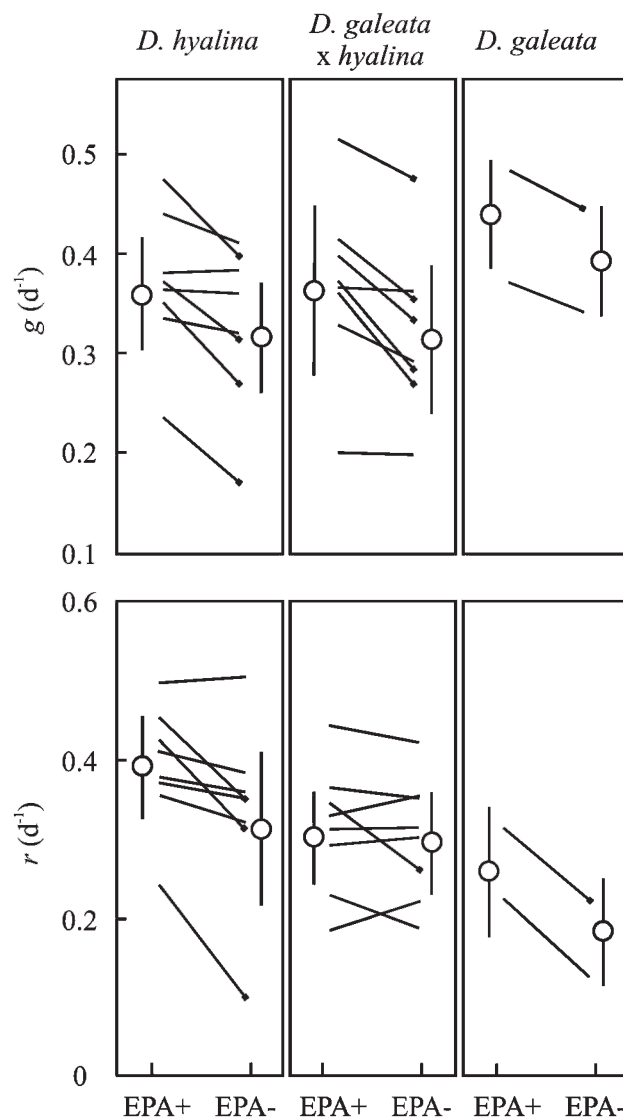


Fig. 1. Juvenile growth rates, g , and intrinsic rates of population increase, r , of taxa from the *D. longispina* complex fed food with (EPA+) or without (EPA-) eicosapentaenoic acid (EPA). Lines represent reaction norms of single clones; circles represent mean values (\pm standard deviation [SD]) for taxa. Squared ends of reaction norms indicate significant differences between the two food treatments (Tukey HSD: $p < 0.01$).

The juvenile growth rates of *D. hyalina* and the hybrid did not differ from each other when fed unsupplemented or supplemented food (Tukey HSD: $p = 0.8$ and $p = 0.9$, respectively) (Fig. 1). When compared to the situation with EPA-supplemented food, EPA depletion was associated with a similar level of inferred suppression of juvenile growth rates in all three taxa (Tukey HSD: $p < 0.001$ for *D. hyalina* and the hybrid, $p < 0.01$ for *D. galeata*) (Fig. 1). This explains the nonsignificant FOOD \times TAXON interaction for g (Table 2).

We found significant differences in the intrinsic rates of population increase, r , in the three taxa grown under the two food treatments (FOOD \times TAXON, Table 2). The

Table 2. Two-way nested general linear model for juvenile growth rate g and intrinsic population growth rate r with 'FOOD' and 'TAXON' as fixed effects, 'CLONE' nested within 'TAXON,' and 'REFERENCE' as a covariable. Asterisks indicate level of significance: * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$. MS indicates mean square.

Source of variation	df	g		r	
		MS	F	MS	F
FOOD	1	0.32	17.9***	1.01	6.84**
TAXON	2	0.23	0.64	1.00	1.52
CLONE	15	0.36	20.1***	0.66	4.45**
FOOD \times TAXON	2	0.002	0.156	0.29	3.10*
FOOD \times CLONE	15	0.018	2.03*	0.14	2.35**
REFERENCE	1	0.001	0.30	0.0002	0.04
Error	72	0.003		0.005	

growth rates, r , of *D. hyalina* and of *D. galeata* were lower when EPA was absent (Tukey HSD: $p < 0.01$ and $p < 0.04$, respectively), whereas the r of the hybrid was not affected by the lack of EPA (Tukey HSD: $p = 0.9$). When EPA was a constituent of the diet, *D. hyalina* showed the highest value for r among the three tested taxa (Tukey HSD: $p < 0.01$ for the hybrid, $p < 0.03$ for *D. galeata*), the intrinsic rate of population increase for the hybrid not differing from that of *D. galeata* (Tukey HSD: $p < 0.9$). In the absence of dietary EPA, the r of the hybrid was not different from that in *D. hyalina* (Tukey HSD: $p < 0.9$), the hybrid and *D. hyalina* both displaying higher population growth rates than *D. galeata* (Tukey HSD: $p < 0.001$ and $p < 0.001$, respectively) (Fig. 1).

Intraspecific differences—We found significant intraspecific differences in reactions to the depletion of EPA (FOOD \times CLONE, Table 2). Where EPA was absent, the juvenile growth rate, g , and intrinsic rate of population growth, r , were significantly lower in the case of only one of the two *D. galeata* clones (Fig. 1); the two clones differed from each other with regard to the absolute values for the juvenile growth rate g in the two food treatments (Tukey HSD: $p < 0.001$) but not for r (Tukey HSD: $p = 0.5$ and $p = 0.9$). Within the two other taxa tested some clones were susceptible to the absence of EPA from their diet, while others were not. Within the hybrids only one out of eight clones responded to the absence of EPA with lower values for both g and r , and three clones showed no differences between the two food treatments (Fig. 1). In the remaining four clones, the responses with respect to g and r were decoupled (Fig. 1). With three out of eight clones of *D. hyalina*, values for both of the tested parameters were significantly lower in the absence of EPA, while in four of the other clones they did not differ from one treatment to another. Finally, in one clone of *D. hyalina* the juvenile growth rate was lower in the absence of EPA, while r values did not differ from one treatment to another (Fig. 1).

Susceptibility to the absence of EPA from the diet—The juvenile growth rate g of the two parental species and of the

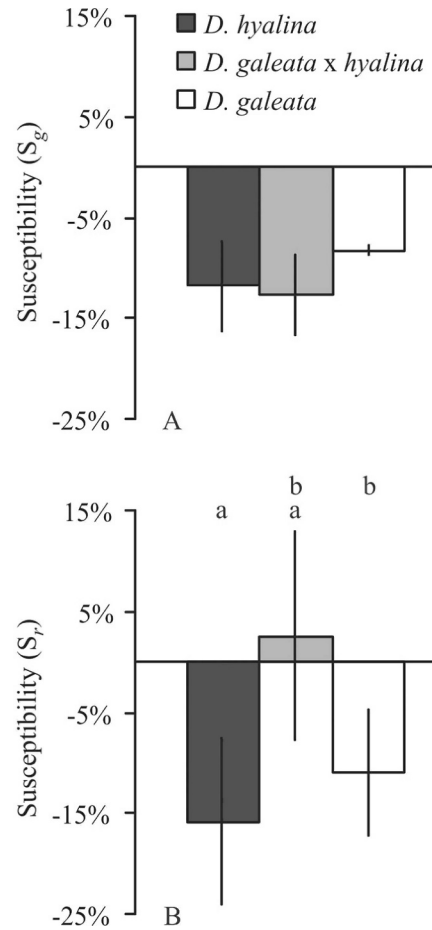


Fig. 2. Susceptibility, S , of taxa from the *D. longispina* complex to the depletion of food algae with eicosapentaenoic acid (EPA). Depicted is the performance on the EPA-free diet related to the performance on the EPA-containing diet for (A) juvenile growth rates (S_g) and (B) the intrinsic population growth rate (S_r). A more negative value of S indicates a higher sensitivity to the absence of EPA from the algal diet. Mean values (\pm standard deviation [SD]). Letters (panel B) denote heterogeneous groups ($p < 0.01$).

hybrid were each lower by 8–13% where EPA was absent, such that all three taxa did not differ in susceptibility to the absence of EPA (Kruskal–Wallis ANOVA: $H_{2,18} = 0.72$, $p = 0.5$) (Fig. 2). When EPA was absent from the diet, the intrinsic rates of population increase r were to a similar degree lower in *D. hyalina* (15%) and *D. galeata* (11%), whereas r of the hybrid was not affected. Hence, the susceptibility of the parental taxa was significantly greater than that of the hybrid (Kruskal–Wallis ANOVA: $H_{2,18} = 12.1$, $p < 0.01$; Fig. 2).

Body size and sensitivity—We found a weak relationship between body size at first reproduction of a particular clone (referred to as M) and the sensitivity of its juvenile growth rate to the absence of EPA ($S_g = 0.612 + 0.176M$; $R^2 = 0.56$, $p < 0.001$). There was no such relationship between body size and sensitivity, S , measured in terms of r ($S_r = 0.803 + 0.26M$; $R^2 = 0.006$, $p = 0.053$).

Discussion

It is well known that the quality of food may affect the success of competing zooplankton species (De Mott 1989). As the results of correlative studies indicate mediation of the quality of natural phytoplankton for herbivorous zooplankton via EPA content, there is a strong indication that its content in the diet may limit the growth and reproduction of daphnids in nature (Müller-Navarra et al. 2000; Wacker and von Elert 2001). Furthermore, as the PUFA content of natural phytoplankton has been shown to fluctuate through the year (Wacker and von Elert 2001), it may be hypothesized that seasonal changes in the clonal (and taxonomic) composition of *Daphnia* assemblages may reflect different susceptibilities of clones (and taxa) to low EPA availability in food. Here we report marked variability in this susceptibility among coexisting clones within the *D. longispina* complex. This finding supports the putative assumption that temporary EPA limitations exert strong selection pressure on natural zooplankton assemblages in which physiological adaptations are involved.

The physiological background of clones' resistance to the depletion of EPA is not clear. We only found a weak relationship between body size and the susceptibility of the juvenile growth rate, S_g , to a lack of EPA, with no such relationship characterizing the susceptibility of the population growth rate S_r . This indicates that body size is of minor importance in terms of the ability to cope with the absence of EPA from the diet. According to von Elert (2002), differences in this resistance may reflect different abilities to use other fatty acids as precursors for the synthesis of EPA. Thus far it remains unclear whether the observed resistance of some parental clones to EPA depletion results from gene exchange between parental species via hybrids and backcrosses or whether it is an immanent characteristic of *D. hyalina*:*D. galeata* populations.

At the taxon level, the EPA-mediated deterioration in food quality resulted in similar inferred lowering of juvenile growth rates among specimens of *D. hyalina*, *D. galeata*, and the hybrid, a finding that would seem to contradict the earlier findings of von Elert (2004), who found that the somatic growth rate, g , of the hybrid was not affected by the dietary level of EPA. However, in our study, the juvenile growth rates (g) of three hybrid clones were also not affected by the availability of EPA, whereas the g values of the remaining five hybrid clones were. This variability in *Daphnia* populations with regard to the susceptibility to EPA limitation underscores the importance of assessing more than one clone of a given species, as evidenced by von Elert (2004).

With regard to the juvenile growth g , *D. galeata* was superior to the two other taxa from the *D. longispina* complex on either food type, which is in accordance with the findings of Weider (1993) and Weider and Wolf (1991). Performance of the hybrids used by Weider (1993) and Weider and Wolf (1991) was intermediate when compared with the parental species, while in our case the hybrid and *D. hyalina* did not differ from each other with regard to g , perhaps reflecting asymmetrical gene flow between the two

taxa (Spaak 1996). Indeed, the genetic structure of the *D. galeata* × *hyalina* hybrids inhabiting Lake Roś is more similar to that of the sympatric *D. hyalina* than to that of *D. galeata* (Brzeziński unpubl. data). The pattern of the relative fitness among the three taxa is different when we consider the intrinsic rate of population growth r . The highest juvenile growth rates (g) of *D. galeata* were not matched by the highest values of r , since *D. hyalina* had the highest fecundity in both food treatments. While effects of food quantity on juvenile growth and reproduction are highly correlated in *Daphnia* (Lampert and Trubetskova 1996), our results indicate a decoupling of effects on growth and reproduction as a result of food quality, as has been shown for mineral limitation (Urabe and Sterner 2001) and for biochemical limitation (Becker and Boersma 2003; Martin-Creuzburg et al. 2005). However, in Lampert and Trubetskova (1996), the first three clutches were taken into account, while only the first clutch was considered in the present study, which renders the results not directly comparable.

In this study we used two different approximations to estimate fitness, the juvenile growth rate, g , and the intrinsic rate of population increase, r . The two different approaches to estimating fitness to some extent reflect the potential performance of the taxa under different mortality scenarios driven by predation. In our study the juvenile growth rate was closely linked to body size at first reproduction ($\ln g = 0.74 [\ln \text{weight}] - 2.49$; $R^2 = 0.59$, $p < 0.0001$); see also Hairston et al. (2001). Since body size is positively related to competitive ability (Gliwicz 1990) and resistance to gape-limited invertebrate predators (Stibor and Lüning 1994), the juvenile growth rate g is a good approximation of fitness in the absence of fish. As larger individuals are more vulnerable to visually oriented predators (Brooks and Dodson 1965), the intrinsic rate of population increase r , calculated on the basis of fecundity at first reproduction, may be a better predictor of fitness in the presence of fish.

Our data show significant genetic variability in *Daphnia* populations with respect to the response to EPA-mediated differences in food quality. Together with reported inter-clonal differences in reaction to varying stoichiometric food quality (Weider et al. 2005), our findings are potentially important to an understanding of how varying food quality maintains genotypic variation in populations. The temporal EPA-mediated heterogeneity of food quality for *Daphnia* is well documented (Müller-Navarra et al. 2000; Wacker and von Elert 2001) and may contribute to the seasonal succession of *Daphnia* genotypes that differ in their susceptibilities to EPA limitation within one lake.

The lack of EPA affected the juvenile growth rates of the three taxa to a similar extent, with no changes in their relative performance, while at the same time reducing the intrinsic rate of population increase r in *D. hyalina* and *D. galeata*, but not in the hybrid. This was accompanied by shifts in relative performance of taxa, as defined by r : from the competitive superiority of *D. hyalina* in EPA-supplemented food to equality of *D. hyalina* and the hybrid in EPA-limited food and from equality of *D. galeata* and the hybrid with supplemented food through to an inferiority of

D. galeata with inferior food. The two parental species differed markedly from each other, and values for the relative fitness of the hybrids oscillated between those for the parental species, following EPA-mediated changes in food quality, indicating that a limited niche overlap exists between the hybrids and the parental species. A similar finding was reported for a food quantity gradient (Weider 1993).

Our results show that when referring to traits related to reproduction, the hybrids within the *D. longispina* complex are less susceptible than are parental species to an EPA-deficient diet, which affects their relative fitness. The parental species do not differ from each other in their ability to deal with EPA limitation; thus, their relative fitness does not change under different EPA availability. In an earlier study (von Elert 2004), only a single clone for each of the three taxa was tested, and these clones were not sympatric. In the present study a large number of clones of coexisting taxa were tested for their susceptibility to EPA limitation, and the conclusion by von Elert (2004) that taxa within the *D. longispina* complex differ in susceptibility of their somatic growth rate to EPA depletion has to be rejected. Although depletion of EPA had a substantial effect on somatic growth of each of the taxa, it did not affect the relative performance with regard to this parameter. However, differences in the susceptibility of the different taxa to the absence of EPA were obvious in traits related to reproduction. Furthermore, we report a high intraspecific variability to cope with the absence of EPA from the diet. Environmental heterogeneity driven by EPA-mediated changes in the quality of food may therefore contribute to the coexistence of hybrids and parental species of the *D. longispina* complex and may favor genotypes that achieve the same fitness with a lower requirement for EPA. This may be an important feedback mechanism that affects the net effect of EPA limitation on zooplankton production and that contributes to the maintenance of genetic diversity in the zooplankton community.

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