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The role of food quality in clonal succession in *Daphnia*: an experimental test

Tomasz Brzeziński · Piotr Dawidowicz · Eric von Elert

Abstract A high genetic variation and recurrent changes in the genetic structure have been found in many pelagic populations. However, evidence that directly links these changes to differences in the ecological performance of particular genotypes is scarce. We hypothesized that within *Daphnia*, the specialization of clones occurring in a particular season to the food quality specific for that time of the year is responsible for the observed changes in the genetic structure of a population. This hypothesis was tested by comparing the fitness of spring and summer clones of the *Daphnia longispina* group, given food of biochemical quality relevant to these seasons. We identified significant intraspecific differences between clones of *Daphnia* that are specific for particular seasons, but there was no evidence that clones are adapted to the food quality available at the respective times of year. Summer clones reproduce at smaller size, and have a lower juvenile specific growth rate as compared to spring clones, irrespective of food quality. Spring clones invest more energy in somatic growth at the cost of reproduction, whereas summer clones invest more energy in reproduction at the cost

of somatic growth. On the basis of the observed differences between spring and summer clones in their patterns of energy allocation, we suggest that other factors, most likely predation, are the major forces driving phenotypic and genetic diversity in the investigated *Daphnia* population of a large lake.

Keywords Seasonal succession · Food quality · Predation · Zooplankton · Population structure

Introduction

Several studies have documented a high degree of genetic variation in populations of clonal animals (Mitter et al. 1979; Weider 1985; De Barro et al. 1995). With regard to pelagic communities, the phenomenon of pronounced genetic diversity, especially within *Daphnia* populations, has been dubbed "the new paradox of the plankton" (Hebert and Crease 1980) and has raised the question of how genetic diversity within taxa is maintained. Some authors have found little ecological difference between clones in permanent populations of *Daphnia*, despite a high level of genetic variation (Mort and Wolf 1986; Lynch et al. 1998). Thus, they have assumed that most of the observed genetic variation may be seen as neutral to natural selection, and that stochastic processes, such as drift (Lynch et al. 1998), random recruitment patterns (Hülsmann 2003) or gene flow between populations (Giessler 1997), may play an important role in maintaining genetic variation. On the other hand, heterogeneity of environmental conditions is considered to be a factor responsible for the maintenance of genetic variation in ecosystems, not only at the species level, but also within a given species (Sommer 1984; Chesson and Huntly 1997). The idea that spatio-temporal

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heterogeneity maintains the coexistence of clones and that this environmental heterogeneity is potentially a driving force for microevolutionary changes in natural populations is supported by the finding that clones within the taxa differ from each other in fitness when exposed to predation (Pijanowska et al. 1993; De Meester et al. 1995) or varying food quality (Weider et al. 2005; Brzezinski and Von Elert 2007).

Under the assumption that a trade-off exists for competitive abilities under different environmental conditions, separate clones are expected to be superior in different microhabitats or at a different time, as resources or environmental conditions change. The frozen niche hypothesis (Vrijenhoek 1978) has been invoked to explain the observed coexistence of different clones within a given population as a result of spatial and/or temporal specialization of clones to specific environmental conditions. Both abiotic and biotic factors vary seasonally in temperate lakes in a relatively recurrent manner, which is reflected by a succession of plankton communities (Sommer et al. 1986). When considering variation in environmental conditions with regard to daphnids, the end of the clear-water phase (period of highest abundance of filter-feeding cladocerans, and lowest phytoplankton abundance during the course of the year) is of special ecological significance, because it divides the season into two distinct periods. Up to this time in spring, daphnids are weakly affected by predation; they are limited to some extent by food quantity, but not by food quality. In summer, after the clear-water phase, daphnids are often subject to strong predation, and they can also suffer from low food quality and quantity (Sommer et al. 1986).

However, it has not been tested if the observed temporal changes in the clonal structure of *Daphnia* (Spaak 1996; Pfrender and Lynch 2000) are due to shifts in the performance of particular clones during the course of the year. Since *Daphnia* clones differ from each other in their sensitivity to low quality food (Brzezinski and Von Elert 2007; Weider et al. 2005), we have focused on food quality as a factor differentiating the performance of clones originating from the same lake but from different seasons: spring and summer. The seasonal succession of phytoplankton species leads to parallel changes in food quality for herbivorous zooplankton, which may result in changes in the taxonomic composition of zooplankton (Sommer et al. 1986; DeMott 1989). The phytoplankton community in spring is dominated by diatoms and chrysophytes, which are rich in essential lipids and easily manageable by daphnids (Ahlgren et al. 1990), whereas major groups of summer phytoplankton are green algae and cyanobacteria, which are suboptimal or poor food for daphnids, as they lack many essential lipids (Ahlgren et al. 1990), or simultaneously show digestion resistance or toxicity (Gliwicz 2003). Since most of the summer phytoplankton in the investigated lake consists of filamentous cyanobacteria, which are scarcely edible for daphnids, green algae represent the vast majority of the edible food supply.

Here we hypothesized that changes in food quality affected the genetic (clonal) structure of a *Daphnia* population during the year. After Vrijenhoek (1978) we expected that specialization of clones with regard to food quality would be seen within a species, i.e. clones occurring in a particular season fit the food quality available then, better than clones occurring at another time of the year. To test this hypothesis we compared the performance of clones of the *Daphnia longispina* complex, isolated in spring or summer, when given algal food specific for spring (cryptophytes) or summer (green algae). Both juvenile specific growth rate (after Lampert and Trubetskova 1996) and fecundity were assumed to reflect the overall fitness in *Daphnia*, we determined the magnitude of these parameters in the tested clones.

Materials and methods

We determined juvenile specific growth rates and fecundity for eight spring clones and eight summer clones of the *D. longispina* group. Four *Daphnia hyalina* and four hybrid *Daphnia galeata × hyalina* ex-ephippial clones were selected as representatives of the spring clones. These were randomly chosen from 96 clones hatched under laboratory conditions from ephippia collected from the sediments of Lake Roś on 22 January 2003. The summer clones were four *D. hyalina* and four *D. galeata × hyalina* clones isolated randomly from pelagic samples taken from Lake Roś on 11 August 2003. Lake Roś (53°43′N 21°52′E, Poland) is a dimictic eutrophic lake of medium size (18.9 km^2). The maximum depth of the lake is 31 m (mean 8.1 m), and the Secchi-disc depth is 2.1 m (average for 2003). The oxygen-rich water layer of the lake is of 8–11 m in thickness, and the hypolimnion is anoxic from May until October, which limits the availability of a deep water refuge for daphnids. The lake is inhabited by abundant populations of planktivorous fish: roach (*Rutilus rutilus*), perch (*Perca fluviatilis*), and smelt (*Osmerus eperlans*) (Jachner 1991; Wołos and Czerwiński 2008). The seasonal pattern of changes in pelagic communities of the lake fit the PEG model of seasonal succession in temperate lakes, as described by Sommer et al. (1986). The spring phytoplankton is dominated by diatoms (*Asterionella formosa*, *Fragillaria crotonensis* and *Melosira* sp., *Tabellaria* sp.—together they constitute up to 86% of the phytoplankton biovolume), with unicellular diatoms and Cryptomonadinae prevailing in the edible size fraction; in summer cyanobacteria and Pyrrophyta build up the net

phytoplankton, while the edible nanoplankton consist almost entirely of the green algae Volvocales and *Scenedesmus* sp. (Witeska 1987; own unpublished data). Substantial seasonal changes occur in the clonal composition within taxa of pelagic *Daphnia* in Lake Roś, indicating the succession of genotypes. Distinct multilocus genotypes (MLGs) dominate particular seasons, and the genetic structure of populations from consecutive periods within a year varies significantly, with an F_{ST} coefficient (the proportion of genetic variation between populations vs. genetic variation within a population) ranging from 0.15 to 0.35 (Brzeziński 2009), which, according to Hartl and Clark (1997), is a major difference.

Allozyme electrophoresis (Hebert and Beaton 1993) was used to examine four loci in each clone: aminoaspartate transferase (AAT; E.C. 2.6.1.1.), aldehyde oxidase (AO; E.C. 1.2.3.1), glucose-6-phosphate isomerase (GPI; E.C. 5.3.1.9) and phosphoglucomutase (PGM; E.C. 5.4.2.2). AAT and AO were used to identify the taxa (Wolf and Mort 1986; Giessler 1997), and GPI and PGM to differentiate the distinct MLGs. See Brzeziński and Von Elert (2007) for a description of the allozyme haplotypes. The proportions of MLGs present in the pelagic population (Brzezinski 2009) and in respective subsamples used in the laboratory experiment did not differ.

The summer food source used in the laboratory experiments was *Scenedesmus obliquus* (strain SAG 276-3a) grown in WC medium (Guillard 1975). When the culture of *S. obliquus* had reached the stationary phase, the cells were harvested by centrifugation (3,000*g*) and resuspended in fresh WC medium. The spring food source was *Cryptomonas* sp. (strain SAG 26.80) grown in WC medium with vitamins (Guillard 1975). In separate experiments we compared the juvenile specific growth rates of a reference *Daphnia* clone [*D. galeata* (Stich and Lampert 1984)] fed *S. obliquus* from a stationary phase, chemostat culture and from a stationary phase culture grown in medium with added vitamins (as for WC medium for *Cryptomonas* culturing), and found no statistically significant differences. Thus we concluded that the media per se had no impact on the performance of the tested clones. The carbon content of the *S. obliquus* and *Cryptomonas* sp. cell suspensions was determined by photometric light extinction at 720 nm for the former and 480 nm for the latter, using previously established carbon-extinction equations. Cryptophyceans of the genus *Cryptomonas* have been shown to be an optimal food source for daphnids (Ahlgren et al. 1990; Bec et al. 2006; Martin-Creuzburg and Von Elert 2009), whereas *S. obliquus* is a green alga lacking certain biochemical components [polyunsaturated fatty acids (PUFAs)] that are essential for daphnids (Von Elert 2002; Becker and Boersma 2005). One of the most important PUFAs is eicosapentaenoic acid (EPA; C20:5n-3), which

regulates the structure and function of membranes and serves as a hormone precursor (Stanley-Samuelson 1994). The strain of *S. obliquus* used in this study has repeatedly been reported as free of EPA (Wiltshire et al. 2000; Von Elert 2004; Becker and Boersma 2005).

The *Daphnia* were fed either *S. obliquus* or *Cryptomonas* sp., added as cell suspensions prepared using membrane-filtered (pore size 0.45 μm) lake water that had been conditioned for 48 h. Experiments were carried out in 0.5-l volumes of conditioned lake water, containing a food level of 2 mg C l^{-1}, that were renewed daily. Each food treatment consisted of three replicates for each clone, each containing 10 to 12 animals. All experiments were run at a constant temperature of 20 ± 0.1°C.

The experimental animals originated from the third broods of mothers raised for three generations under non-limiting *S. obliquus* food concentrations. Synchronized cohorts of 14- to 24-h-old neonates were used to start the growth experiments. The initial weight of the *Daphnia* was measured in two subsamples of 20 individuals. The experiments were run until the animals released their first clutch into the brood chamber. They were then transferred to pre-weighed aluminium boats and dried at 60°C, before being weighed on a Mettler UMT2 electro-balance to the nearest 0.1 μg. Juvenile specific 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 of individuals at the end of the experiment, M_0 represents their initial body mass and t is the duration of the experiment. Eggs in the brood chambers were counted under a dissecting microscope.

Since it was not possible to run single growth experiment involving all of the selected clones, four separate ones were performed, each including the reference clone *D. galeata* grown on pure *S. obliquus*. For each of the experimental clones, all of the different food treatments were run within the same experiment.

Statistical analyses

To meet the assumptions of normality and homogeneity of variances, the dependent variables were $\log_n$ transformed prior to analysis. A mixed general linear model was used, which included taxon (a two-level factor: *D. hyalina* and *D. hyalina* × *galeata*), food (a two-level factor: spring and summer food), season (a two-level factor: spring and summer clones), and clone (a four-level factor). The clone factor was set as a random factor, and it was nested in both season and taxon factors. The juvenile specific growth rate and fecundity of the reference clone were used as co-variables to account for the fact that the experimental clones were examined in separate growth experiments. The tested parameters were normalized to the values for the reference clone, using proportion of the maximum and

assay-specific values of this clone. Differences between means were tested using Tukey's test.

We expected that summer clones fed summer food (green algae) should perform better than spring clones fed green algae. A significant interaction of food × season would indicate that the summer and spring clones differed in their adaptation to season-dependent food quality.

We calculated coefficients of variation (CV) for juvenile specific growth rate and fecundity of spring and summer clones. The variances within groups of spring and summer clones were compared using Bartlett's tests.

A significance level of α < 0.05 was applied to all statistical analyses. For Bartlett's tests, sequential Bonferroni adjustment was applied (Rice 1989). The analyses were performed using SAS 9.2 (SAS Institute) software.

Results

Daphnia fed *Cryptomonas* sp. had a higher juvenile specific growth rate and fecundity than those fed the green alga *Scenedesmus obliquus* (Table 1; Fig. 1). There were marked differences in both juvenile specific growth rate and fecundity between the summer and spring clones. The juvenile specific growth rate of spring clones was higher than that of summer clones, irrespective of whether they were fed summer (*S. obliquus*; $P < 0.0001$) or spring (*Cryptomonas* sp.; $P < 0.0001$) food (Fig. 1). Similarly, spring clones achieved a larger body size at first reproduction with both food types ($P < 0.01$). There was no significant food × season interaction with regard to juvenile specific growth rate, fecundity or size at first reproduction (Table 1), which indicates that food quality had no effect on the relative fitness of spring and summer clones. Interestingly, but contrary to our expectations, the juvenile specific growth rate of summer clones was lower than that of spring clones when both were fed summer food (*S. obliquus*; $P < 0.0001$), and there were no differences in fecundity between summer and spring clones fed spring food (*Cryptomonas* sp.; $P > 0.07$; Fig. 1).

There were differences between *D. hyalina* and *D. hyalina* × *galeata* hybrids originating from the two seasons in their patterns of reaction to varying food quality, as indicated by significant taxon × food × season interactions with regard to juvenile specific growth rate and SFR (Table 1). This is because: (1) juvenile specific growth of spring *D. hyalina* declined when the food supply was switched from spring food (*Cryptomonas* sp.) to summer food (*S. obliquus*), which was not observed in spring hybrids; and (2) in summer hybrids, juvenile specific growth rate declined when the food supply was changed from *Cryptomonas* sp. to *S. obliquus* food, which was not the case in summer *D. hyalina* (Fig. 2). In both spring and summer

Table 1 General linear model results for analyses of juvenile specific growth rate, fecundity, and size at first reproduction in summer and spring clones from the *Daphnia longispina* complex fed summer (*Scenedesmus obliquus*) or spring (*Cryptomonas* sp.) food

|	SS	df	MS	F	P
Juvenile specific growth rate					
Taxon	0.11	1	0.11	15	0.002
Season	0.29	1	0.29	42	0.0001
Food	0.46	1	0.46	66	0.0001
Taxon × season	0.11	1	0.11	15	0.002
Taxon × food	0.01	1	0.01	1.5	0.2
Season × food	0.006	1	0.006	0.8	0.4
Taxon × season × food	0.06	1	0.06	8.1	0.006
Clone (taxon × season)	5.3	11	0.49	69	0.0001
Food × clone (taxon × season)	5.8	24	0.24	99	0.0001
Ref	0.00	1	0.00	0.03	0.8
Error	0.52	76	0.007		
Fecundity					
Taxon	0.25	1	0.25	8.9	0.004
Season	0.004	1	0.004	0.13	0.7
Food	1.4	1	1.4	49	0.0001
Taxon × season	0.05	1	0.05	1.8	0.2
Taxon × food	0.005	1	0.005	0.18	0.7
Season × food	0.03	1	0.03	1.0	0.3
Taxon × season × food	0.00	1	0.00	0.00	0.9
Clone (taxon × season)	9.7	11	0.87	31	0.0001
Food × clone (taxon × season)	12	24	0.48	24	0.0001
Ref	0.00	1	0.00	0.00	0.9
Error	2.1	76	0.03		
Size at first reproduction					
Taxon	0.002	1	0.002	6.4	0.01
Season	0.008	1	0.008	28	0.0001
Food	0.009	1	0.009	29	0.0001
Taxon × season	0.005	1	0.005	12	0.001
Taxon × food	0.007	1	0.007	24	0.0001
Season × food	0.0003	1	0.0003	1.0	0.3
Taxon × season × food	0.002	1	0.002	5.8	0.02
Clone (taxon × season)	0.06	11	0.005	17	0.0001
Food × clone (taxon × season)	0.81	24	0.03	172	0.0001
Ref	0.00	1	0.00	0.03	0.8
Error	0.02	76	0.0003		

D. hyalina, SFR did not change when the food supply was switched from *Cryptomonas* sp. to *S. obliquus*, while in both spring and summer hybrids, SFR was reduced (Fig. 2).

Contrary to our expectations SFR and juvenile specific growth rate of both hybrid and *D. hyalina* clones were lower in summer clones than in spring clones, irrespective of whether they were fed spring (*Cryptomonas* sp.) or
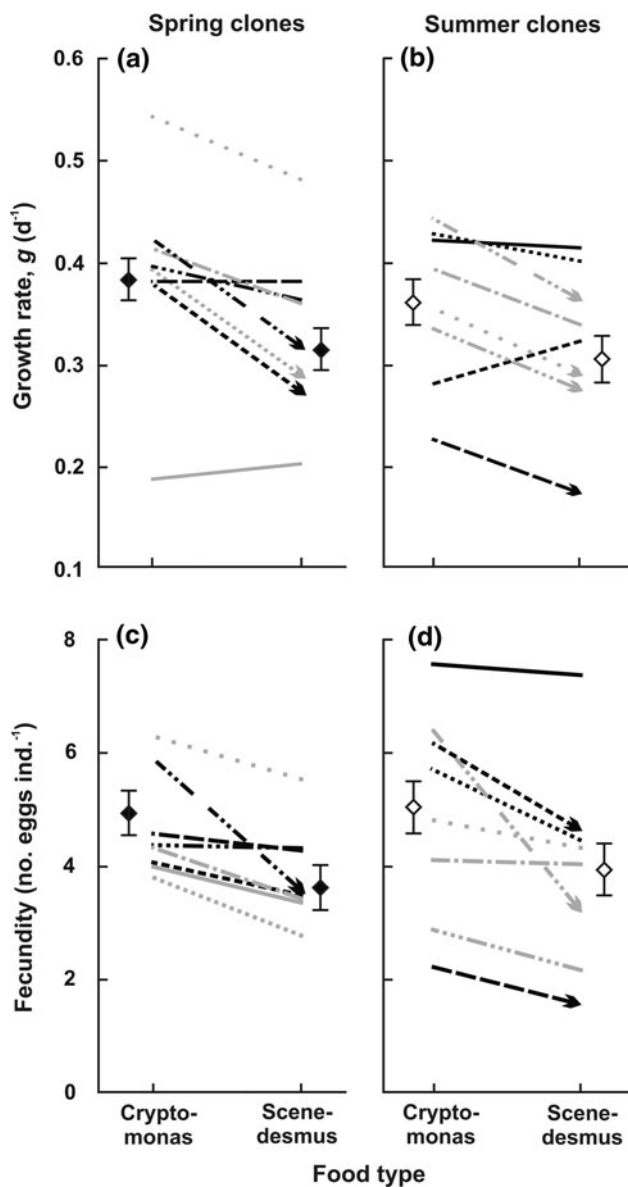
Fig. 1 Reaction norms of **a, b** juvenile specific growth rate (*g*) and **c, d** fecundity within spring (**a, c**) and summer (**b, d**) clones of *Daphnia hyalina* (*black lines*) and *D. hyalina × galeata* (*grey lines*) fed *Cryptomonas* sp. (spring food) or *Scenedesmus obliquus* (summer food). *Each line* represents a different clone. *Arrows* at the end of a reaction norm curve indicate a significant difference between the two treatments of the respective clone (Tukey's test, *P* < 0.05). *Diamonds* represent mean ± 95% confidence intervals for spring or summer clones (*D. hyalina* and *D. hyalina × galeata* pooled together) fed spring or summer food

summer (*S. obliquus*) food (Fig. 2). Within both taxa, spring clones fed spring food tended to have higher or at least the same juvenile specific growth rate and SFR compared with those fed summer food, and the same pattern was apparent for summer clones (Fig. 2).
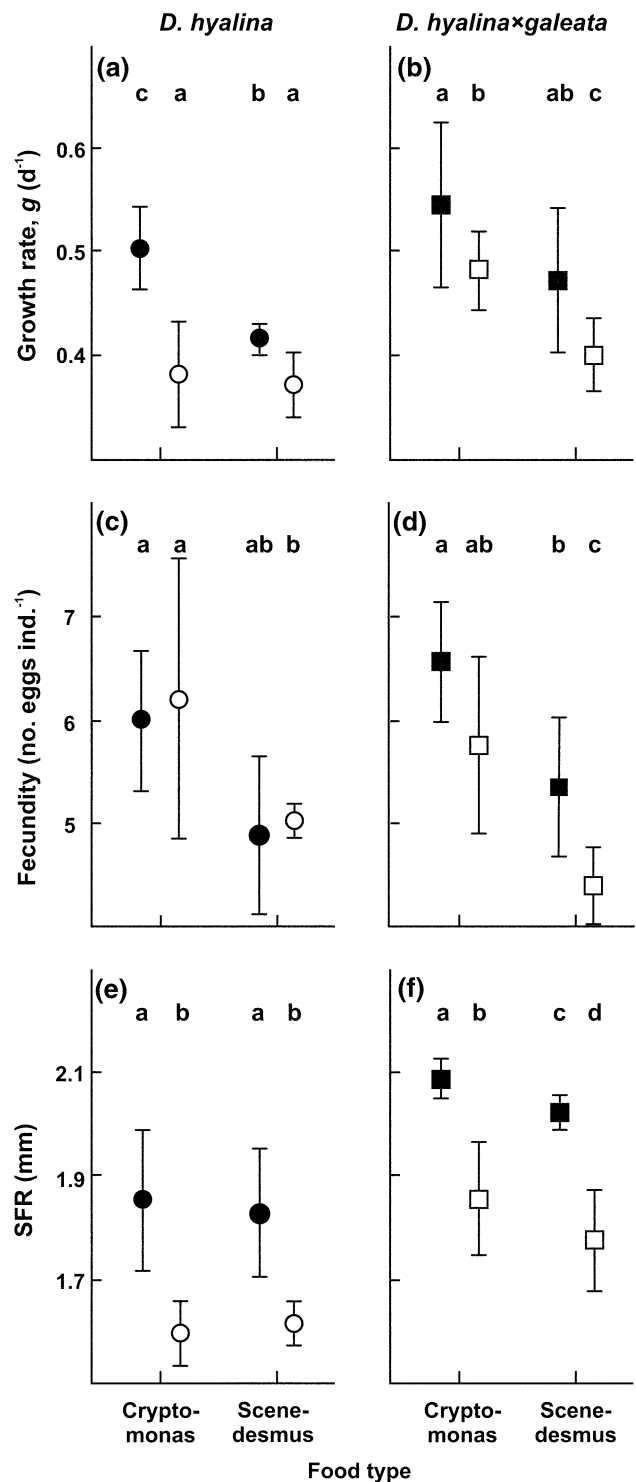
Fig. 2 **a, b** Juvenile specific growth rate, **c, d** fecundity, and **e, f** size at first reproduction (*SFR*) of spring (*filled symbols*) and summer (*open symbols*) clones of *D. hyalina* (*circles*) and *D. hyalina × galeata* (*rectangles*) fed *Cryptomonas* sp. (spring food) or *Scenedesmus obliquus* (summer food), means ± 95% confidence intervals. *Letters* indicate homogeneous groups (Tukey's test, *P* > 0.05)

There was no significant taxon × food × season interaction with regard to fecundity (Table 1); however, differences between *D. hyalina* and hybrids originating from the two seasons occurred in response to the change in food supply. The fecundity of spring hybrids declined when the food supply was switched from *Cryptomonas* sp. to *S. obliquus*, which was not the case in spring *D. hyalina* (Fig. 2). The fecundity of summer hybrids was lower than that of spring hybrids when both were fed summer food (*S. obliquus*), whereas the fecundity of spring and summer *D. hyalina* did not differ when both were fed summer food (*S. obliquus*; Fig. 2).

The hybrids always achieved higher SFR than *D. hyalina*, when compared within a given food treatment and origin ($P < 0.0001$). The fecundity of hybrids was not different from that of the respective *D. hyalina* ($P > 0.8$). Summer hybrids had a higher juvenile specific growth rate than summer *D. hyalina*, regardless of whether they were fed *Cryptomonas* sp. ($P < 0.0001$) or *S. obliquus* ($P < 0.02$). The juvenile specific growth rate of spring hybrids and spring *D. hyalina* did not differ, irrespective of the food type ($P > 0.1$).

There were marked interclonal differences in juvenile specific growth rate and fecundity within both groups of clones in response to varying food quality [a significant food × clone (taxon × season) effect; Table 1]. In some clones, both juvenile specific growth rate and fecundity increased with spring food; in other clones, only one of the tested parameters increased; while in others, no differences between the food treatments were found (Fig. 1).

Within *D. hyalina*, the variation in the fecundity of summer clones was higher than that of spring clones (Table 2; Fig. 3). Variation in the juvenile specific growth rate of the spring hybrids was higher than that of the

Table 2 Bartlett's tests' results for comparisons of variances between spring and summer clones of the *D. longispina* complex, and between contemporary *D. hyalina* and *D. hyalina* × *galeata*

	χ^2	df	P
Spring clones versus summer clones			
Juvenile specific growth rate *D. hyalina*	3.1	1	>0.08
Juvenile specific growth rate *D. hyalina* × *galeata*	9.4	1	<0.002*
Fecundity *D. hyalina*	15	1	<0.00008*
Fecundity *D. hyalina* × *galeata*	1.3	1	>0.3
D. hyalina × *galeata* versus *D. hyalina*			
Juvenile specific growth rate spring	13	1	<0.0003*
Juvenile specific growth rate summer	1.4	1	>0.2
Fecundity spring	1.2	1	>0.3
Fecundity summer	3.6	1	>0.06

Asterisks indicate significant differences after Bonferroni correction
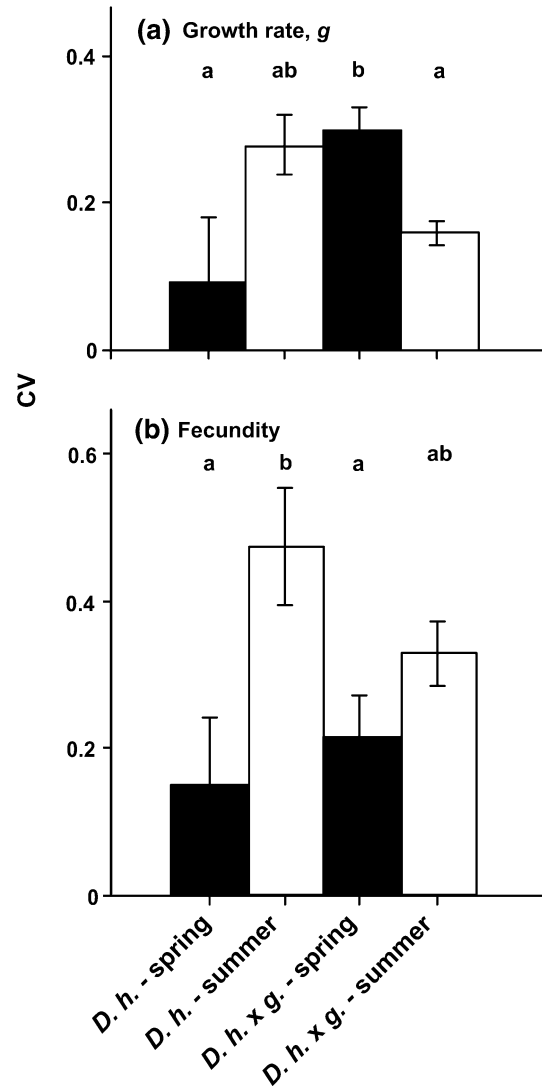
Fig. 3 The coefficients of variation (*CV*; ± maximum, minimum) in **a** juvenile specific growth rate, and **b** fecundity in spring (*filled bars*) and summer (*open bars*) clones of the *Daphnia longispina* group, *D. hyalina* (*D.h.*) and *D. hyalina* × *galeata* (*D.h* × *g.*). Letters indicate groups of similar variances (Bartlett's tests, $P > 0.02$—non-significant after Bonferroni correction)

summer hybrids, and higher than that of spring *D. hyalina* (Fig. 3).

Discussion

Intraspecific differences within zooplankton species in response to varying food quality have been observed in several studies (Repka 1996; Brzezinski and Von Elert 2007). In a microcosm experiment, Weider et al. (2005) showed that stoichiometric food quality affected competition between *Daphnia* clones: when given food with a high C:P ratio, one clone dominated, whereas when the dietary

C:P ratio was low, coexistence of clones was achieved. The results of Weider et al. (2005) indicate the possible role of food quality in causing microevolutionary changes that are reflected in changes in the genetic structure of populations. Long-term changes in the genetic structure of a population resulting in an increase in the resistance of *Daphnia* to toxic cyanobacteria have also been reported (Hairston et al. 2001).

The conceptual framework of the PEG model of succession in temperate lakes (Sommer et al. 1986) indicates that changes in zooplankton should be visible within 1 year, not only in species composition, but also in the genetic structure of the population. There is some evidence for such changes in the genetic population structure, correlated with changes in predation pressure, habitat availability, or competition strength (Geedey et al. 1996; Hembre and Megard 2006). However, there has been no direct empirical evidence for the role of food quality in the seasonal succession of clones within the population of a given species.

We confirmed that microevolutionary changes in the genotype composition of a *Daphnia* population, which can be seen within the course of a year in Lake Roś (Brzezinski 2009), are reflected in differences in the performance of the clones. On average, the performance of clones isolated in spring differed from that of summer clones. However, the effect of food quality on the genetic composition of the investigated *Daphnia* population seemed to be of minor importance, since we found no significant food × time interaction. The summer clones appeared not to be specifically adapted to the low PUFA content of summer food, as their juvenile specific growth rates were lower than those of the spring clones given food of quality typical for the summer. However, despite their lower juvenile specific growth rates, the fecundity of summer clones was at least comparable to that of the spring clones, regardless of food quality. This contributes to the differences between spring and summer clones in their patterns of energy allocation (Fig. 4): spring clones appeared to invest energy in fast somatic growth at the cost of fecundity, which according to Gliwicz (1990) and Lampert and Trubetskova (1996), increases their competitive abilities, while in summer clones, fecundity was boosted at the cost of somatic growth.

It should be noted that despite the finding that spring and summer hybrids differed from contemporary groups of *D. hyalina* in their reactions to varying food quality, we found evidence within both the hybrids and *D. hyalina* to reject the hypothesis that clones are specifically adapted to the food quality specific for the season of their origin. In both taxa, summer clones did not perform better than spring clones when fed summer food. In both taxa the reduction in body size observed in summer clones, compared
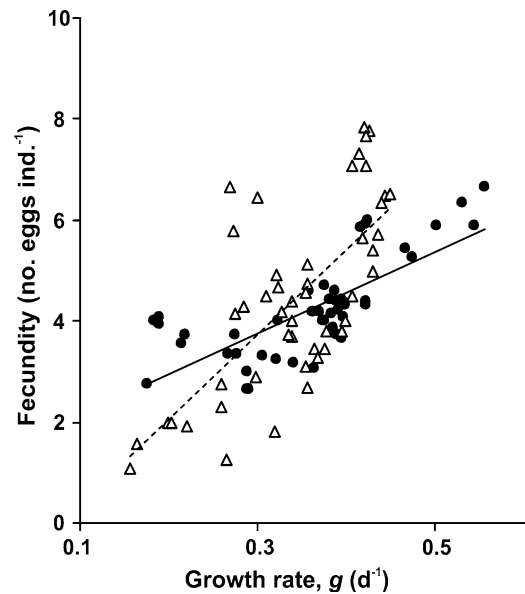
Fig. 4 The relationship between juvenile specific growth rate and fecundity in spring (*filled circles*, *solid line*) and summer (*open triangles*, *dashed line*) clones of the *D. longispina* group. Each clone is represented by six points on the graph (2 food treatments × 3 replicates). The slopes of the regressions differ significantly ($P < 0.0001$)

with spring ones (Fig. 2), may reflect the impact of size-selective predation, rather than changes in food quality.

Based on patterns of energy allocation for growth and reproduction, we suggest that predation by planktivorous fish, rather than food quality, is the main factor responsible for the differences in genetic structure observed by Brzezinski (2009) between spring and summer populations of *D. longispina* in Lake Roś. Due to their higher juvenile specific growth rate and larger body size, the spring clones are better competitors for food than summer clones, regardless of the quality of this food. Up until the clearwater phase, the spring clones should be weakly affected by predation. The smaller body size of the summer clones can be seen as the result of positive size-selection by fish (Brooks and Dodson 1965), which is highest in June/July in Lake Roś (Jachner 1991). Furthermore, the differences between the spring and summer clones in their energy allocation patterns closely resemble the trade-offs in the reactions of daphnids exposed to fish exudates: reduced somatic growth and body size, earlier reproduction and increased clutch size (Machacek 1991; Gliwicz and Boavida 1996).

Tessier and Woodruff (2002) noted that daphnid species are specifically adapted to exploit habitats of rich or poor food quality such that under low food quality genotypes with high competitive abilities are favoured, whereas under high food quality genotypes with a high reproductive effort are favoured. However, they noted also, that

increased resource availability was associated with an increased mortality risk due to predation. Thus they were not able to distinguish between effects of predation and effects of food quality on daphnids. In concordance with Gliwicz (2003), who underlines the importance of the run for life over the run for food, we hypothesize that differences in competitive abilities of daphnids derived from environments of different food conditions might be a by-product of simultaneous selection under changing predation regimes.

It is unlikely that these changes are due to abiotic factors, such as UV radiation, temperature etc., as these factors are non-selective with regard to investigated individual life history parameters (Gliwicz and Pijanowska 1989; Gliwicz 2003). The impact of parasites on daphnids in Lake Roś during summer should not be strong, as abundances of *Daphnia* are low (Brzeziński 2009), which reduces transmition rate of parasites between hosts (Little and Ebert 2001), moreover fish predation, which is strong in Lake Roś (Jachner 1992), has been reported to reduce the impact of parasites on their planktonic hosts (Chiavelli et al. 1993).

Intraspecific variation CV

We expected to find the greatest variation among the spring clones (and there to be lower variation among spring *D. hyalina* than among spring hybrids), as the spring clones represent genotypes untouched by selection. A burst of variation in spring hybrids, followed by its reduction in the summer, reflects the emergence of daphnids from amphigonic resting eggs, which is restricted to the spring (Brendonck and DeMeester 2003; Brzezinski 2009), and further elimination of lines due to selection and branching processes (Jagers 1995). As hybrids originate from the gene pools of two species, they are expected to show greater variation than the parental species, and this was found to be the case on comparing variances in spring hybrids and spring *D. hyalina* (Fig. 3; Table 2).

Interestingly, the changes in the mean performance of the spring and summer clones were not accompanied by a reduction in variance of the latter compared to the former, with the mentioned exception of the hybrids (Fig. 3; Table 2). Such a pattern of increased variation in traits over time is predicted by the frozen niche hypothesis as the result of specialization of clones and niche partitioning in a heterogeneous environment (Vrijenhoek 1978). With regard to zooplankton, this may reflect the impact of predation, which promotes the coexistence of multiple combinations of behavioural, morphological and life history characteristics (De Meester et al. 1995; Boersma et al. 1998; Sakwinska and Dawidowicz 2005).

Hybrids versus *D. hyalina*

Despite the lack of evidence that changes in food quality are responsible for differences in the performance of spring and summer clones, pronounced variation in sensitivity to inferior food was observed in both of these groups (Fig. 2). This variation to some extent reflects differences in the performance of *D. hyalina* and *D. hyalina × galeata*. As demonstrated by significant taxon × food × time interactions within groups of spring and summer clones, the taxa differ from each other in their sensitivity (at least with regard to juvenile specific growth rate and SFR) to changing food quality conditions. Our results confirm earlier reports that hybrids within the *D. longispina* complex cope better than parental species with a deterioration in food quality (Von Elert 2004; Seidendorf et al. 2007). Summer hybrid clones performed better on summer food than summer *D. hyalina* clones. This may contribute to the increase in hybrid abundance observed in Lake Roś during the course of the year (Brzezinski 2009).

It is also noteworthy that when spring clones were considered, hybrids did not differ from *D. hyalina* in their performance, despite the fact that the *D. hyalina* clones were on average smaller, whereas over the course of the year *D. hyalina* diverged from hybrids and its own spring source population (at least with regard to juvenile specific growth rate). This highlights the fact that *D. hyalina* and the hybrids followed different selection trajectories.

It should be noted that the group of spring clones studied here was probably a mixture of real spring clones and animals that were destined to become summer clones. Since *D. hyalina* does not overwinter in Lake Roś and the population is not abundant in early spring, individuals hatched from resting eggs isolated from this lake were used as spring clones. However, the genetic structure of the hatchling pool, as determined by allozyme patterns, closely resembled that of the pelagic population in spring ($F_{ST} = 0.01$; Brzeziński 2009), which suggests that the use of ex-ephippial clones as the spring population did not affect the results of this study.

Our results do not support the hypothesis that variation in food quality, as expressed by the PUFA content, is important for the succession of clones within a *Daphnia* population. Of course the biochemical content of food is not the only factor affecting food quality. *S. obliquus* was grown under non-limiting conditions so that stoichiometric constraints on the food quality can be excluded. This may not reflect natural lake conditions; however, the food quality of the seston was found to be correlated with the EPA content, but not with the P content, over a whole season in lake Constance (Wacker and Von Elert 2001). Cyanobacteria are a major constituent of summer phytoplankton in Lake Roś, and their occurrence may

additionally lower the quality of phytoplankton available in summer due to inadequate biochemical content, increased resistance to digestion or toxicity (Porter and McDonough 1984; Von Elert et al. 2003). However, the presence of cyanobacteria can alter the realized juvenile growth rate, but not the potential juvenile growth rate—the juvenile growth rate of daphnids in lake Constance is depressed under the presence of cyanobacteria, but despite this daphnids "resurrected" from ephippia that had been deposited during the period of eutrophication and heavy cyanobacterial blooms have higher juvenile growth rates than daphnids "resurrected" from ephippia that had been deposited before the period of eutrophication (Hairston et al. 2001). Thus, cyanobacteria cannot be responsible for the observed differences between spring and summer clones, although the latter were certainly exposed to cyanobacteria in Lake Roś.

In this study we have demonstrated significant intraspecific differences between clones of *Daphnia* that are specific for particular seasons of the year. The effects of their different abilities to cope with varying biochemical food quality, which can be seen in particular *Daphnia* clones, seem to be overridden by adaptations to minimize the risk of mortality by predation. Therefore, we suggest that the major driving force of phenotypic and genetic diversity in the investigated *Daphnia* population of a large lake is predation.

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