Single dietary amino acids control resting egg production and affect population growth of a key freshwater herbivore

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Abstract The enormous success of the genus *Daphnia* in freshwater ecosystems is at least partially due to their cyclical parthenogenetic life cycle, in which asexual and sexual reproduction alternate periodically. This temporal change between reproductive strategies allows for (1) rapid population growth via subitaneously developing eggs when environmental conditions are appropriate and (2) the maintenance of genetic diversity via sexual reproduction and the production of resting eggs when environmental conditions deteriorate. We show here that dietary amino acids are involved in triggering the switch between reproductive modes in *Daphnia pulex*. Supplementation experiments demonstrate that specific dietary amino acids, in particular arginine and histidine, avert crowding-induced resting egg production, enhance subitaneous reproduction by increasing algal food quality and, as a combined effect of both processes, increase population growth rates. These findings suggest that the availability of single dietary amino acids potentially affects the seasonal dynamics and long-term persistence of *Daphnia* populations in the field, which may have consequences for the efficiency of carbon transfer and thus the trophic structure of freshwater food webs.

Keywords *Daphnia* · Food quality · Reproductive mode · Parthenogenesis · Resting eggs

Introduction

The elemental and biochemical composition of plants and algae significantly affects the performance of herbivores. For example, the mineral content and primary and secondary metabolite contents of plants and algae can influence herbivore foraging behaviour (Berner et al. 2005; Schatz and McCauley 2007), somatic growth and reproduction (Sternner and Elser 2002; Von Elert 2002; Martin-Creuzburg et al. 2009), life history strategy (Twombly et al. 1998), immune function (Bukovinszky et al. 2009; Smilanich et al. 2009), anti-predator defence (Kazana et al. 2007) and genetic diversity (Weider et al. 2008). As a consequence, the elemental and biochemical composition of herbal food sources potentially affects the population dynamics of herbivores and thus the efficiency with which carbon and organic matter are transferred to higher trophic levels (Müller-Navarra et al. 2000; Wacker and Von Elert 2001; Sternner and Elser 2002).

Seasonal dynamics of the freshwater keystone herbivore *Daphnia* spp. (Crustacea, Anomopoda, Daphnidae) strongly affect the efficiency of carbon transfer from primary producers to higher trophic levels. This is due both to the high grazing impact daphnids impose upon phytoplankton and to the fact that daphnids are the principal prey of many invertebrate predators and planktivorous fish species. As a result, demographic changes in *Daphnia* spp. and its population dynamics can have profound effects on the trophic structure and function of freshwater food webs (Lampert 1987; Leibold 1989; Carpenter et al. 2001). The life cycle of daphnids is usually characterised by a cyclical

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parthenogenesis, i.e. asexual (parthenogenetic) reproduction alternates periodically with sexual reproduction. In the field, *Daphnia* populations consist primarily of parthenogenetic females reproducing via subitaneously developing eggs. Sexual reproduction, which in *Daphnia* is associated with the production of resting stages (so-called ephippia, containing up to two diapausing eggs), is induced by deteriorating environmental conditions and often restricted to specific times of the year, such as during short periods in early summer and late autumn (Cáceres 1998; Jankowski and Straile 2004). This temporal change between reproductive strategies allows for both rapid population growth via parthenogenesis when environmental conditions are appropriate, thereby avoiding the costs of producing males (Innes et al. 2000), and the maintenance of genetic diversity via sexual reproduction and the production of resting eggs when environmental conditions deteriorate (Hairston 1996; Jankowski and Straile 2004).

Resting egg production is an important life history trait which enables survival during unfavourable environmental conditions, such as low temperature, intense predation, desiccation or starvation (Schwartz and Hebert 1987; Ślusarczyk 1995; Alekseev and Lampert 2001), and facilitates dispersal in time and space (Hairston and Kearns 2002; Panov et al. 2004; Louette and De Meester 2005). However, the production of resting eggs is supposed to be associated with higher costs compared to the production of subitaneously developing eggs because during each molt cycle, a single female can either produce one clutch of several subitaneously developing eggs or one ephippium containing only one or two resting eggs. This trade-off in quantity between subitaneously developing eggs and resting eggs, together with the costs associated with male production, presumably have favoured the evolution of an inducible rather than a fixed switch between reproductive modes. The inducibility of resting egg production in *Daphnia* has been extensively studied, and several environmental factors have been shown to induce the switch between reproductive modes, including population density, food concentration, photoperiod or fish kairomones (Stross and Hill 1965; Kleiven et al. 1992; Ślusarczyk 1995, 2001).

In past decades, a considerable amount of research focused on the effects of food quality on the performance of *Daphnia* species and the associated consequences for food web processes. In particular, a low availability of dietary phosphorus (Sternner and Elser 2002), polyunsaturated fatty acids (PUFAs) (Müller-Navarra et al. 2000; Wacker and Von Elert 2001; Von Elert 2002) and sterols (Von Elert et al. 2003; Martin-Creuzburg et al. 2005, 2009) has been shown to impair the growth and reproduction of *Daphnia* species. However, up to now, food quality studies have focused almost exclusively on the parthenogenetic part of the *Daphnia* life cycle, thereby neglecting the

effects of food quality on resting egg production. In fact, only one published study has focused on this aspect, documenting that food quality can have pronounced effects on the frequency of resting egg production (Abrusán et al. 2007).

In a previous publication, we demonstrated that dietary proteins are involved in triggering the switch between subitaneous reproduction and resting egg production in two *Daphnia* species, *D. galeata* and *D. pulex*. (Koch et al. 2009). This finding led us to suggest that the availability of dietary proteins may significantly affect long-term persistence and dispersal and, potentially, also the genetic diversity of *Daphnia* populations in the field. Moreover, our data revealed that protein supplements can improve parthenogenetic fecundity of the herbivore, which implies that a deficiency in dietary proteins is an important food quality constraint. In the study reported here, we investigated whether the observed effects are mediated by single amino acids rather than unspecific proteins. By supplementing the nutritionally mediocre green alga *Scenedesmus obliquus*, which has been shown to cause resting egg production in crowded daphnids (Koch et al. 2009), with ten presumably essential amino acids, we tested the potential of single amino acids to control the switch between reproductive modes in a clone of *Daphnia pulex*.

Materials and methods

Cultivation of food organisms

The green alga *Scenedesmus obliquus* (SAG 276–3a) and the cryptophyte *Cryptomonas* sp. (SAG 28.80) were cultured semi-continuously in aerated 5-L vessels at a dilution rate of 0.25 day^{-1} (20°C; illumination at $146 \mu\text{mol quanta s}^{-1}\text{m}^{-2}$). *S. obliquus* was grown in 'Cyano' medium (Jüttner et al. 1983) and *Cryptomonas* sp. in WC medium supplemented with vitamins (Guillard 1975). Preliminary experiments revealed that the vitamins used to cultivate *Cryptomonas* sp. did not affect ephippia production in the *D. pulex* feeding on *S. obliquus*.

Daphnia growth experiments

Experiments were conducted with an obligate parthenogenetic *D. pulex* clone, which is characterised by a naturally high allocation to ephippia production. The low density at which ephippia production can be induced in this clone [35–50 individuals (ind.) L^{-1}] minimises competition and interference among individuals in the experimental beakers. Besides crowding, all other known stimuli for resting egg production were excluded, i.e. food limitation, fish kairomones or changes in photoperiod. The

pre-experimental daphnids were raised in filtered lake water (pore size of membrane filter 0.45 µm) with saturating concentrations of *S. obliquus* at constant densities and continuous dim light for at least two generations. Experiments were conducted with female third-brood juveniles born within 12 h.

All experiments lasted 16 days, i.e. a time intervals which allowed for three subitaneous or two ephippial broods, and were run under continuous dim light at 20°C in glass beakers filled with 200 mL of filtered lake water (pore size 0.45 µm) containing saturating food concentrations (2 mg C L⁻¹). Each jar contained ten animals to stimulate crowding-induced ephippia production (four replicates per treatment). Animals were transferred each day into new beakers with freshly prepared food suspensions, and the number of released ephippia and neonates was determined. During the experiments, daphnids were able to produce at most three subitaneous or two ephippial broods, respectively. Ephippia were usually released 4 days after the previous reproduction, whereas subitaneous reproduction could occur every second day. Allocation to resting egg production was determined as the number of produced ephippia; allocation to subitaneous reproduction was determined as the cumulative number of produced neonates. Population growth rates (*r*) were estimated iteratively using the Euler–Lotka equation:

$$1 = \sum_{x=0}^n l_x m_x e^{-rx}$$

wherein l_x is the age-specific survivorship, m_x is the age-specific fecundity (number of neonates per individual) and x is the age at reproduction (in days). The probability of survival (l_x) was estimated from the mortality that occurred during the experiments.

Supplementation of amino acids

Amino acids (AA) were dissolved in ultrapure water and stored at -20°C. Aliquots of these stock solutions were added to the experimental beakers containing 2 mg C L⁻¹ of *S. obliquus*. The ten amino acids [Essential L-Amino Acid kit LAA1 (Sigma, St. Louis, MO) containing arginine (arg), histidine (his), isoleucine (ile), leucine (leu), lysine (lys), methionine (met), phenylalanine (phe), threonine (thr), tryptophane (try), valine (val)] were supplemented separately or in combination. In a first set of experiments, a mixture of all ten amino acids was initially supplemented to the experimental beakers, resulting in a concentration of 25 µM per amino acid in each beaker. Subsequently, each amino acid was provided in a concentration-dependent manner, i.e. 25, 12.5, 6.25, 3.125 and 0 µM amino acid. In a second set of experiments, amino acids were tested

separately at a concentration of 25 µM amino acid. In addition, arginine and histidine were tested in different concentrations (50, 25, 0 µM) either separately or in combination.

Amino acid analyses

For the analysis of particulate combined amino acids, approximately 1 mg particulate organic carbon (POC) of *Cryptomonas* sp. or *S. obliquus* were concentrated on precombusted GF/F filters (25 mm; Whatman, New York, NY) and stored at -80°C. Prior to analysis the samples were hydrolysed with 6 M HCl at 155°C for 1 h and neutralised with 6 M NaOH. Filtered aliquots were derivatised with *o*-phthaldialdehyde (OPA) and analysed in a high-performance liquid chromatography (HPLC) system (HP1000; Agilent Tech, Santa Clara, CA) equipped with a fluorescence detector (Grossart et al. 2007). With this method, arginine and citrulline could not be separated from each other. Particulate organic carbon, nitrogen and phosphorus were analysed as described in Martin-Creuzburg et al. (2009).

Statistical analyses

Cumulative numbers of produced ephippia and neonates were analysed using one-way analyses of variance (ANOVA) with post hoc comparisons (Tukey's HSD). Dose-dependent responses to amino acids were analysed using non-linear regressions: an exponentially declining function was fitted to the data to describe the functional response of ephippia production, and a modified Michaelis–Menten function with a non-zero *y* intercept was fitted to describe the functional response of neonate production and population growth rate. Multiple regression analyses with amino acid supplementation and ephippia production as independent variables were used to disentangle the direct (improvement of food quality) and indirect (switch in reproductive mode) effects of amino acid supplementation on neonate production and population growth rate. Pairwise comparisons using one-way ANOVAs were used to compare single amino acid treatments with unsupplemented *S. obliquus*. Dose-response effects of arginine and histidine were analysed using analysis of covariance (ANCOVA).

Results

The green alga *Scenedesmus obliquus* and the flagellate *Cryptomonas* sp. caused different responses in crowded *Daphnia pulex*: animals fed with *S. obliquus* showed a high investment in resting egg production, whereas those fed

with *Cryptomonas* sp. reproduced only subitaneously (Fig. 1). The molar carbon to nitrogen (C:N) and carbon to phosphorus (C:P) ratios of *S. obliquus* [mean $\pm$ standard deviation (SD): C:N 5.4 ± 0.1 ; C:P 86.8 ± 15.7] and *Cryptomonas* sp. (C:N 5.6 ± 0.1 ; C:P 117.1 ± 15.7) were low, indicating a high nitrogen and phosphorus content of the food suspensions. Thus, a limitation of *D. pulex* by nitrogen or phosphorus is rather unlikely.

Amino acid analyses revealed that *Cryptomonas* sp. contained considerably higher amounts of each amino acid analysed in this study than *S. obliquus* (Table 1). Supplementation of a *S. obliquus* food suspension with a mixture of ten presumably essential amino acids (25 μ M each) inhibited ephippia production of *D. pulex* and accordingly increased the production of subitaneous offspring (Tukey's HSD, $p < 0.05$; following ANOVA, $F_{2,9} = 39.5$ for ephippia production, $F_{2,9} = 32.8$ for subitaneous offspring, $p < 0.001$; Fig. 1).

Ephippia production declined exponentially ($y = e^{-0.58x}$, $F_{1,18} = 53.5$, $p < 0.0001$) when increasing amounts of the amino acid mixture were added to the diet. Ephippia production was close to zero at a concentration of 6.25 μ M of each amino acid (Fig. 2a). Both neonate production [Fig. 2a; $y = 22.4 + 60 \times [x/(x + 22.8)]$, $F_{3,17} = 22.3$, $p < 0.0001$] and population growth rates [Fig. 2b; $y = 0.23 + 0.11 \times [x/(x + 3.1)]$, $F_{3,17} = 27.0$, $p < 0.0001$] increased with increasing amino acid supplementation following a saturation function. Note that neonate production as well as population growth rates increased further after ephippia production had ceased—i.e. at an amino acid concentration of $>6.25 \mu$ M (simple regression models for neonates: $R^2 = 0.68$, $F_{2,12} = 21.33$,

$p < 0.001$; for population growth rate: $R^2 = 0.48$, $F_{2,12} = 9.28$, $p < 0.02$). A multiple regression model indicated that for the full range of amino acid supplementations, neonate production (no. ind $^{-1}$) and population growth rates r (day $^{-1}$) increased with declining ephippia production (no. ind $^{-1}$) and increasing amino acid supplementation (μ M AA $^{-1}$):

1. neonates = $31.2 - 11.2 \times \text{ephippia} + 0.9 \times \text{AA}$; ($R^2 = 0.82$, $F_{3,17} = 38$, $p < 0.0001$)
2. $r = 0.3 - 0.07 \times \text{ephippia} + 0.001 \times \text{AA}$; ($R^2 = 0.94$, $F_{3,17} = 149$, $p < 0.0001$).

Note that both ephippia production and amino acid supplementation contributed significantly to both models (all $p < 0.005$).

The experiments involving supplementation of a *S. obliquus* food suspension with a single amino acid at a concentration of 25 μ M indicated that arginine and histidine were particularly effective in reducing ephippia production and increasing neonate numbers (Fig. 3). Pairwise comparisons using one-way ANOVAs between the two amino acid treatments and *S. obliquus* without amino acid addition revealed significant differences (arginine: $F_{1,6} = 6.5$, $p < 0.05$ for ephippia production, $F_{1,6} = 7.6$, $p < 0.05$ for subitaneous neonate production; histidine: $F_{1,6} = 11.8$, $p < 0.05$ for ephippia production, $F_{1,6} = 15.9$, $p < 0.05$ for subitaneous neonate production).

Increasing supplementation with arginine and histidine (either separately or in combination) decreased ephippia production and accordingly increased neonate production and therewith population growth rate (Fig. 4). The ANCOVA with the supplemented concentration (25 or 50 μ M) as a covariate and the specific amino acid (arginine, histidine, or arginine and histidine) as a categorical factor revealed a significant influence of the supplemented amino acid concentration as well as significant differences between treatments (Table 2). However, no significant interaction effects (all $p > 0.1$) were detected for ephippia and neonate production or for population growth rate. Tukey's HSD suggested that histidine was less efficient in reducing ephippia production than arginine or a combination of arginine and histidine ($p < 0.05$). In contrast, the combined addition of arginine and histidine yielded a higher neonate production and higher population growth rates than the addition of arginine and histidine individually ($p < 0.05$).

Discussion

The results of our study demonstrate that specific dietary amino acids suppress resting egg production and

Fig. 1 Ephippia and neonate production of *Daphnia pulex* fed *Cryptomonas* sp. (Crypto), *Scenedesmus obliquus* (Scen), or *S. obliquus* supplemented with a mixture of ten amino acids (Scen + AA) in a concentration of 25 μ M AA $^{-1}$. Data are presented as the cumulative numbers of ephippia and neonates produced per individual within 16 days. Error bars Mean + standard error (SE). Significant differences are labeled with different letters based on Tukey's HSD at $p < 0.05$ following analysis of variance (ANOVA): A, B, C Ephippia production, A', B', C' neonate production

Table 1 Content of selected amino acids in *Cryptomonas* sp. and *Scenedesmus obliquus*

	Amino acids analysed ^a	<i>Cryptomonas</i> sp. ($\mu\text{mol mg C}^{-1}$)	<i>Scenedesmus obliquus</i> ($\mu\text{mol mg C}^{-1}$)
	Essential amino acids ^b		
Data are expressed as the mean value ($\mu\text{mol mg C}^{-1}$) $\pm$ standard deviation ($n = 3$) ^a The abbreviations of the amino acids are given in the "Materials and methods" ^b His, Arg, Thr, Met, Val, Phe, Ile and Leu were assumed to be essential and used in the supplementation experiments; lysine was not measured	His	0.574 $\pm$ 0.072	0.211 $\pm$ 0.021
	Arg/Cit	0.709 $\pm$ 0.087	0.252 $\pm$ 0.021
	Thr	0.711 $\pm$ 0.095	0.306 $\pm$ 0.028
	Met	0.641 $\pm$ 0.113	0.073 $\pm$ 0.005
	Val	1.078 $\pm$ 0.130	0.498 $\pm$ 0.026
	Phe	0.748 $\pm$ 0.084	0.366 $\pm$ 0.023
	Ile	0.932 $\pm$ 0.117	0.391 $\pm$ 0.016
	Leu	1.021 $\pm$ 0.116	0.489 $\pm$ 0.026
	Sum of essential amino acids	6.416 $\pm$ 0.815	2.585 $\pm$ 0.167
	Asp	0.973 $\pm$ 0.105	0.381 $\pm$ 0.037
	Glu	1.308 $\pm$ 0.173	0.552 $\pm$ 0.050
	Ser	0.485 $\pm$ 0.080	0.175 $\pm$ 0.016
	Gly	0.484 $\pm$ 0.089	0.200 $\pm$ 0.024
	Ala	0.723 $\pm$ 0.078	0.309 $\pm$ 0.025
	Tyr	0.788 $\pm$ 0.088	0.186 $\pm$ 0.009
	Sum of total amino acids	11.176 $\pm$ 1.374	4.388 $\pm$ 0.324

accordingly enhance the production of subitaneously developing offspring in crowded *D. pulex*, suggesting that specific dietary amino acids are involved in triggering the switch between reproductive modes in the freshwater keystone herbivore *Daphnia*.

In eukaryotes, amino acids are required for a multitude of different physiological processes, such as muscle generation, synthesis of enzymes and peptide hormones, precursors of neurotransmitters or vitellogenesis (e.g. Harrison 1990; Lafont 2000). In principle, it is assumed that some amino acids are essential across the animal kingdom, i.e. that vertebrates and arthropods presumably share the same requirements for dietary amino acids (Cowey and Forster 1971; Peters and De Bernardi 1987; Guisande et al. 1999). It has been shown that the availability of dietary amino acids significantly affects the reproduction of terrestrial insects, such as butterflies (Bauerfeind and Fischer 2005; Mevi-Schütz and Erhardt 2005) or the cyclical parthenogenetic aphids (e.g. Dixon et al. 1993; Simpson et al. 1995). It has also been suggested that dietary amino acids promote high fecundity in marine copepods (Kleppel et al. 1998; Guisande et al. 1999, 2000; Helland et al. 2003). As yet, however, the nutritional requirements of the freshwater herbivore *Daphnia* for single amino acids have not been studied.

Our supplementation experiments reveal that the amino acids arginine and histidine are of particular importance for *Daphnia*. In a decapod crustacean, arginine and histidine were the principal free amino acids detected during embryogenesis (Rosa et al. 2005) and, in a juvenile

marine shrimp species, a certain dietary arginine level was determined that supports optimal growth (Chen et al. 1992). Moreover, it has been reported that egg production in a marine copepod is significantly related to the arginine and histidine content of its diet (Kleppel et al. 1998). A potential arginine limitation of marine copepods was suggested by Anderson et al. (2004) based on comparisons between amino acid profiles of consumers (copepods) and their food (seston). Together with the finding that the free amino acid pool of *Daphnia magna* is dominated by arginine, histidine, alanine and glutamic acid (Gardener and Miller 1981), these examples imply that arginine and histidine are important nutrients which play significant roles in the metabolism of crustaceans. A high metabolic requirement for arginine and histidine in crustaceans would also explain why the low arginine and histidine content of *S. obliquus* influenced the reproductive mode and the neonate production of *Daphnia* in our experiments. However, additional amino acids also seem to be important, at least when supplied in combination with arginine and histidine. For example, the threshold concentration of arginine and histidine for suppressing ephippia production was 6.25 μM when supplied in combination with eight other amino acids (Fig. 2), but 25 μM were not sufficient to completely stop ephippia production when arginine and histidine were supplied individually (Fig. 4). These results suggest that dietary amino acids provoke complex synergistic effects on *Daphnia* reproduction and may support the assumption that these amino acids are essential and not substitutable,

Fig. 2 Cumulative numbers (mean $\pm$ SE) of ephippia and neonates produced per individual (a) and population growth rates (r) (b) within 16 days of *D. pulex* being fed *S. obliquus* and supplemented with increasing amounts of an amino acid mixture containing ten different amino acids, leading to concentrations of 0–25 $\mu\text{M AA}^{-1}$

as supplementation of only one amino acid may lead to a deficiency in a different essential amino acid.

In our experiments two scenarios of amino acid up-take by daphnids can be considered: (1) directly from the surrounding water, or (2) via the ingestion of food algae that may have concentrated amino acids at their surface by adsorption or in their cells by the direct uptake of dissolved amino acids. A direct uptake of the amino acid leucine by *S. obliquus* was described by Kamjunke and Tittel (2008). To the contrary, it has been demonstrated that nauplii of *Artemia franciscana* are able to take up dissolved free amino acids directly from the surrounding water, even though the uptake of particle-bound amino acids has been shown to be more efficient (Tonheim et al. 2000). This latter result is in accordance with our data which show that amino acids associated with *Cryptomonas* sp. were much more efficient in reducing ephippia production than dissolved free amino acids supplemented to the water.

During a single molt cycle daphnids can produce either one ephippium or one clutch of several neonates. Furthermore, the production of an ephippium delays the

Fig. 3 Cumulative numbers of ephippia (a) and neonates produced per individual (b) within 16 days of *D. pulex* being fed *Cryptomonas* sp. (Crypto), *S. obliquus* (Scen), or *S. obliquus* supplemented with ten different amino acids (concentration 25 $\mu\text{M AA}^{-1}$; see "Materials and methods" for AA abbreviations). Error bars Mean $\pm$ SE. Arrows indicate the most effective amino acids, i.e. minimum ephippia production and maximum neonate production

production of subsequent clutches. The costs of producing a single ephippium have been estimated by Lynch (1983) to be equivalent to the cost of producing nine neonates; these calculations were based on dry mass differences between neonates and one ephippium of *D. pulex*. Our data from the regression analysis suggest a cost of 11.2 ± 3.2 neonates, respectively, a growth rate reduction of $0.07 \pm 0.006 \text{ day}^{-1}$ per ephippium produced. Consequently, the reduction of ephippia production due to amino acid supplementation resulted in a substantial enhancement of neonate production and the population growth rate of *D. pulex*. However, neonate production and population growth rate (r) further increased after ephippia production had ceased, suggesting that food quality, i.e. the dietary amino acid content, affects the production of offspring in a manner independent of the switch between reproductive modes. Following the switch between reproductive modes, population growth rates and neonate production further increased by 8 and 60%, respectively, when amino acid supplementation was enhanced from 6.25 to 25 $\mu\text{M AA}^{-1}$. The effect of amino acid supplementation on neonate

Fig. 4 Cumulative numbers (means $\pm$ SE) of ephippia (a), neonate production produced per individual (b) and population growth rates (r) (c) within 16 days of *D. pulex* being fed *Cryptomonas* sp. (Crypto), or *S. obliquus* (Scen) supplemented with 1, 25 and 50 $\mu\text{M AA}^{-1}$, respectively, of arginine (arg) and histidine (his) separately or combined

production was more pronounced than that on population growth rates because neonate numbers showed the largest increase in the third clutch, which affects the calculation of population growth rates using the Euler–Lotka equation less than earlier clutches.

It has been reported that the comparatively poor food quality of *S. obliquus* for *Daphnia* spp. is due to a deficiency in essential long chain PUFAs (Weers and Gulati 1997; Von Elert 2002; Brzeziński and Von Elert 2007). Taking Liebig's law of the minimum into consideration, this explanation appears to be incompatible with our data, which imply that the performance of daphnids feeding on *S. obliquus* is constrained by a deficiency in specific amino acids. Recently, however, it has been shown that the

growth and reproduction of the herbivore *Daphnia* can be limited by more than one nutrient simultaneously (Martin-Creuzburg et al. 2009), which suggests a colimitation of *Daphnia* spp. feeding on *S. obliquus* by specific fatty acids and amino acids.

In nature, a major factor determining the growth and reproduction of *Daphnia* is the composition of its food, which undergoes pronounced seasonal changes due to the succession of algal species and seasonally varying ratios of algal to detritus biomass (Sommer 1985). We used crowding conditions and high food quantities in our experimental study to enhance ephippia production. Thus, our experimental set-up represents a scenario with high *Daphnia* abundance and high food availability, which occurs in nature at least on a seasonal basis. In Lake Constance, the start of ephippia production in early summer is just after the peak of the spring algae bloom (Jankowski and Straile 2004), which is a time characterised by strong changes in phytoplankton composition (Sommer 1985) and, consequently, in food quality for *Daphnia* (Wacker and Von Elert 2001); however, food concentrations are still high, and there is, therefore, an energy supply for resting egg production. Abrusán et al. (2007) reported that, at strongly limiting food concentrations, resting egg production in *D. pulicaria* is potentially constrained by a dietary deficiency in essential PUFAs, which suggests that food quality, in terms of PUFAs, in conjunction with environmental stimuli indicating deteriorating growth conditions affect the induction of resting egg production. In contrast, we show here that the presence of single dietary amino acids can suppress environmentally induced ephippia production at high food concentrations. Thus, the start of ephippia production in *Daphnia* and the number of mothers switching to resting egg production in nature are apparently a result of a complex interplay between inducing and suppressing environmental cues. Further studies are required to assess the relative significance of single cues in triggering the switch between reproductive modes under field conditions.

In conclusion, our results demonstrate that the growth and reproduction of *D. pulex* is significantly affected by the availability of dietary amino acids, particularly by arginine and histidine. We show that these amino acids are involved in triggering the switch between reproductive modes, i.e. they suppress resting egg production in crowded *Daphnia* and accordingly enhance parthenogenetic fecundity. The effect of amino acids on ephippia production differs qualitatively from that of fatty acids as the latter do not affect the switch between reproductive modes, but they are an important resource for the production of resting eggs (Abrusán et al. 2007; Koch et al. 2009). In our experiments, we used an obligate parthenogenetic *D. pulex* clone. Obligate parthenogenetic *Daphnia* clones are common in

Table 2 Results of the analysis of covariance (ANCOVA) with the supplemented concentration (25 or 50 µM) of amino acid as a covariate and the specific amino acid (arginine, histidine, or arginine and histidine) as a categorical factor

Covariate/categorical factor	Model	Significance
Ephippia production	(Model $R^2 = 0.61$, $p < 0.001$)	
AA concentration	$F_{1,20} = 10.43$	$p = 0.004$
AA treatment	$F_{2,20} = 10.78$	$p < 0.001$
Neonate production	(Model $R^2 = 0.78$, $p < 0.001$)	
AA concentration	$F_{1,20} = 12.78$	$p = 0.002$
AA treatment	$F_{2,20} = 29.45$	$p < 0.001$
Population growth rate	(Model $R^2 = 0.75$, $p < 0.001$)	
AA concentration	$F_{1,20} = 19.80$	$p < 0.001$
AA treatment	$F_{2,20} = 20.04$	$p < 0.001$

AA, Amino acid

No significant interaction effects (all $p > 0.1$) were detected for ephippia and neonate production or for population growth rate

natural systems and often coexist with sexual clones of the same species (Innes and Singleton 2000; Innes et al. 2000; Omilian et al. 2006). In an earlier study, we demonstrated that protein supplementation can suppress ephippia production in the asexual *D. pulex* clone used here, as well as in a sexual *D. galeata* clone (Koch et al. 2009), suggesting that amino acids are also involved in triggering the switch between reproductive modes in sexual *Daphnia* clones. However, further studies are necessary to determine potential clone- and/or species-specific differences in resting egg production in response to dietary amino acid availability.

Hence, in addition to minerals (Sterner and Elser 2002), PUFAs (Müller-Navarra et al. 2000; Wacker and Von Elert 2001; Von Elert 2002) and sterols (Von Elert et al. 2003; Martin-Creuzburg et al. 2005, 2009), dietary amino acids have to be considered as potentially limiting resources which constrain the performance of *Daphnia*. Our data imply that the availability of single dietary amino acids may affect seasonal dynamics, long-term persistence, dispersal in and among ecosystems and, consequently, the genetic diversity of *Daphnia* populations in the field.

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