Ecol Res
DOI 10.1007/s11284-014-1240-4

ORIGINAL ARTICLE

Claudio A. Reyes · Rodrigo Ramos-Jiliberto
Javier González-Barrientos

Temporal variability of food determines the outcome of pesticide exposure in *Daphnia*

Received: 23 July 2014 / Accepted: 25 December 2014

Abstract In nature the effect that pollutants exert on exposed organisms could depend on the state and dynamics of natural environmental factors as well as on the internal state of the exposed organisms. In this study we evaluated how level and variability of food, as well as the age of exposure can modify the effects of the pesticide deltamethrin on the freshwater crustacean *Daphnia magna*. The effects of the pesticide were measured on life history and nutritional traits (lipid storage) of the test organisms, which were exposed at different juvenile ages under constant (high and low) food as well as food shortage treatments. Our results show that deltamethrin exerts significant effects on all evaluated traits of *Daphnia*, and several responses are shaped by deltamethrin interacting with food and age of exposure. Two novel results are remarkable. First, at constant food treatments the effects of the pesticide were stronger on younger individuals, whereas in food shortage treatments the effects were stronger on older individuals. Second, we observed that deltamethrin exerted stronger effects on certain traits (survival, body growth and median consumption time of lipids) in *Daphnia* exposed to food shortage, as compared to constant low food treatments. Our results highlight the importance of the dynamics of resources in freshwater systems for shaping the vulnerability of herbivores to pollutants released to the ecosystem and improve our understanding of how the organismal responses to environmental stress are determined by the ecological condition of the organisms at the instant of being exposed to perturbations.

Keywords Zooplankton · Pyrethroids · Deltamethrin · Age · Ecotoxicology · Lipids

Introduction

Aquatic ecosystems are recurrent recipients of pollutants released by human activities (Kreuger 1998; Voeltz et al. 2005). They also exhibit wide variations in natural physicochemical variables such as temperature or salinity (Aladin and Potts 1995; Reichwaldt et al. 2004), as well as in biological factors, such as food availability (McCauley et al. 1990) and predation (Fulford et al. 2006). The assessment of effects of stressors on aquatic biota are commonly carried out by means of controlled experiments in which the environment is kept nearly constant, exposing test organisms to a focal stressor during part of their life cycle. In nature, nevertheless, the effect that pollutants exert on exposed organisms could be dependent on the state and dynamics of natural environmental factors (Heugens et al. 2001; Jonker et al. 2004). Food availability is a critical ecological factor that often exhibits wide fluctuations over time and space in aquatic environments (Sommer et al. 2012). In addition, a finite budget of energy and nutrients gathered by organisms from food has to be allocated to different vital functions including development, reproduction, reparation and maintenance of body components. Thus, food limitation is expected to increase the organisms' sensitivity to chemical stressors through decreasing their energy budget available for physiological process leading to reparation and detoxification (Smolders et al. 2005). The nutritional status of an aquatic organism can significantly modify the toxicity of chemicals, such it has been reported for fishes and cladocerans (Antunes et al. 2003; Holmstrup et al. 2010). Furthermore, differences in maternal nutrition can give rise to divergences in offspring tolerance to stressors (Enserink et al. 1990;

Electronic supplementary material The online version of this article (doi:10.1007/s11284-014-1240-4) contains supplementary material, which is available to authorized users.

C. A. Reyes (✉) · R. Ramos-Jiliberto · J. González-Barrientos
Centro Nacional del Medio Ambiente, Fundación de La Universidad de Chile, Av. Larraín 9975 La Reina, Santiago, Chile
E-mail: doble.reyes@gmail.com
Tel.: +56-2-29275589

R. Ramos-Jiliberto
Instituto de Filosofía y Ciencias de la Complejidad, IFICC, Los Alerces 3024, Santiago, Chile

Pieters et al. 2006). On the other hand, the nature and strength of the effects that stressors exert on the exposed organisms are commonly dependent on their developmental stage. For example, several studies addressing the effects of radiation, metals and organic compounds have found an age-specific or stage-specific sensitivity of aquatic organisms (Ringwood 1990; Forget et al. 1998; Petersen and Kristensen 1998; Klein 2000; Schulz and Liess 2001; Medina et al. 2002; Ramos-Jiliberto et al. 2004; Bielmeyer et al. 2005; Hoang and Klaine 2007; Manyin and Rowe 2010). Consideration of multiple natural stressors is one step to improve the effects assessment especially of toxic compounds. Folt et al. (1999) highlight the potentially strong effects of temperature to zooplankton taxa and the degree of enhanced stress that is likely when episodes of low food or toxic exposure are combined.

Also, cumulative additive effects and non-additive interactions of natural antagonists as parasitism and predation have shown to interact with pollutants and exert considerable impacts on ecologically relevant parameters (Coors and De Meesters 2008). Natural variables are common experimental factors in ecological research, but the interplay between variation in natural factors and stressor exposure is still poorly understood. For a deeper understanding of the effects of stressors in natural aquatic ecosystems appears necessary to examine a wide set of interactions between the exposure to stressors, variations in environmental factors and the internal state of the exposed organisms. In this vein, in this study our aim is to assess whether the effects of a pesticide depend on the temporal variation of food availability and the age at which organisms are exposed. We hypothesize that irrespective of exposure age, we would expect stronger effects of deltamethrin on those individuals growing under constant low food densities, weaker effects under constant high food levels and effects of intermediate strength on individuals exposed to decreasing (from high to low) food levels.

We chose deltamethrin as a model stressor, since it is a widely used type II pyrethroid in the formulation of products against insect pests in agriculture and in the treatment of infections by ectoparasites in salmoniculture. Depending on its concentration this pyrethroid has been shown to exert lethal or sublethal effects on crustaceans (Xiu et al. 1989; Beketov 2004; Toumi et al. 2014). As response variables we considered a set of physiological and life history traits of the freshwater zooplankter *Daphnia magna*.

Methods

Experimental organisms and culture conditions

Strains of *D. magna* and *P. subcapitata* were obtained from cultures maintained at the Biodiversity Laboratory of the National Center for the Environment (CENMA), Santiago, Chile. From a stock culture of 15 adults L^{-1}, juveniles (<72 h) of a single *D. magna* clone were extracted and placed individually into 50 mL beakers filled with 40 mL of reconstituted hard water (ASTM 1980). This clone line has been maintained for many generations and it has been utilized periodically for ecotoxicity testing. Test organisms (neonates < 24 h) were obtained from the second clutch of the previously selected *Daphnia* individuals. The food was a suspension of the microalgae *Pseudokirchneriella subcapitata* at a density of 10^5 cell mL^{-1} and the medium for *D. magna,* including food, was renewed daily. Temperature was kept at 20 ± 1 °C, pH at 7.8 ± 0.1 and photoperiod 16:8 (L:D). In the experiments, test organisms were randomly allocated to the treatments.

Test chemical

A saturated stock solution of deltamethrin (CAS 52918-63-5, Merck) was prepared by adding 10 mg of the compound to 1 L of acetone, refrigerated at 5 ± 1 °C and kept in darkness. For each experiment it was prepared an aqueous solution of deltamethrin following Medina et al. (2002) by adding 500 µL of the stock solution into a glass bottle and then allowing the solvent to evaporate completely, leaving a crystalline residue of deltamethrin on the glass. Subsequently, the container was filled to 0.5 L with reconstituted hard water and mixed for 2 days at 10 °C on an orbital shaker. This procedure allows solubilizing the test chemical in water, eliminating acetone. Nevertheless, in a low dose acetone has been previously reported as a harmless substance for *D. magna* (LeBlanc and Surprenant 1983).

The deltamethrin concentration to be used in the main experiments was 5×10^{-3} µg L^{-1}. This level was calculated to be the one needed to kill 15 % of the test organisms (i.e., 24 h-LC_{15}). We choose this level because we wanted to perform our experiments with pesticide concentrations causing only subtle effect, and this is known to occur closely above the LC_{10} (Kast-Hutcheson et al. 2001). The toxicity test was performed modifying the OECD (2004) procedure, with five replicates for each of the test concentrations, each one containing four newborns in 40 mL of medium. The modification to the standard test consisted of feeding the individuals during the test with *P. subcapitata* at 10^6 cell mL^{-1}, in order to mimic conditions of the main experiments.

Experimental design

Main experiments were carried out at two food densities: 10^4 cell mL^{-1} (low food) and 10^6 cell mL^{-1} (high food) of *P. subcapitata*. These food densities were below and above the incipient limiting concentration of food for *D. magna* individuals, respectively (Lampert 1987). Two main experiments were performed: the first one with constant food treatments and the second one with food shortage treatments. Each experiment lasted for 7 days, the time needed for the first brood to be released. This period chosen for experimental observations is considered to be enough to capture the reproductive variability among treatments (see for example Guilhermino et al. 1999), and resembles many natural conditions, where *Daphnia* only survives short after its first reproduction due to size-selective predation by vertebrates (Pijanowska et al. 2006).

In both experiments, each treatment included five replicates with four *D. magna* neonates (<24 h) in a 50 mL beaker filled with 40 mL of medium that was renewed daily.

Each experiment included a pulsed (24 h) exposure to 5×10^{-3} μg L^{-1} of deltamethrin. Pulsed exposures of pesticides are commonly observed in the field, especially in aquatic systems due to high precipitation and runoff events (Liess et al. 1999). This is expected to be the rule particularly for deltamethrin, given its fast decay rates in water (Maguire et al. 1989).

In the first experiment (constant high and constant low food treatments), the test organisms were exposed to a single 24-hour pulse of deltamethrin at ages of either 0, 1 or 2 days. In addition, two control treatments were run without deltamethrin exposure, one in high food and one in low food. For the second experiment, a shift in food density from high to low density was exerted 24 h after the pulsed exposure to deltamethrin. Hereafter we refer to those treatments as "food shortage". Thus, the treatments exposed to deltamethrin at ages 0, 1 and 2 days were exposed to a decrease in food density at ages 1, 2 and 3 days, respectively. Control treatments were run, without deltamethrin exposure but with a food shortage at the time described above.

Response variables

We evaluated life-history and physiological traits of *D. magna* as response variables. For each replicate consisting in a jar with four test individuals, we measured and then analyzed mean values of the following life-history traits: the ability to survive for 7 days (hereafter seven-day survival), body length and fertility (clutch size). These variables were measured daily. In order to minimize manipulation of animals in the course of the experiments, body length measures (from the top of the head to the base of the tail spine) were made from digital images obtained with a digital camera attached to a microscope, keeping individuals at the same focal point. The precision of the length measurements was 10^{-4} mm. For each replicate, data of mean body length versus age (days) were fitted to the following sigmoid model by means of least-squares non-linear regression:

$$L(a) = \left(\frac{L_{max} - L_{min}}{1 + (a/t_h)^k} \right) + L_{min}$$

where $L(a)$ = body length at age a, L_{max} = asymptotic body length (i.e., projected body length at infinite age), L_{min} = initial body length, t_h = age at which the middle value between L_{min} and L_{max} is reached, and k = abruptness of the curve. Parameter k was not considered for further analyses, so we focused on the responses of L_{max} and t_h. We also evaluated physiological traits related to the storage of lipid reserves. Lipids (tryacylglycerol) are a mayor storage product of cladoceran (Goulden and Hornig 1980). These lipids are stored as droplets arranged laterally and ventrally to the gut. The measurement of the amount of stored lipids allows the assessment of the nutritional state of zooplankton (Bychek et al. 2005). These reserves were evaluated daily, and we took the average values over the four individuals in each replicate. Reserve measurements in each individual were performed through processing digital images with the software Biopix IQ 2.0 (Gothenburg, Sweden) to separate the colored lipid droplets stored around the gut. This method based on image analysis can be considered to be a modification of the well-known lipid index, based on the evaluation of visible lipid droplets in the body (Goulden and Hornig 1980). Even though our method only represents a rough approximation to measurements made by conventional analytical methods (Folch et al.1957), it has the advantage of allowing perform repeated measurements on a single individual and minimizes the manipulation time. The lipid amount was calculated as the total area of these droplets in the body of individuals. Figure S1 illustrates the image processing used for this measurement. From the assessed temporal change of lipid amount in the experimental individuals we determined the following traits: (a) cumulative lipid amount (hereafter "TL"), calculated as the area under the curve between days 0 and 5 (start of the experiment and the appearance of eggs in the brood chamber, respectively), (b) maximal lipid amount reached within the period indicated above (hereafter "MLA"), and (c) time employed by an individual to consume 50 % of its maximal lipid amount (hereafter "CT_{50}") calculated by linear regression using the MLA as 100 % of lipid amount stored. See figure S2 for a graphical explanation of these measurements.

Statistics

We used two-way MANOVA for analyzing our results. In the first experiment food and deltamethrin exposure were used as factors. In the second experiment, deltamethrin exposure and age at exposure were used as factors. Furthermore, to verify the univariate MANOVA results a two-way randomization ANOVA was performed in each experiment, running 1500 randomizations over the original values of the variables. Randomization ANOVA is robust against data departures from parametric assumptions and small sample sizes. The MANOVA analyses were performed using the R Project for Statistical Computing (http://www.r-project.org/). Randomization ANOVA was performed using the software RT 2.1 (Manly 1997).

Results

Constant food treatments

The MANOVA analysis showed that the overall effect of food (d.f. = 1, F = 2123.54, $p < 0.0001$), age at pesticide exposure (d.f. = 3, F = 6.34, $p < 0.0001$) and their interaction (d.f. = 3, F = 5.05, $p < 0.0001$) were significant on physiological and life history traits of *D. magna*.

Subsequent univariate analysis showed that food and deltamethrin exposure significantly affected the seven-day survival of *D. magna* (Table 1; Fig. 1a) when food density was kept constant. Randomized ANOVA rendered the same qualitative results than univariate components of MANOVA. Post hoc analysis among deltamethrin exposure treatments revealed that exposure at age 0 reduced significantly their seven-day survival as compared to exposure at older ages. In high food treatments, only exposure at age 0 differed from the control, while in low food treatments seven-day survival was lower than the control under all exposure treatments (Fig. 1a). The fertility (clutch size) of *D. magna* was affected by food, deltamethrin exposure and their interaction (Table 1). In high food treatments, deltamethrin affected clutch size only in individuals exposed at age 0, whereas in low food treatments offspring production was canceled by deltamethrin exposure (Fig. 1b). Body growth closely matched a sigmoid curve of body length versus age. The nonlinear regressions were all significant ($R^2 > 0.96$; $P < 0.05$ in all cases; Figs. S3, S4). For body length, parameter L_{max} was significantly affected by the interactive effect of food availability and deltamethrin exposure (Table 1). At both food treatments, exposure at ages 0 and 1 reduced L_{max}, being stronger when individuals were exposed earlier in their ontogenies and in high food treatments (Fig. 1c). Body growth rate parameter t_h, which indicates the time required to achieve the half of maximum growth, was affected additively by food and deltamethrin exposure (Table 1). Nevertheless, an increase in t_h was observed only when individuals were exposed at age 0 (Fig. 1d).

Total lipids (TL) and Maximal lipid Amount (MLA) were affected significantly by the interaction between food and deltamethrin exposure (Table 2). In high food treatments TL decreased significantly with deltamethrin exposure at age 0. Conversely, at age 2 a significant increase in TL respect to control was observed. In low food treatments deltamethrin exposure decreased TL with negligible differences among exposure ages (Fig. 1 e). Deltamethrin exposure decreased significantly MLA in high food treatments only at age 0. However, under low food treatments MLA was decreased at all exposure treatments (Fig. 1f). Finally, CT_{50} was affected additively by food level and deltamethrin exposure (Table 2), with stronger decreases of this variable when individuals were exposed at age 0 (Fig. 1g).

Table 1 Summary of univariate results from the 2-way multivariate analysis of variance (MANOVA) performed for testing the effects of age at deltamethrin exposure and food level on life history and physiological traits of *Daphnia magna* under constant (high and low) food treatments

Trait	Source	d.f.	F	*P*
Seven-day survival	Food level (F)	1	34.78	< 0.0001
	Age at exposure (A)	3	16.23	< 0.0001
	F × A	3	0.93	0.44
	Error	32		
Clutch size	Food level (F)	1	3464.30	< 0.0001
	Age at exposure (A)	3	85.67	< 0.0001
	F × A	3	55.12	< 0.0001
	Error	32		
Asymptotic body length (L_{max})	Food level (F)	1	3387.01	< 0.0001
	Age at exposure (A)	3	44.26	< 0.0001
	F × A	3	6.68	< 0.01
	Error	32		
Age to half asymptotic body length (t_h)	Food level (F)	1	625.16	< 0.0001
	Age at exposure (A)	3	25.14	< 0.0001
	F × A	3	0.86	0.47
	Error	32		
Total lipids (TL)	Food level (F)	1	4702.58	< 0.0001
	Age at exposure (A)	3	43.60	< 0.0001
	F × A	3	19.79	< 0.0001
	Error	32		
Maximal lipid amount(MLA)	Food level (F)	1	1338.02	< 0.0001
	Age at exposure (A)	3	27.75	< 0.0001
	F × A	3	6.14	< 0.001
	Error	32		
Consumption time of lipids (CT_{50})	Food level (F)	1	132.71	< 0.0001
	Age exposure (A)	3	82.91	< 0.0001
	F × A	3	2.55	0.07
	Error	32		

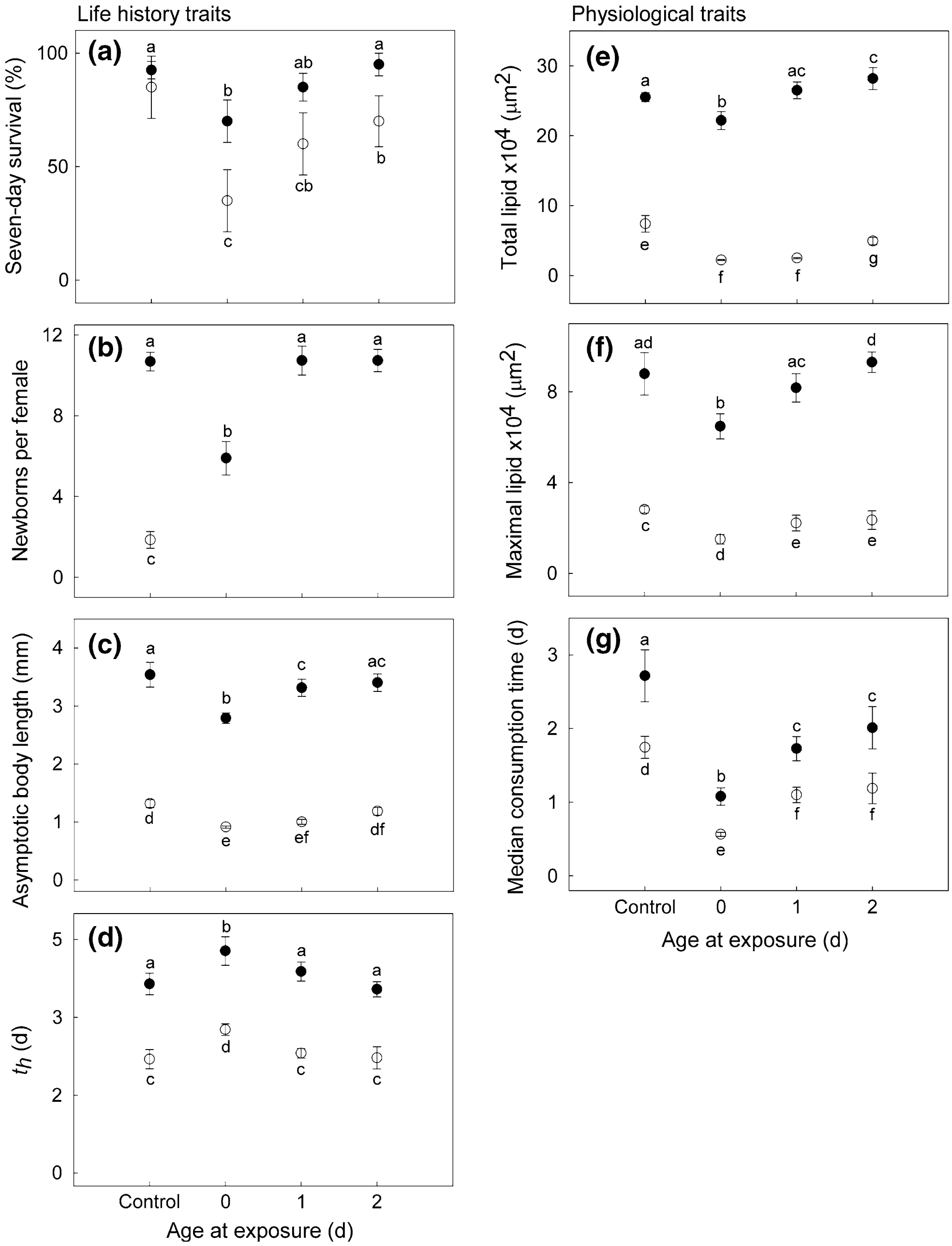

Fig. 1 Effects of deltamethrin exposure at different ages under constant food treatments, on life history (*left panel*) and physiological (*right panel*) traits of *Daphnia magna*. Life history traits are: **a** seven-day survival, **b** clutch size, **c** asymptotic body length ($L_{\max}$) and **d** age to half asymptotic body length (t_h). Physiological traits are: **e** total lipid amount (TL), **f** maximal lipid amount (MLA) and **g** median consumption time (CT_{50}). Each symbol in low food (*white circles*) and high food (*black circles*) treatments represent mean values ± SD. *Different letters* above symbols indicate significant differences among treatments (post hoc test, $P < 0.01$)

Food shortage treatments

The MANOVA analysis showed that the overall effect of deltamethrin exposure (d.f. = 1, F = 97.04, $p < 0.0001$), age of disturbance exposure (d.f. = 2, F = 29.49, $p < 0.0001$) and their interaction (d.f. = 2, F = 3.43, $p < 0.002$) in food shortage treatments were significant on physiological and life history traits of *D. magna*. The univariate analysis showed that deltamethrin exposure and age at disturbance exposure (either shortage in food density or shortage in food density followed by exposure to deltamethrin) additively affected seven-day survival (Table 2). Deltamethrin decreased seven-day survival at all ages of exposure with stronger effects on younger individuals (Fig. 2a). Clutch size was affected interactively by deltamethrin exposure and age at disturbance exposure (Table 2). Deltamethrin decreased the clutch size in older exposed individuals (of age 2). Individuals disturbed at earlier ages (age 0 and 1 under deltamethrin exposure and age 0 without delta-

Table 2 Summary of univariate results from the 2-way multivariate analysis of variance (MANOVA) performed for testing the effect of deltamethrin exposure and age at disturbance exposure on life history and physiological traits of *Daphnia magna* under food shortage treatments

Trait	Source	d.f.	F	*P*
Seven-day survival	Deltamethrin (D)	1	53.33	< 0.0001
	Age at exposure (A)	2	10.53	< 0.0001
	D × A	2	1.73	0.20
	Error	24		
Clutch size	Deltamethrin (D)	1	183.56	< 0.0001
	Age at exposure (A)	2	289.62	< 0.0001
	D × A	2	86.68	< 0.0001
	Error	24		
Asymptotic body length (L_{max})	Deltamethrin (D)	1	47.38	< 0.0001
	Age at exposure (A)	2	258.76	< 0.0001
	D × A	2	7.34	< 0.005
	Error	24		
Age to half asymptotic body length (t_h)	Deltamethrin (D)	1	92.01	< 0.0001
	Age at exposure (A)	2	46.80	< 0.0001
	D × A	2	4.59	0.021
	Error	24		
Total lipids (TL)	Deltamethrin (D)	1	197.66	< 0.0001
	Age at exposure (A)	2	99.36	< 0.0001
	D × A	2	27.55	< 0.0001
	Error	24		
Maximal lipid amount (MLA)	Deltamethrin (D)	1	221.61	< 0.0001
	Age at exposure (A)	2	100.84	< 0.0001
	D × A	2	29.82	< 0.0001
	Error	24		
Consumption time of lipids (CT_{50})	Deltamethrin (D)	1	149.27	< 0.0001
	Age exposure (A)	2	48.28	< 0.0001
	D × A	2	4.58	0.021
	Error	24		

methrin exposure) had no offspring (Fig. 2b). Body length (L_{max}) and body growth rate (t_h) parameters were also affected interactively by deltamethrin and age of disturbance exposure (Table 2), with stronger effects of deltamethrin at older ages of disturbance exposure (Fig. 2c, d).

Physiological parameters TL and MLA were significantly affected by the interaction between deltamethrin exposure and age of disturbance exposure (Table 2). For both traits, their values were lower as the disturbance was applied earlier in the ontogeny of *Daphnia*, although the isolated effect of deltamethrin was slightly stronger when older individuals suffered the disturbance (Fig. 2e, f). Consumption of lipid was also affected interactively by deltamethrin and age at disturbance exposure (Table 2). *Daphnia* consumed their lipid storage faster under deltamethrin exposure and when disturbed earlier in their development. The effect strength of deltamethrin increased with the age at disturbance exposure (Fig. 2g). As in the first experiment, randomized ANOVA rendered the same qualitative results than univariate components of MANOVA. In addition, for both experiments we also performed the analysis of lipids using the lipidic fraction (lipid amount/body area), and the results remained the same than those reported in Tables 1 and 2.

Finally we assessed the effect strength $((\bar{x}_T - \bar{x}_C)/\bar{x}_C)$ of pesticide exposure on each trait under the different food regimes, for all ages merged, where $\bar{x}_T$ and $\bar{x}_C$ represent mean values of a given trait under pesticide and control treatments, respectively. These results are shown in Fig. 3. As expected, the lowest effects of pesticide exposure were observed in the high food treatments. Interestingly, the strongest effects of pesticide exposure were observed under the food shortage treatments on seven-day survival, body growth and lipid storage traits (TL, MLA and CT_{50}).

A similar analysis was made to assess age specific effect strength, shown in Fig. 5S. Under both constant food treatments, younger individuals (age 0) exhibited the largest effect strength on all traits excepting clutch size and TL. In contrast, under food shortage treatments, the older individuals (age 2) exhibited the largest effect strength on all traits excepting seven-day survival and clutch size (Fig. 5S).

Discussion

The results of this study expand our understanding of how the organismal responses to environmental stress are determined by the ecological condition (internal plus environmental factors) of the organisms at the time of being exposed to the source of stress. Here we were mainly interested in evaluating the role of two dimensions of the organisms' ecological condition: variability of food availability and age at stress exposure. As stress agent we used the pyrethroid pesticide deltamethrin, and

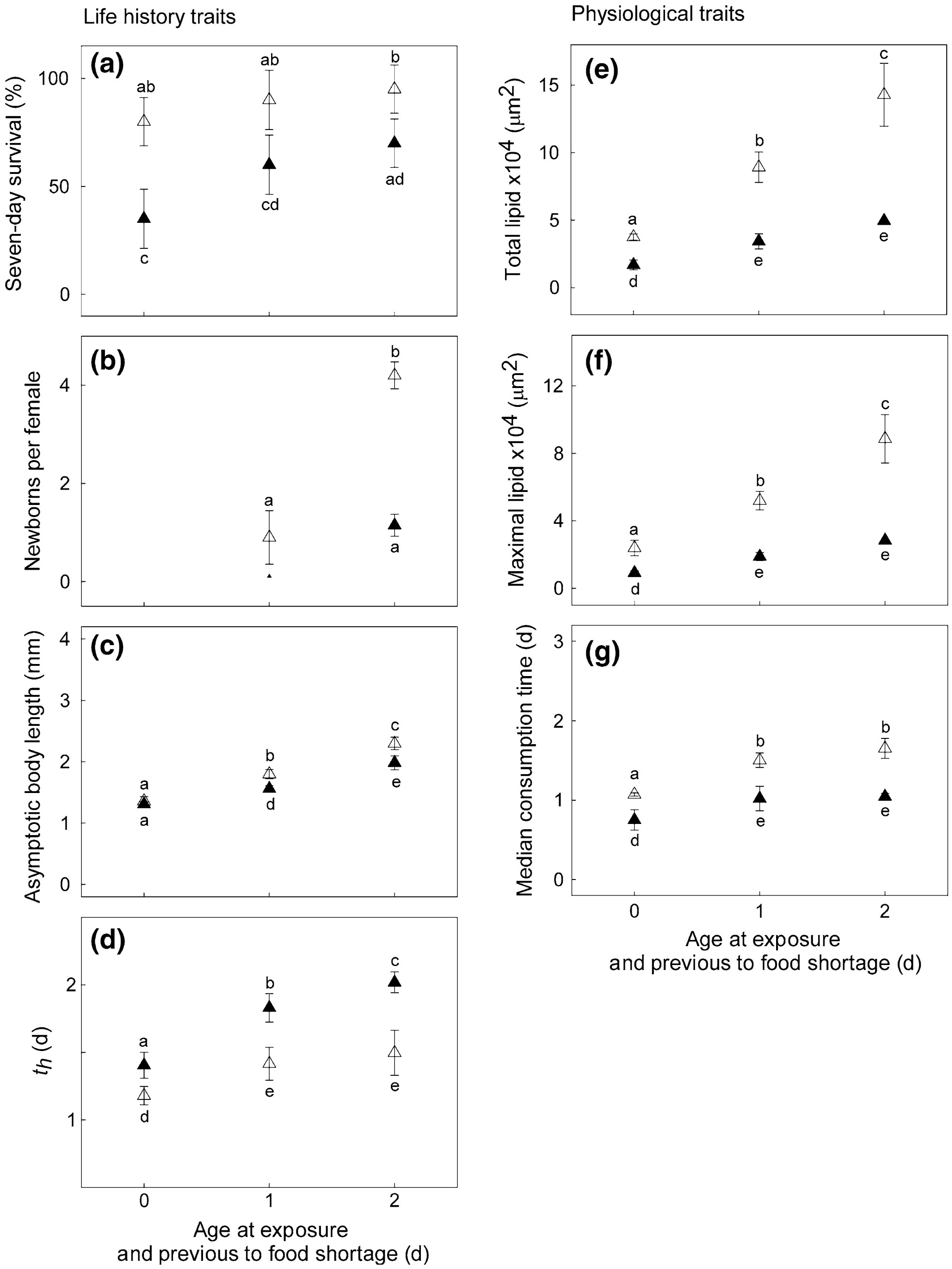

Fig. 2 Effects of deltamethrin exposure at different ages under food shortage treatments, on life history (*left panel*) and physiological (*right panel*) traits of *Daphnia magna*. Life history traits are: **a** seven-day survival, **b** clutch size, **c** asymptotic body length ($L_{\max}$) and **d** age to half asymptotic body length (t_h). Physiological traits are: **e** total lipid amount (TL), **f** maximal lipid amount (MLA) and **g** median consumption time (CT_{50}). Each symbol in control (*white triangles*) and exposure (*black triangles*) treatments represent mean values ± SD. *Different letters* above symbols indicate significant differences among treatments (post hoc test, $P < 0.01$). In all cases, food shortage was conducted 1 day after deltamethrin exposure

as response variables we measured a number of life history traits directly related to fitness (seven-day survival, reproduction, body growth) and a set of physiological parameters related to energy (lipid) storage. Stored lipids are an important source of metabolic energy and their amount in the body has been used for decades as a measure of feeding success and resistance to stress in cladoceran species (Tessier et al. 1983). Clearly, our study was restricted to a single study species (*D. magna*) and a single clone of this species. This sacrifices generality for replicability, but is a valid choice as a first step for assessing interaction effects among multiple factors.

Pyrethroids have a neurotoxic mode of action (Xiu et al. 1989) and have been used widely to control insect pests on crops due to its limited persistence. The exposure of freshwater crustaceans, to low pyrethroid levels lead to immobility, reduction in coordination movements and thus filtering activity (Christensen et al. 2005; Reynaldi et al. 2006). Like many other synthetic pyrethroids, deltamethrin has been shown to be toxic also to fish (Viran et al. 2003).

Our results showed that deltamethrin exposure exerted significant effects on all evaluated traits of *Daphnia*. Furthermore, parameters of body growth, lipid storage and reproduction were affected by the interaction between deltamethrin exposure and food treatments. Overall, deltamethrin exposure exerted detrimental effects on seven-day survival, clutch size and body length on *Daphnia* under constant—both low and high food—as well as under food shortage treatments, which confirm the toxicity of this pesticide to *Daphnia* and its detrimental effects on fitness components. In addition, lipid consumption time was accelerated by deltamethrin exposure, which indicated that energy expenditure increased for compensatory mechanisms. On the other hand, negative effects on lipid storage were observed at constant food and food shortage treatments. However, a positive effect was obtained for individuals exposed at age 2 in high food treatments. Such responses cannot be explained without further research, but it is possible that the deltamethrin exposure under high food availability could promote higher energy demand and allow higher uptake for detoxification and reparation. Overcompensatory responses of this kind have been reported previously for aquatic invertebrates such as *D. magna* (De Coen et al. 2001; Duquesne et al. 2006).

The age of test organisms at the time of exposure was an important determinant of deltamethrin toxicity but, more interestingly, the relative age-specific sensitivity to the pesticide varied qualitatively according to food variability. Under constant food availability, the younger individuals exhibited a greater sensitivity to deltamethrin. This response was exhibited through seven-day survival, reproduction, body growth, total lipid storage and its consumption. Predictably, these effects were stronger under low food treatments. In contrast, under food shortage treatments, older individuals exhibited a greater deltamethrin sensitivity through seven-day survival, body growth, total lipid storage and lipid consumption (Fig. S5).

In line with our results for constant food densities, previous studies showed that pyrethroid pesticides exert their neurotoxic activity preferentially on early developmental stages of invertebrates (Xiu et al. 1989). Often, body size is believed to explain the higher toxicant sensitivity exhibited by earlier stages, particularly to pyrethroids. The usual mechanism is an increased mass-specific uptake and bioconcentration of toxicants displayed by smaller-sized individuals (Day and Kaushik 1987). Similar findings have been reported for larval stages of invertebrates, where a brief pyrethroid exposure was able to reduce survival and developmental rate (Forbes and Cold 2005; Pieters et al. 2006). Nevertheless, the physiological mechanisms responsible for a higher sensitivity of older organisms under food shortage treatments are unclear, being necessary to perform new research to uncover such causes.

Conversely to our hypotheses, we observed that deltamethrin exposure exerted stronger effects on *Daphnia* seven-day survival, body growth and parameters of lipid storage in food shortage treatments, as compared to *Daphnia* growing in constant low food treatments (Fig. 3).

The causes of this effect cannot be determined from our experiments, but we guess that they could rest on two main mechanisms. A first plausible mechanism is that the physiological condition of zooplankters would be weakened more severely by an abrupt decrease in food densities, as compared to any regime of constant food density, which impede organisms to compensate the harmful effects of the toxicant. A second plausible mechanism is that decreasing food availability triggers consumption of food reserves gained during an initial phase of high food availability and thereby the release of lipophilic pollutants stored in the adipose tissues. This increases the plasmatic concentration of the toxicant, reaching and poisoning critical organs through the circulation. For testing the first mechanism we would need independent measures of physiological performance or stress under decreasing versus constant food availability. For the second proposed mechanism, which has been proven to occur in humans (Chevrier et al. 2000; Imbeault et al. 2001, 2002; Hue et al. 2006; Lim et al. 2011), we would need measures of plasmatic concentration of the toxicant. Nevertheless, our results provide partial evidence in support of the second mechanism, since the consumption of lipid reserves in the deltamethrin-exposed organisms showed to be faster under food short-

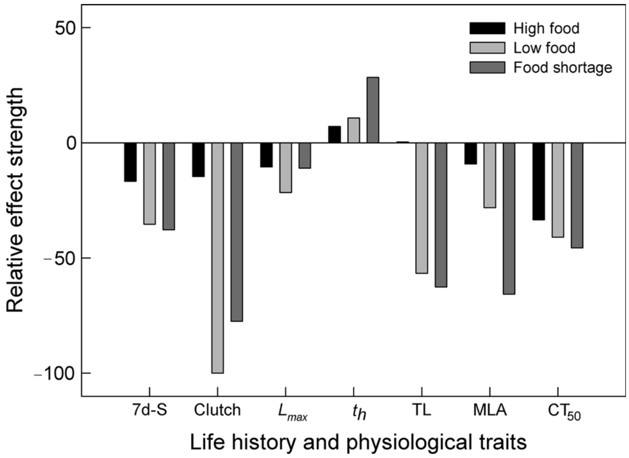

Fig. 3 Relative effect strength $((\bar{x}_T - \bar{x}_C)/\bar{x}_C)$ of deltamethrin exposure under high (*black*), low (*light gray*) and food shortage (*dark gray*) treatments on life history and physiological traits of *Daphnia magna*. Mean values for pesticide ($\bar{x}_T$) and control ($\bar{x}_C$) treatments were computed as the mean over all evaluated ages. Each bar shows the mean effect strength on seven-day survival (7d-S), clutch size, asymptotic body length (L_{max}), age to half asymptotic body length (t_h), total lipid amount (TL), maximal lipid amount (MLA) and median consumption time (CT_{50})

age treatments, as compared to constant food treatments.

Whatever the underlying mechanisms, our findings suggest that the phenological phase in which a pollutant is released to ecosystems strongly determines the strength of the ecological outcome, with stronger adverse effects on certain traits if the pollutant is released short before a shortage in food density.

Marked temporal and spatial variation of food availability is a common phenomenon in aquatic ecosystems. The phenological succession of communities in temperate freshwaters (Cloern and Jassby 2008; Sommer et al. 2012) generate patterns of severe decrease of population size of primary producers in a short time, as in the clear-water-phase or in a longer time due to the end of the vegetative season. On the other hand, the diel vertical migration of zooplankters in response to predator cues (Haupt et al. 2009) imposes herbivores to sharply shift the availability of their food on an hourly basis. Thus, our study suggests that the dynamics of food resources may determine a high vulnerability of herbivores to certain pollutants released to the ecosystem, and that the analysis of the outcome of pollution in natural communities should consider the effects of this ecological dynamics, whose deep understanding and forecasting is still evasive to science.

Acknowledgments This study was supported by FONDECYT grant 1120958 to R. R. -J. C. A. R and J. G-B acknowledge graduate scholarships from CONICYT. We thank M. Fernandez-González for his help in performing lab experiments and M. Arancibia for technical lab assistance.

References

Aladin NV, Potts WTW (1995) Osmoregulatory capacity of the cladocera. J Comp Physiol B164:671–683

Antunes SC, Castro BB, Goncalves F (2003) Effect of food level on the acute and chronic responses of daphnids to lindane. Environ Pollut 127:367–375

ASTM (1980) Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. American Standards for Testing and Materials, Philadelphia

Beketov MA (2004) Comparative Sensitivity to the insecticides Deltamethrin and Esfenvalerate of some aquatic insect larvae (Ephemeroptera and Odonata) and *Daphnia magna*. Russ J Ecol 35:200–204

Bielmeyer GK, Brix KV, Capo TR, Grosell M (2005) The effects of metals on embryo-larval and adult life stages of the sea urchin, *Diadema antillarum*. Aquat Toxicol 74:254–263

BioPix (2008) BioPixsoftware, ver. iQ 2.0, computer program, BioPix Software, Gothenburg, Sweden

Bychek EA, Dobson GA, Harwood JL, Guschina IA (2005) *Daphnia magna* can tolerate short-term starvation without major changes in lipid metabolism. Lipids 40:599–608

Chevrier J, Dewailly E, Ayotte P, Mauriege P, Despres JP, Tremblay A (2000) Body weight loss increases plasma and adipose tissue concentrations of potentially toxic pollutants in obese individuals. Int J Obes 24:1272–1278

Christensen BT, Lauridsen TL, Ravn HW (2005) A comparison of feeding efficiency and swimming ability of *Daphnia magna* exposed to cypermethrin. Aquat Toxicol 73:210–220

Cloern JE, Jassby AD (2008) Complex seasonal patterns of primary producers at the land-sea interface. Ecol Lett 11:1294–1303

Coors A, De Meester L (2008) Synergistic antagonistic and additive effects of multiple stressors: predation threat parasitism and pesticide exposure in *Daphnia magna*. J Appl Ecol 45:1820–1828

Day K, Kaushik NK (1987) Short-term exposure of zooplankton to the synthetic pyrethroid fenvalerate, and its effects on rates of filtration and assimilation of the alga, *Chlamydomonas reinhardtii*. Arch Environ Cont Tox 16:423–432

De Coen WM, Janssen CR, Segner H (2001) The use of biomarkers in *Daphnia magna* toxicity testing V. In vivo alterations in the carbohydrate metabolism of *Daphnia magna* exposed to sublethal concentrations of mercury and lindane. Ecotox Environ Safe 48:223–234

Duquesne S, Reynaldi S, Liess M (2006) Effects of the organophosphate paraoxon-methyl on survival and reproduction of *Daphnia magna*: importance of exposure duration and recovery. Environ Toxicol Chem 25:1196–1199

Enserink L, Luttmer W, Maas-Diepeveen H (1990) Reproductive strategy of *Daphnia magna* affects the sensitivity of its progeny in acute toxicity tests. Aquat Toxicol 17:15–26

Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509

Folt CL, Chen CY, Moore MV, Burnaford J (1999) Synergism and antagonism among multiple stressors. Limnol Oceanogr 44:864–877

Forbes VE, Cold A (2005) Effects of the pyrethroids fenvalerate on life-cycle traits and population dynamics of *Chironomus riparius*-Importance of exposure scenario. Environ Toxicol Chem 24:78–86

Forget J, Pavillon JF, Menasria MR, Bocquené G (1998) Mortality and LC50 values for several stages of the marine copepod *Tigriopus brevicornis* (Muller) exposed to the metals arsenic and cadmium and the pesticides atrazine, carbofuran, dichlorvos and malathion. Ecotox Environ Safe 40:239–244

Fulford RS, Rice JA, Miller TJ, Binkowski FP, Dettmers JM, Belonger B (2006) Foraging selectivity by larval yellow perch (*Perca flavescens*): implications for understanding recruitment in small and large lakes. Can J Fish Aquat Sci 63:28–42

Goulden CE, Hornig LL (1980) Population oscillations and energy reserves in planktonic c1adocera and their consequences to competition. P Natl Acad Sci USA 77:1716–1720

Guilhermino L, Sobral O, Chastinet C, Ribeiro R, Goncalves F, Silva MC, Soares AMVM (1999) A *Daphnia magna* first-brood chronic test: an alternative to the conventional 21-day chronic bioassay? Ecotox Environ Safe 42:67–74

Haupt F, Stockenreiter M, Baumgartner M, Boersma M, Stibor H (2009) *Daphnia* diel vertical migration: implications beyond zooplankton. J Plankton Res 31:515–524

Heugens EH, Hendriks AJ, Dekker T, Van Straalen NM, Admiraal W (2001) A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. Cr Rev Toxicol 31:247–284

Hoang TC, Klaine SJ (2007) Influence of organism age on metal toxicity to *Daphnia magna*. Environ Toxicol Chem 26:1198–1204

Holmstrup M, Bindesbol AM, Oostingh GJ, Duschl A, Scheil V, Kohler HR, Loureiro S, Soares AM, Ferreira AL, Kienle C, Gerhardt A, Laskowski R, Kramarz PE, Bayley M, Svendsen C, Spurgeon DJ (2010) Interactions between effects of environmental chemicals and natural stressors: a review. Sci Total Environ 408:3746–3762

Hue O, Marcotte J, Berrigan F, Simoneau M, Dore J, Marceau P et al (2006) Increased plasma levels of toxic pollutants accompanying weight loss induced by hypocaloric diet or by bariatric surgery. Obes Surg 16:1145–1154

Imbeault P, Chevrier J, Dewaill E, Ayotte P, Despres JP, Tremblay A et al (2001) Increase in plasma pollutant levels in response to weight loss in humans is related to in vitro subcutaneous adipocyte basal lipolysis. Int J Obes 25:1585–1591

Imbeault P, Tremblay A, Simoneau JA, Joanisse DR (2002) Weight loss-induced rise in plasma pollutant is associated with reduced skeletal muscle oxidative capacity. Am J Physiol-Endoc M 282:E574–E579

Jonker D, Freidig AP, Groten JP, de Hollander AEM, Stierum RH, Woutersen RA, Feron VJ (2004) Safety evaluation of chemical mixtures and combinations of chemical and non-chemical stressors. Rev Environ Health 19:83–139

Kast-Hutcheson K, Rider CV, LeBlanc GA (2001) The fungicide propiconazole interferes with embryonic development of the crustacean *Daphnia magna*. Environ Toxicol Chem 20:502–509

Klein B (2000) Age as a factor influencing results in the acute daphnid test with *Daphnia magna* Straus. Water Res 34:1419–1424

Kreuger J (1998) Pesticides in stream water within an agricultural catchment in southern Sweden, 1990-1996. Sci Total Environ 216:227–251

Lampert W (1987) Feeding and nutrition in *Daphnia*. In: Peters RH, Bernardi R de (eds) *Daphnia*. Mem Ist Ital Idrobiol 45:143–192

LeBlanc GA, Surprenant DC (1983) The acute and chronic toxicity of acetone, dimethylformamide, and triethylene glycol to *Daphnia magna* (Straus). Arch Environ Con Tox 12:305–310

Liess M, Schulz R, Liess MHD, Rother B, Kreuzig R (1999) Determination of insecticide contamination in agricultural headwater streams. Water Res 33:239–247

Lim S, Son HK, Park SK, Jacobs DR, Lee DH (2011) Inverse associations between long-term weight change and serum concentrations of persistent organic pollutants. Int J Obes 35:744–747

Maguire RJ, Carey JH, Hart JH, Tkacz RJ, Lee H-B (1989) Persistence and fate of deltamethrin sprayed on a pond. J Agric Food Chem 37:1153–1159

Manly BFJ (1997) A program for randomization testing. Centre for Applications of Statistics and Mathematics University of Otago, Dunedin

Manyin T, Rowe C (2010) Reproductive and life stage-specific effects of aqueous copper on the grass shrimp, *Palaemonetes pugio*. Mar Environ Res 69:152–157

McCauley E, Murdoch WW, Nisbet RM, Gurney WSC (1990) The physiological ecology of *Daphnia:* development of a model of growth and reproduction. Ecology 71:703–715

Medina M, Barata C, Telfer T, Baird D (2002) Age-and sex related variation in sensitivity to the pyrethroid cypermethrin in the marine copepod *Acartia tonsa Dana*. Arch Environ Con Tox 42:17–22

OECD (2004) OECD guidelines for testing of chemicals. Guideline 202: *Daphnia sp*., Acute immobilisation test, adopted April 2004

Petersen GI, Kristensen P (1998) Bioaccumulation of lipophilic substances in fish early life stages. Environ Toxicol Chem 17:1385–1395

Pieters BJ, Jager T, Kraak MHS, Admiraal W (2006) Modeling responses of *Daphnia magna* to pesticide pulse exposure under varying food conditions: intrinsic versus apparent sensitivity. Ecotoxicology 15:601–608

Pijanowska J, Dawidowicz P, Howe A, Weider LJ (2006) Predator-induced shifts in *Daphnia* life-histories under different food regimes. Arch Hydrobiol 167:37–54

Ramos-Jiliberto R, Dauelsberg P, Zuñiga LR (2004) Differential tolerance to ultraviolet-B light and photo enzymatic repair in cladocerans from a Chilean lake. Mar Freshwater Res 55:193–200

Reichwaldt ES, Wolf ID, Stibor H (2004) Effects of a fluctuating temperature regime experienced by *Daphnia* during diel vertical migration on *Daphnia* life history parameters. Hydrobiologia 543:199–205

Reynaldi S, Duquesne S, Jung K, Liess M (2006) Linking feeding activity and maturation of *Daphnia magna* following short-term exposure to fenvalerate. Environ Toxicol Chem 25:1826–1830

Ringwood AH (1990) The Relative Sensitivities of Different Life Stages of *Isognomon californicum* to Cadmium Toxicity. Arch Environ Contam Toxicol 19:338–340

Schulz R, Liess M (2001) Toxicity of aqueous-phase and suspended particle-associated fenvalerate: chronic effects after pulse-dosed exposure of *Limnephilus lunatus* (Trichoptera). Environ Toxicol Chem 20:185–190

Smolders R, Baillieul M, Blust R (2005) Relationship between the energy status of *Daphnia magna* and its sensitivity to environmental stress. Aquat Toxicol 73:155–170

Sommer U, Adrian R, De Senerpont Domis L, Elser JJ, Gaedke U, Ibelings B et al (2012) Beyong the plankton ecology group (PEG) model: mechanisms driving plankton succession. Ann Rev Ecol Evol S 43:429–448

Tessier AJ, Goulden CE (1982) Estimating food limitation in cladoceran populations. Limnol Oceanogr 27(4):707–717

Tessier AJ, Henry LL, Goulden CE, Durand MW (1983) Starvation in *Daphnia*: energy reserves and reproductive allocation. Limnol Oceanogr 28:667–676

Toumi H, Bournaiza M, Immel F, Sohm B, Felten V, Ferard JF (2014) Effect of deltamethrin (pyrethroid insecticide) on two clones of *Daphnia magna* (Crustacea, Cladocera): a proteomic investigation. Aquat Toxicol 148:40–47

Viran R, ÜnlüErkoç F, Polat H, Koçak O (2003) Investigation of acute toxicity of deltamethrin on guppies (*Poecilia reticulata*). Ecotox Environ Safe 55:82–85

Voeltz NJ, Zuelling RE, Shieh SH, Ward V (2005) The effects of urban areas on benthic macroinvertebrates in two Colorado plains rivers. Environ Monit Assess 101:175–202

Xiu R, Xu Y, Gao S (1989) Toxicity of the new pyrethroid insecticide, deltamethrin, to *Daphnia magna*. Hydrobiologia 188–189:411–413