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A study on the effects of food quantity and quality on glutathione S-transferase (GST) activity and growth rate parameters of *Daphnia carinata* varying in age

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Abstract The adverse influences of insufficient food and toxins on *Daphnia carinata*'s body growth, reproduction and tolerance were investigated in the laboratory. Different concentrations of *Scendesmus obliquus* and a mixture of *S. obliquus* and microcystin (MC)-containing *Microcystis aeruginosa* PCC7820 were used to feed *D. carinata*. Glutathione S-transferase (GST) activity towards five chemical compounds (substrates) was measured and used as an indicator of their tolerance. Body growth rate and clutch size of *D. carinata* decreased with declined concentration or decreased proportion of *S. obliquus* in the diet. GST activity decreased with ageing in *D. carinata*. However, GST activity to several chemical compounds increased when food quantity or food quality decreased. Adult *D. carinata* had a lower GST activity towards *p*-nitrophenenzyl chloride (PNBC) than juveniles and exhibited a sharp decline ($P < 0.001$) in GST activity towards PNBC as the animals aged. It is suggested that the age-specific decrease in GST activity is a possible mechanism for the high morality of adult *D. carinata* in the summer of eutrophic lakes.

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Introduction

Size- or age-dependent mortality rates of cladocerans are important not only in relation to predation, but also in relation to food conditions (Vijverberg 1976; Hovenkamp 1989; Trabeau et al. 2004). In eutrophic lakes, the occurrence of relatively less green algae and blooms of *Cyanobacteria* would result in unfavourable food conditions that invariably result in a high mortality in *Daphnia* (Hülsmann and Weiler 2000). Juvenile daphnids are known to be particularly vulnerable to a decrease in food conditions (Hovenkamp 1989), whereas mortality of adult daphnids in the life-table experiments is negligible (Hovenkamp 1990). Adult mortality is known to increase due to interactions between age-specific and starvation-induced mortality (De Bernardi 1974; Hovenkamp 1989; Boersma et al. 1996). Age-specific mortality in *Daphnia* as suggested by Hülsmann (2003) was either based on a fortnightly sampling interval and lack of demographic data. Hülsmann and Weiler (2000) reported that adult daphnids not only have a higher mortality than juveniles, but also induce the decline of the *Daphnia* population.

In eutrophic lakes and reservoirs with cyanobacterial blooms, *Cyanobacteria* produce toxins such as microcystin (MC) and exert toxic effects on many animals. Summer-blooms of toxic *Cyanobacteria* contribute to poor food conditions to daphnids (Ferrão-Filho and Azevedo 2003). MC seems to be an effective factor for tolerance against toxins of *Daphnia* because it works even if the biomass of the MC-containing *Microcystis* is only a small fraction of the whole algal community (Ferrão-Filho et al. 2000).

In several previous studies, glutathione S-transferase (GST) was called an indicator of tolerance against toxins in many animals (Habig et al. 1974; Sagara and Sugita 2001; Sen and Semiz 2007). Microcystin concentrations in animal tissue decreased because of its high GST activity, therefore, its high capacity to biotransform MCs (Pflugmacher et al. 1998, 2005). Previous studies reported increase in GST activity in many animals exposed to microcystins for a short time (Wiegand et al. 1999; Best et al. 2002; Pinho et al. 2003), suggesting their substantial roles in the toxicity reduction of some toxins, as indicated by increase of LD_{50}s and decline of mortality in various insects (Hayaoka and Dauterman 1982; Vontas et al. 2001). Chen et al. (2005) reported that GST activity (to CDNB) of *Daphnia magna* decreased when it was exposed to microcystins for several weeks. The decline in GST activity (to CDNB) of *D. magna* seems to contradict the mechanism of detoxification (Pflugmacher et al. 1998; Chen et al. 2005). An investigation into changes in GST activity to different chemical compounds in *D. carinata* would explain the contradiction. Otherwise, identification of a relationship between GST activity and different chemical substrates is needed to provide a preliminary indication of a direct protective role of GST among organisms exposed to different toxic levels of MCs (Leblanc and Cochrane 1985; Sagara and Sugita 2001). The GST activity levels towards 1-chloro-2, 4-nitrobenzene (CDNB) were comparable in *Daphnia magna* and *Ceriodaphnia reticulata* for estimating their tolerance against toxins (Leblanc and Cochrane 1985; Chen et al. 2005). Seven GST isoenzymes were isolated from *Daphnia magna* (Leblanc et al. 1988; Baldwin and Leblanc 1996), which exhibited nearly twofold higher activity with ethacrynic acid (EA) than in *Ceriodaphnia* (Leblanc and Cochrane 1985).

The decrease in nutrient-sufficient algae (such as *S. obliquus*) and the increase in nutrient-poor food algae (such as *M. aeruginosa*) often occur when *Daphnia* populations declines in eutrophic lakes and reservoirs (Ferrão-Filho and Azevedo 2003). In our studies, such food conditions were designed to culture *Daphnia carinata* collected from a eutrophic lake, and daphnids were divided into adults and juveniles. We measured GST activity of the animals exposed to different chemical compounds to evaluate their tolerance to adverse effects from insufficient food and toxins and addressed the following two questions: (1) do adult daphnids have a lower GST activity than juveniles? and (2) what are the effects of food quantity and quality on individual life history parameters such as body growth, reproduction, and tolerance to adverse effects from insufficient food and toxins?

Methods

Study site

Lake Chaohu is located in Anhui Province in the southeastern China, is among the five largest freshwater lakes in China. It is a subtropical lake with a surface area of 760 km^2, a mean depth of 3.06 m, and a mean water retention time of 136 days. During the past decades, the lake has witnessed a steady increase in eutrophication, as evident from a regular occurrence of cyanobacterial surface blooms (mainly composed of *Microcystis* spp. and *Anabaena* sp.) during the warmer periods of each year (Deng 2004). The toxicity of *Microsystis aeruginosa* in Chaohu was confirmed for the first time by Carmichael et al. (1988). During the spring clear water phase (from February to May) in lake Chaohu, the density of Chlorophyta was about 2.2×10^4 cells/ml and the density of *Cyanobacteria* ranged from 7.4×10^3 cells/ml to 2.6×10^4 cells/ml (Zhao et al. 2002), the *D. carinata* density in Chaohu was varied between 1.2 and 42.5 ind/L (Deng 2004). There was a significantly positive correlation between the density of small-bodied cladocerans (such as *Bosmina* and *Ceriodaphnia cornuta*) and the biomass of *Microcystis* and *Anabaena,* while large-bodied cladocerans (including *D. pulex, D. hyaline* and *D. carinata*) declined or even disappeared during cyanobacterial blooms. There was a significant negative relationship between the densities of large-bodied cladocerans and

colonial *Cyanobacteria.* It was also experimentally confirmed that *Microcystis* inhibited the growth and reproduction of *D. pulex* and *D. carinata* (Deng 2004).

Daphnia and algae

Daphnia carinata was collected from Lake Chaohu, and one of the clones was cultured for more than a year in the laboratory prior to the experiments and fed with *Scenedesmus obliquus* once every two days. Aerated tap water was used as medium. Eight experimental aquaria (68 × 52 cm each) were used, and 100-L medium was added to each aquarium. All experiments were carried out at 25 ± 1°C and maintained at about 30 μmol photons m^{-2} s^{-1} of 14:10 h light/dark cycle. *Microcystis aeruginosa* PCC7820 was cultured in the medium BG11, and *S. obliquus* was cultured in the medium HB-6. The concentration of algae was counted in the laboratory using a compound microscope (at a magnification of 100×).

The microcystins from *M. aeruginosa* PCC7820 were extracted and analysed using high-performance liquid chromatography (HPLC) following Zheng et al. (2004). The per unit biovolume of microcystin-LR was 1.34×10^{-10} μg/μm^3.

Substrates and determination of GST activity

Sixty individuals of *D. carinata* (20–800 μg soluble protein fractions) were homogenized in 0.1 M sodium phosphate buffer, pH 7.0 and centrifuged for 10 min at 5,000 g, and then the supernatant was centrifuged for 10 min at 10,000 g. Absorbance at different wavelengths was determined with a spectrometer (Habig and Jakoby 1981).

The six chemical compounds used in our experiments for GST activity analysis were 1-chloro-2,4-nitrobenzene (CDNB), 1,2-dichloro-4-nitrobenzene (DCNB) (No activity), *p*-Nitrophenethyl bromide (PNBB), *p*-Nitrophenenzyl chloride (PNBC), trans-4-Phenyl-3-buten-2-one (Trans), and ethacrynic acid (EA) (Habig and Jakoby 1981). GST activities were assayed spectrophotometrically by measuring the rate of glutathione-chemical compound conjugate formation (Habdous et al. 2002). Assays with CDNB were performed at a concentration of 1.0 mM and a glutathione concentration of 1.0 mM. Assays with PNBB were performed at a concentration of 0.1 mM and a glutathione concentration of 5.0 mM. Assays with PNBC were performed at a concentration of 1.0 mM and a glutathione concentration of 5.0 mM. Assays with EA were performed at a concentration of 0.2 mM and a glutathione concentration of 0.25 mM. Assays with Trans were performed at a concentration of 0.05 mM and a glutathione concentration of 0.25 mM. All assays were conducted at pH of 6.5. Protein concentrations in the homogenates were determined according to Bradford (1976) with bovine serum albumin as a standard. GST-specific activity was quantified as nano moles of the compound conjugated per milligram of soluble protein (nmoles/min/mg) at 25°C.

The body length (L) of 6- and 8-day-old *D. carinata* carrying eggs was measured, and the biomass of *D. carinata* was derived from L to W (–weight) regression equations of Huang (1999) and the mean daily growth rate (GWR) and decrease rate of GST activity (GDR) were calculated as:

$$\mathrm{GWR} = \left(W_f - W_i\right)/\Delta_t(\mu\mathrm{g/day})$$
$$\mathrm{GDR} = \left(G_f - G_i\right)/\Delta_t(\mathrm{nmoles/min/mg/day})$$

where GWR is the mean daily growth rate (gw, μg/day), W_f and W_i are the final and initial weight, respectively. GDR is the decrease rate of GST activity, G_f and G_i are the final and initial GST activity, respectively. Δ_t is time for the culture.

Experiments using different food quantities

The neonates of *D. carinata* (<24-h old) were used in our experiments at *S. obliquus* densities ranging from 8 to 10 ind/l. The food was refreshed every 2 days, keeping the concentrations of *S. obliquus* as: 1000, 2000, 4000, 8000, and 20,000 cells/ml (1000 cells/ml = 9.74×10^5 μm^3/mL, equivalent to ~0.05 mg C L^{-1}; 20,000 cells/ml, equivalent to ~1 mg C L^{-1}). The number of eggs in the brood chamber was counted in sixty *D. carinata* every two days; for each adult female, the length from top of the head to the end of the carapace was measured under a dissecting microscope. Thirty daphnids in duplicate ranging in size from 0.7 mm to 1.8 mm were stored at −80°C before analysis of GST activity. The individuals tested were chosen at random from the conditioned population in cultures. Eight-day growth experiments then were run

using the same food combinations and concentrations, and the body growth rates were measured at two-day intervals.

Experiments using different food quality

This experiment was designed similarly to the food quantity experiments; however, the foods were mixtures of *M. aeruginosa* PCC7820 and *S. obliquus*. The mixtures included 0, 20, 40, and 60% *M. aeruginosa* with a total algal concentration of 20,000 cells/ml in each treatment. Body length and eggs per brood were quantified as above, and the GST activity was analysed at 2-d intervals. The influences of food and interactions on *D. carinata* were assessed with a repeated-measures ANOVA.

Results

Effects of food quantity and food quality on body growth rate

Body growth rate of *D. carinata* was clearly affected by the decrease in *S. obliquus* concentration and the presence of *Microcystis* in the diet (Fig. 1). The quantity and quality of food had a significant effect on body growth rate. When the concentration of *S. obliquus* increased, the body growth rate increased most rapidly ($F = 60.80$, $P < 0.001$), body growth rate decreased with increasing proportions of *M. aeruginosa* PCC7820 in the diet ($F = 108.11$, $P < 0.001$). These results suggest that MC-containing *M. aeruginosa* PCC7820 had a negative effect on the body growth of *D. carinata*.

Effects of food quantity and quality on clutch size

Clutch size that was measured as an estimate of reproductive capacity in *D. carinata* (Fig. 2) differed in concentrations of *S. obliquus* ($F = 1425.58$, $P < 0.001$), and the clutch size decreased significantly when food quality decreased ($F = 226.86$, $P < 0.001$). The average clutch size ranged from 0.20 to 9.50 eggs per adult female in *S. obliquus* concentrations varying from 1000 to 20,000 cells/ml. Thus, even though increased *S. obliquus* concentration had positive effects on reproductive capacity, the reproductive capacity of *D. carinata* decreased with increasing proportions of *M. aeruginosa* in the diet (Fig. 2).

Effects of food quantity and quality on GST activity

A repeated-measure ANOVA over all food concentrations, from 1000 cells/ml to 20,000 cells/ml *S. obliquus* concentration, indicated different age effects on the GST activity towards CDNB, PNBB, PNBC, EA, and Trans. The GST activity towards the five compounds decreased as the *D. carinata*'s age increased when *D. carinata* was fed with 20000 cells/ml ($F = 18.57$, $P < 0.001$), 8000 ($F = 35.85$, $P < 0.001$), 4000 cells/ml ($F = 46.23$, $P < 0.001$), and 2000 cells/ml ($F = 90.00$, $P < 0.001$) of *S. obliquus* concentrations. Only in the 1000 cells/ml *S. obliquus* treatment, did the GST activity towards these compounds except PNBC not decrease as the animal's age increased ($F = 1.69$, $P > 0.05$; Fig. 3).

Repeated-measure ANOVA on GST activity for CDNB, PNBB, PNBC, EA, and Trans in 0% to 60% proportions of *Microcystis* treatments indicated a

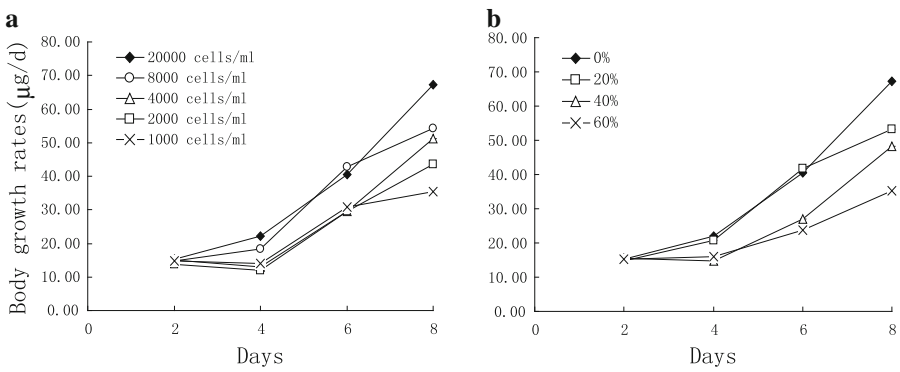

Fig. 1 The body growth rate of *Daphnia carinata* in different concentrations of *Scenedesmus obliquus* (**a**) and different proportions of MC-containing *Microcystis aeruginosa* PCC7820 in the diet (**b**). *Vertical bars* show standard deviations ($n = 60$)

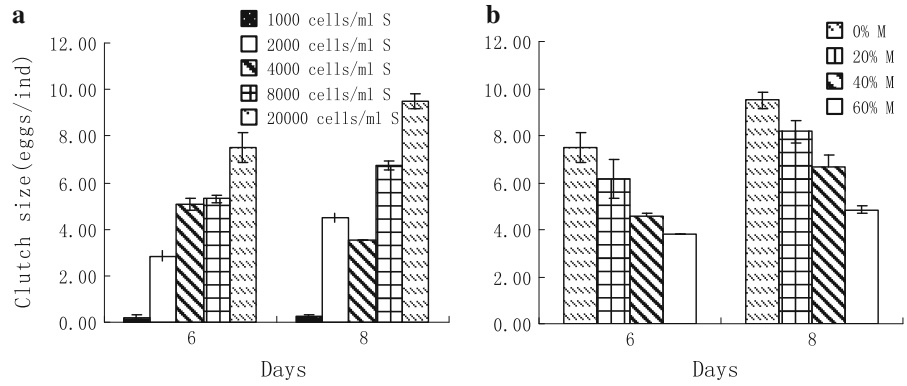

Fig. 2 The clutch size of *Daphnia carinata* in different concentrations of *Scenedesmus obliquus* (**a**) and different proportions of MC-containing *Microcystis aeruginosa* PCC7820 in the diet (**b**). *Vertical bars* show standard deviations

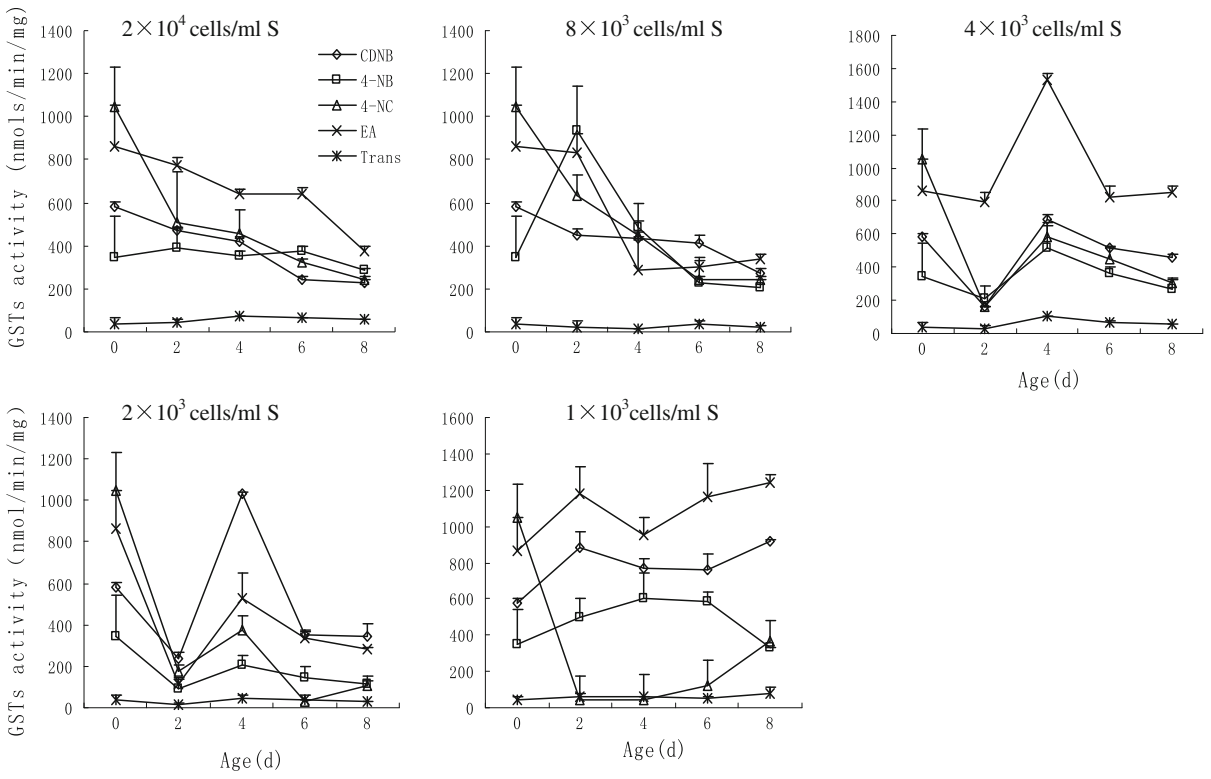

Fig. 3 Glutathione S-transferase (GST) activity in *Daphnia carinata* towards five substrates in different concentrations of *Scenedesmus obliquus* (cells/ml S). *Vertical bars* show standard deviations. Substrates were *CDNB* 1-chloro-2,4-nitrobenzene, *DCNB* 1,2-dichloro-4-nitrobenzene, *PNBB* *p*-Nitrophenethyl bromide, *PNBC* *p*-Nitrophenenzyl chloride, *Trans* trans-4-Phenyl-3-buten-2-one, and *EA* ethacrynic acid

significant age effect on the GST activity. The GST activity towards five chemical substrates decreased significantly as *D. carinata*'s age increased during the absence of *Microcystis* in the diet ($F = 18.57$, $P < 0.001$). GST activity in case of exposure to the five compounds significantly decreased (Age > day 4) when *D. carinata* was fed 20% *Microcystis* ($F = 25.69$, $P < 0.001$), 40% *Microcystis* ($F = 50.26$, $P < 0.001$) and 60% *Microcystis* ($F = 50.26$, $P < 0.001$) as *D. carinata*'s age increased (Fig. 4).

Comparison in adult and juvenile daphnids of GST activities for PNBC and CDNB

The age had an insignificant effect on the activity of GST for CDNB in *D. carinata* fed different

concentrations of *S. obliquus* ($F = 5.39$, $P > 0.05$). Daphnids (day 0) were recognized as an indicator of juveniles, and daphnids (day 8) were recognized as an indicator of adults. GST activity in case of the juveniles exposed to CDNB (day 0, 579.30 ± 24.46 nmoles/min/mg) was much higher than in adults (day 8, 230.18 ± 15.47 nmoles/min/mg) in 20,000 cells/ml *S. obliquus* treatment. However, in treatment involving 1000 cells/ml, juveniles (day 0, 579.30 ± 24.46 nmoles/min/mg) were not higher than adults (day 8, 915.58 ± 14.71 nmoles/min/mg). The age of *D. carinata* exhibited a significant effect on the activity of GST towards CDNB in different proportions of *M. aeruginosa* treatment ($F = 88.05$, $P < 0.001$). The activity of GST towards CDNB in adult daphnids was lower than in juveniles in 40% *Microcystis* treatment (T-test, $t = 4.84$, $P < 0.05$). For example, the GST activity in case of CDNB of the adults (466.45 ± 54.84 nmoles/min/mg) in 60% *M, aeruginosa* treatment was higher than for adults (230.18 ± 15.47 nmoles/min/mg) in 0% *M. aeruginosa* treatment, but much lower than GST activity (579.30 ± 24.46 nmoles/min/mg) for juveniles exposed to CDNB (Fig. 5).

The GST activity in case of exposure to PNBC decreased markedly as age increased; it was regardless of different food concentrations ($F = 39.49$, $P < 0.001$) or food quality ($F = 33.55$, $P < 0.001$). The activity of GST in PNBC-exposed adult daphnids (day 8) was significantly lower than those in the juveniles (day 0) exposed to this compound. The GST activity of *D. carinata* on days 0 and 8 for the ages differed: the GST activity of adult daphnids exposed to PNBC (day 8) was significantly lower than that in juveniles (day 0) when fed with different concentrations of *S. obliquus* ($t = 17.181$, $P < 0.001$); GST activity of the PNBC-exposed juveniles (1048.47 ± 185.35 nmoles/min/mg) was nearly 3 times higher than that of the adults (367.98. ± 111.52 nmoles/min/mg). Lower GST activity in the adults exposed to PNBC than in the juveniles also occurred in *D. carinata* in the animal fed *M. aeruginosa* ($t = 9.186$, $P < 0.001$), such as GST activity for juveniles (day 0) exposed to PNBC was 1048.47 ± 185.35 nmoles/min/mg, whereas in the adults (day 8), it was about 277.59 ± 15.39 nmoles/min/mg in 60% *Microcystis* treatment. The low GST activity towards PNBC may be related to a lower specific tolerance for

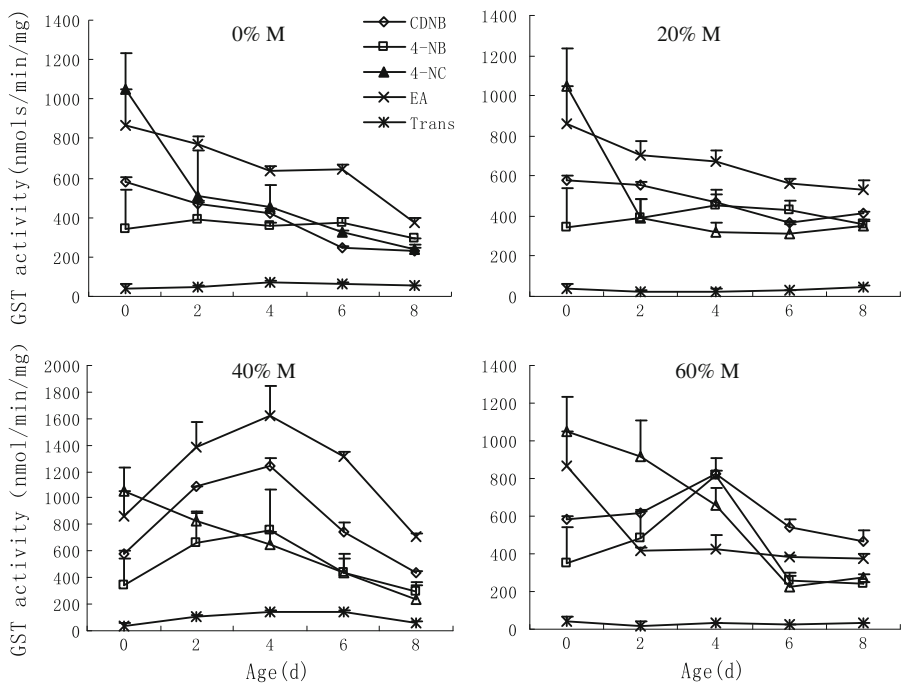

Fig. 4 Glutathione S-transferase (GST) activity towards five substrates of *Daphnia carinata* in different proportions of MC-containing *Microcystis aeruginosa* PCC7820 (%M) in the diets (20,000 cells/ml). *Vertical bars* show standard deviations. Substrate abbreviations as in Fig. 3

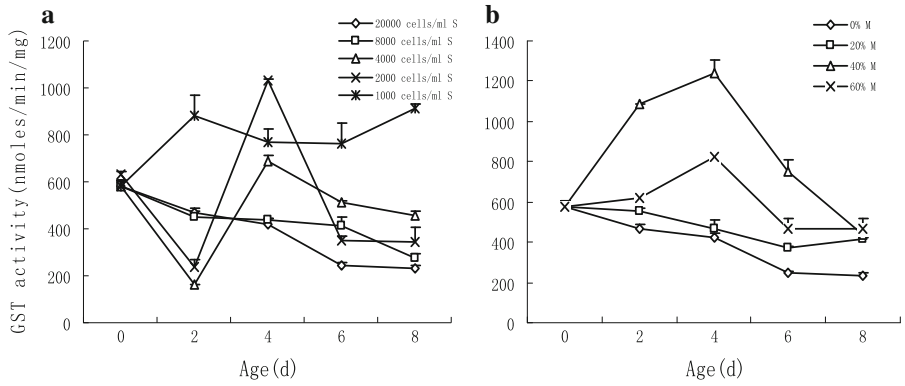

Fig. 5 Glutathione S-transferase (GST) activity towards 1-chloro-2,4-nitrobenzene (CDNB) of *Daphnia carinata* in different concentrations of *Scenedesmus obliquus* (**a**) and in different proportions of *Microcystis aeruginosa* PCC7820 in total cell concentrations of 20,000 cells/ml (**b**). *Vertical bars* show standard deviation

adverse effects from insufficient food and toxins of *D. carinata* (Fig. 6).

Growth rate decrease and changes in clutch size of daphnids exposed PNBC

A decrease in growth rate and changes of clutch size in animals exposed to PNBC are shown in Tables 1 and 2, respectively. In animals exposed to PNBC, both the mean daily growth rate (GWR, from 14.76 ± 0.56 μg/day to 50.99 ± 13.25 μg/day) and the clutch size (from 4.18 ± 2.77 eggs per female to 7.29 ± 2.01 eggs per female) increased, and GST activity (GDR, from 370.85 ± 36.43 nmoles/min/mg/day to 87.76 ± 19.12 nmoles/min/mg/day) decreased with an increased age of *D. carinata.* Different concentrations of *S. obliquus* as well as mixture of *S. obliquus* and MC-containing *M. aeruginosa* PCC7820 were used as food to feed *D. carinata*.

Discussion

In the present study, the mean number of eggs per female in the low *S. obliquus* concentration was significantly less than that in the high *S. obliquus* concentration (the effects of food quantity). Moreover, with an increasing proportion of the toxic alga in the diet, body growth rate and reproduction of *D. carinata* decreased (the effects of food quality). The low body growth and reproduction resulted in a low recruitment of *D. carinata* population and partly resulted in the decline *D. carinata* population (Benndorf et al. 2001). Both food quantity and

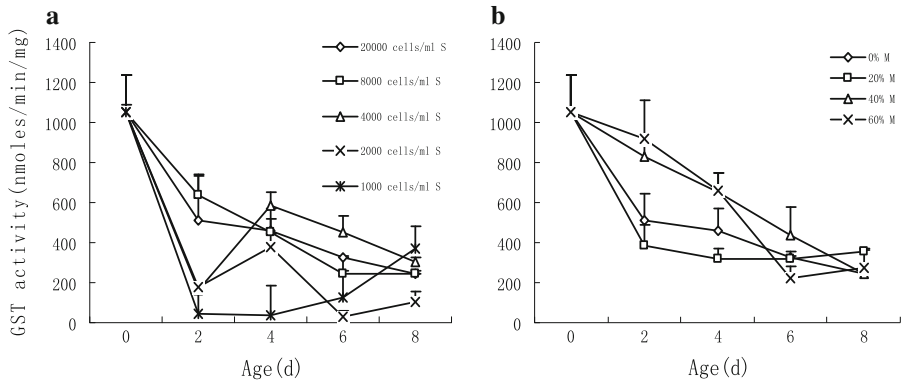

Fig. 6 Glutathione S-transferase (GST) activity towards *p*-Nitrophenenzyl chloride (PNBC) of *Daphnia carinata* in different concentrations of *Scenedesmus obliquus* (**a**) and with different proportions of *Microcystis aeruginosa* PCC7820 in total cell concentrations of 20,000 cells/ml (**b**). *Vertical bars* show standard deviations

Table 1 Mean daily growth rate (GWR), decrease rate of GST activity (GDR) towards PNBC and clutch size in *Daphnia carinata* over different concentrations of *Scenedesmus obliquus* in different physiological age

Age (day)	Mean daily growth rate (μg/day)	Decrease rate of GST activity towards PNBC (nmoles/min/mg/day)	Clutch size (eggs per female)
2	14.76 ± 0.56	370.85 ± 36.43	–
4	15.82 ± 4.25	203.66 ± 32.14	–
6	34.64 ± 6.48	111.12 ± 14.39	4.18 ± 2.77
8	50.39 ± 11.94	99.85 ± 10.54	4.89 ± 3.48

Table 2 Mean daily growth rate (GWR), decrease rate of GST activity (GDR) towards PNBC and clutch size in *Daphnia carinata* over different proportions of MC-containing *Microsystis aeruginosa* PCC7820 in different physiological age

Age (day)	Mean daily growth rate (μg/day)	Decrease rate of GST activity towards PNBC (nmoles/min/mg/day)	Clutch size (eggs per female)
2	15.18 ± 0.33	193.40 ± 43.04	–
4	18.36 ± 3.54	180.69 ± 17.53	–
6	33.22 ± 9.18	87.76 ± 19.12	5.52 ± 1.65
8	50.99 ± 13.25	96.26 ± 6.53	7.29 ± 2.01

quality have important effects on *Daphnia* populations (Lampert 1986; Sommer et al. 1986; Lürling 2003). Food quality for *Daphnia* is frequently poor in eutrophic lakes with cyanobacterial blooms (Trabeau et al. 2004). A drastic decline of differently sized *Daphnia* species was observed soon after food conditions became poor (Gliwicz et al. 1981). Other studies also indicate that decreases of food quantity and quality could result in a slow body growth rate and low fecundity in daphnids leading to a decline of *Daphnia* population (Vijverberg 1976; Lampert 1981; Lynch 1989; Lürling 2003), which is in agreement with our results.

Further, our experiments show that adult *D. carinata* often have a lower GST activity than juveniles as suggested by Chen et al. (2005) in *D. magna*. GST activity in adult *D. carinata* exposed to PNBC, regardless of changes in food quantity and quality, seems to remain lower than in juveniles, suggesting that adult daphnids have a lower age-specific tolerance. It seems to be more so if adults are exposed to insufficient food and toxins for longer durations. In previous studies, the timing between enhanced mortality in *Daphnia* populations due to senescence on one hand and predation on the other hand are both higher in adult daphnids (Vijverberg 1976; Hovenkamp 1990; Hülsmann and Weiler 2000). Especially, daphnids >1.2 mm have occasionally a higher mortality in some populations (Hülsmann and Weiler 2000). In the present study, demographic GST activity of *D. carinata* in a generational data reveal that the adult (day 8, >1.2 mm) *D. carinata* always have a lower GST activity for the PNBC-exposed animals. Hülsmann and Weiler (2000) suggest that the decline of *Daphnia* populations in lakes is attributable to relatively higher mortality of the adults, while such higher mortality seems not to be caused by changing temperature, food conditions, or predation by vertebrates or invertebrates. In previous studies, high mortality of animal possibly was attributed to the low GST activity in animal (Hayaoka and Dauterman 1982; Vontas et al. 2001). We observed that GST activity of *D. carinata* for the PNBC-exposed animals depends on the animal's age, *i.e.* a high adult mortality that then can trigger the initiation of the midsummer decline of *D. carinata* population in the field.

We examined the effects of food conditions on the life history of daphnids to test how the life history parameters change in response to interaction of a set of food factors. Daphnids usually have a slow growth rate and a low reproductive capacity as a defence mechanism if food conditions deteriorate (Threlkeld 1979; Lampert 1986; Lynch 1989; DeMott 1999; Reinikainen et al. 1999; Repka et al. 1999). The induction of GST activity on exposure to CDNB, PNNB, EA, and Trans in *D. carinata* is considered to be an effective defence mechanism (Wiegand et al. 1999; Best et al. 2002; Pinho et al. 2003; Gustafsson

and Hansson 2004; Pflugmacher et al. 2005), especially in treatment involving low food quantity (such as 1000 cells/ml *S. obliquus* concentration in the present study); GST towards several substrates in adults is much higher than juveniles. From the work of Vijverberg (1976), it seems that if daphnids are fed enough edible food, large numbers of small offspring are produced and these offspring have a lower tolerance to adverse effects of insufficient food and toxins. In contrast, Walls et al. (1997) demonstrate that in poor food conditions, large and highly tolerant offspring are produced. We find that low concentration of green algae has little effects on tolerance to adverse effects of insufficient food and toxins in *D. carinata*. Low food conditions appear to mainly result in severe food limitation, leading to both low body growth and reproduction and therefore to a low recruitment of *Daphnia* population.

A large amount of variation observed in zooplankton species is attributed as a response to differences in algal resources (Lampert 1981; Fulton and Paerl 1987b). Body growth, reproduction, and age-specific tolerance (GST activity towards a special substrate as a marker) always differ if energy strategies are compared in different cladoceran species (Fulton and Paerl 1987b). Small-bodied zooplankton, such as *Ceriodaphnia* and *Bosmina*, always exhibit more flexible responses to changing food conditions and stronger resistance to toxic *Cyanobacteria*, which cause great mortality in large-bodied zooplankton species such as *D. carinata* (Guo and Xie 2006). These small-bodied zooplankters may have high GST activity in animals exposed to PNBC, so that *Daphnia* spp. are replaced as dominant taxa when food conditions deteriorate in eutrophic lakes (DeMott and Kerfoot 1982; Ferrão-Filho and Azevedo 2003). This needs to be clarified in our future studies.

We designed our experimental conditions such that they are comparable with those prevailing in a eutrophic lake Chaohu during the spring clear water phase: food density of 20,000 algal cells/ml, and the mean *D. carinata* density of about 8–10 ind/l in Chaohu (Deng 2004). We note that a probable explanation for the decline or disappearance of large-bodied cladocerans (*D. carinata*) during cyanobacterial blooms is a decrease in tolerance to adverse effects from insufficient food and toxins in this animal. In the present study, CDNB EA and other substances were treated as basic substrates towards GST, though these compounds are not present in Lake Chaohu. Leblanc and Cochrane (1985) first found GST activity towards different substrates was biochemically separated into different protein fractions suggesting the existence of different distinct isozymes. Our results suggested at least five distinct isozymes existed in *D. carinata*, and GST activity towards PNBC really was first detected. Glutathione S-transferase activity towards PNBC is uninducible; the age-specific GST activity towards PNBC exhibited a lower tolerance in adult daphnids. All of our experiments were conducted in a large tank with 100-l medium. We did not consider some factors in our experiments, such as competition for food between zooplankton taxa (Fulton and Paerl 1987a), and the possible effects of filamentous and colony-forming *Cyanobacteria* (Lampert 1981; Ferrão-Filho and Azevedo 2003). The measurement of GST activity seems to provide a good indication of the level of tolerance in cladocerans, and it is likely that quantitative changes of GST activity include more physiological information than most field investigators can provide (LeBlanc et al. 1988; Baldwin and Leblanc 1996).

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