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ORIGINAL ARTICLE

Allelopathic interactions between the macrophyte *Egeria densa* and plankton (alga, *Scenedesmus acutus* and cladocerans, *Simocephalus* spp.): a laboratory study

Cristian A. ESPINOSA-RODRÍGUEZ,[1] Ligia RIVERA-DE LA PARRA,[2] Aurora MARTÍNEZ-TÉLLEZ,[1] Gisela C. GÓMEZ-CABRAL,[1] S.S.S. SARMA,[3*] Sarma NANDINI[3]

[1]Postgraduate Programme in Marine Sciences and Limnology, National Autonomous University of Mexico, Ciudad Universitaria, Av. Universidad 3000, 04510 México City; [2]Postgraduate Programme in Biological Sciences, National Autonomous University of Mexico, Postgraduate Studies Building, CU, Coyoacán, 04510 Mexico City; [3]Laboratory of Aquatic Zoology, Division of Research and Postgraduate Studies, National Autonomous University of Mexico, Campus Iztacala, Av. de los barrios # 1, Los Reyes Iztacala, 54090 Tlalnepantla, México
*Corresponding author: sarma@unam.mx

ABSTRACT

Allelopathic interactions between macrophytes and zooplankton are important to understand the plankton dynamics in shallow waterbodies. Egeria densa *is a native, perennial, submerged macrophyte in the tropical and subtropical zones of South America. It has been introduced to Central and North America and is now common in many Mexican lakes. This macrophyte produces chemical substances that negatively affect some phytoplankton species. However, it is not clear how zooplankton species adapt different life history strategies in the chemical presence of this macrophyte. Here, we tested the direct and indirect effects of allelochemicals released by* E. densa *on the population growth of* Scenedesmus acutus *and on the demographic variables of three species of* Simocephalus, S. exspinosus, S. serrulatus *and* S. mixtus *(via alga exposed to the macrophyte allelochemicals). To quantify the effect of* E. densa *on* S. acutus *we set up four treatments: control, artificial* Egeria*, natural* Egeria *and allelochemicals from* Egeria*. To test the allelochemical effects on* Simocephalus *species, we compared four treatments: Control, indirect effect (using* S. acutus *grown on* Egeria*-allelochemicals), direct effect (using* Egeria*-conditioned medium) and together with direct and indirect effects.* Scenedesmus *had the highest cell density in the presence of allelochemicals from* Egeria*, followed by controls. The specific algal growth rate (μ) between control and allelochemicals treatment was not significant (P< 0.05). However, the μ of alga in the presence of artificial or natural* Egeria *was significantly lower than in controls or in treatments involving allelochemicals. The age-specific survivorship of the three cladoceran species was longer in treatments containing* Egeria*-conditioned medium. Cladocerans receiving* Egeria *conditioned-medium and algae cultured on macrophyte-allelochemicals also had a longer survivorship. Daily fecundity of* S. serrulatus *increased after reaching mid-age while* S. expinosus *and* S. mixtus *showed continuous reproduction starting from the first week. In general,* Egeria*-allelochemicals enhanced the age-specific reproductive output for all the three cladoceran species. The average lifespan of the three* Simocephalus *varied from 17 to 46 days, depending on the cladoceran species and treatment.* S. serrulatus *had lower lifespan compared to other two cladoceran species. For the three species, lifespan significantly increased in treatments containing macrophyte-conditioned medium + algae grown on the plant-allelochemicals; also under these conditions, both gross and net reproductive rates were significantly enhanced. This stimulatory effect was also evident in generation time (about 50% higher). The rate of population increase ranged from 0.23 to 0.38 per day for the three tested* Simocephalus *species but there were no significant differences (P> 0.05) among treatments. Our results suggest that the biological activity as well as physical structure of* E. densa *had negative effects on* S. acutus *population growth but had stimulatory effects on the demography of* Simocephalus*.*

Key words: Allelopathy; life-table variables; zooplankton; allelochemicals; phytoplankton.

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INTRODUCTION

Many freshwater lakes around the world are small, shallow and display two strongly contrasting states (Scheffer *et al*., 1993): one dominated by macrophytes and low turbidity and the other dominated by phytoplankton and high turbidity (Scheffer, 2004). In shallow waterbodies there exists an antagonistic relationship between phytoplankton and macrophytes. In macrophytic zones, low water turbulence, reduced light intensity and low nutrient concentrations in water limit phytoplankton growth (Lürling *et al*., 2006; Muylaert *et al*., 2010). However, macrophytes also affect plankton through the release of allelochemicals into the medium. Macrophytes belonging to the genera *Elodea*, *Stratiotes*, *Chara* and *Myriophyllum* release chemical substances that alter the abundance of phytoplankton (Körner and Nicklish, 2002; Gross, 2003; Hilt, 2006), and have effects on zooplankton behavior (Pennak, 1973; Meerhoff *et al*., 2006) and lifetable variables of cladocerans (Burks *et al*., 2000; Cerbin *et al*., 2007; Gutierrez and Paggi, 2014). Most studies concerning the allelopathic effects of macrophytes have been on

phytoplankton in temperate systems, while such works from subtropical and tropical zones are limited (Meerhoff *et al*., 2006; Vanderstukken *et al*., 2011; Dong *et al*., 2013). In addition, the information available regarding the effect of chemical cues from macrophytes on many genera of cladocerans including *Simocephalus* that inhabit lake areas with aquatic vegetation is inadequate (Hilt, 2006).

The effect of chemical stress provoked by pesticides, heavy metals and chemical cues on cladocerans has been evaluated using different approaches such as somatic growth rate, population growth, feeding and filtration rates, diet selection, survivorship and life table changes (Lass and Spaak, 2003). In a review, Sarma and Nandini (2006) summarized some of the ecotoxicological studies on cladocerans using demographic variables. Since demographic changes are sensitive to different kinds of biotic and abiotic factors and are easily quantifiable, most workers have used the life table method to quantify the effects of chemicals on zooplankton (Conde-Porcuna, 1998; Doksaeter and Vijverberg, 2001; Nandini *et al*., 2004). Some stressful conditions such as low light intensity, herbivory, predation and high temperatures are determining components in the production and release of chemical cues, in this way the allelopathic effects of macrophytes on zooplankton vary considerably (Machacek, 1991; Gilbert, 2009; Gutierrez and Paggi, 2014). Here we tested the effect of *E. densa*-conditioned medium on selected zooplankton species.

Egeria densa is a native, perennial, submerged macrophyte in parts of South America (Uruguay-Paraguay-Brazil); it has been introduced, due to aquaculture, into several water bodies around the world (Lot and Novelo, 2004; Santos *et al*., 2011) and has thus become a nuisance in temperate, subtropical and tropical ecosystems because of its rapid growth (Duarte *et al*., 1999). Moreover, this submerged macrophyte produces allelopathic substances which affect phytoplankton adversely (Nakai *et al*., 1999; Mulderij *et al*., 2007; Vanderstukken *et al*., 2011). These allelochemicals also affect the demography of *Daphnia* in different ways (Cerbin *et al*., 2007).

Some studies have focused on effects of allelopathic substances produced by macrophytes on cladocerans, especially *Daphnia*, as the main bioassay organism which are typically limnetic. On the other hand, *Simocephalus* is usually found in the littoral/macrophyte zones where it could be affected in different ways due to its preference for the littoral regions. Thus, we selected three *Simocephalus* species of which *S. serrulatus* coexists with *E. densa* in nature.

We evaluated the effects of allelochemicals released by *Egeria densa* on the growth of *Scenedesmus acutus* and on the survivorship (average lifespan and age-specific survival) and on the demographic variables (age-specific fecundity, gross and net reproductive rates, generation time and rate of population increased (*r*) per day) of three *Simocephalus* species: *S. exspinosus*, *S. serrulatus* and *S. mixtus* exposed to macrophyte-allelochemicals, directly (conditioned-medium), indirectly (algal food exposed to allelochemicals), and both (direct and indirect effects). We supposed that the summarized effects of allelochemicals from *Egeria densa* would have stronger effects on life table variables of *Simocephalus* species than isolated effects (direct or indirect).

METHODS

Plankton cultures

We used three cladoceran species of the same genus *Simocephalus*: *S. exspinosus, S. serrulatus* and *S. mixtus.* These species were isolated from three different shallow water bodies from the State of Mexico (Mexico), because they rarely co-exist (Orlova-Bienkowskaja, 2001). The alga *Scenedesmus acutus,* was obtained from the University of Texas and *Egeria densa* and *S. serrulatus* were collected from Benito Juárez reservoir (Mexico City). *S. acutus* was batched-cultured in Bold's Basal medium (Borowitzka and Borowitzka, 1988) in 2L transparent glass bottles using continuous fluorescent light and aeration. The medium was supplemented with 3 mM $NaHCO_3$ as a source of carbon. The algae were harvested after 8 days, centrifuged and resuspended in distilled water. The density of algae was estimated using a haemocytometer. All the cultures and experiments were maintained at a temperature of 22±1°C.

Each of the three *Simocephalus* species was separately cultured for several months using moderately hard water, the EPA medium, and fed *Scenedesmus acutus* (about 0.5 $\times 10^6$ cells mL^{-1}). EPA medium was prepared by adding 96 mg of $NaHCO_3$, 60 mg of $CaSO_4$, 60 mg of $MgSO_4$ and 4 mg of KCl to 1L of distilled water (Weber, 1993). The cladoceran cultures were transferred to fresh medium containing the specified concentration of fresh *Scenedesmus* from culture (0.5×10^6 cells mL^{-1}) every second day by filtering the culture using a 100 µm mesh. For obtaining the macrophyte-conditioned medium, *Egeria* was carefully washed using aged-tap water, then treated with ionized silver (0.082%) for 40 min. to minimize any interference from organisms that use this macrophyte as substrate, and finally rinsed a few times with distilled water. Thereafter, 750 g of wet *E. densa* was transferred to a transparent jar containing 9 L of EPA medium and placed under continued-diffuse light and constant mild-aeration. After 24 h, the conditioned-medium was collected by filtering it through a Whatman Polycap filter (0.45 µm) (for obtaining allelochemicals liberated by the macrophyte without particulate organic matter).

Effects of *Egeria* on *Scenedesmus*

To test the effect of allelochemicals from the natural plant and its physical presence on the algal specific

growth rate, we inoculated 16 (four treatments X four replicates for each treatment) transparent jars of 2 L capacity containing 1.6 L of Bold's Basal medium with *S. acutus* at an initial density of 0.125×10^6 cells mL^{-1}. The treatment details are as follows:

Treatment 1: this comprised controls, with only Bold medium and *Scenedesmus* grown in this medium.

Treatment 2: we added plastic *Egeria*-like plants in bio-volume similar to the live *E. densa* (1.6 DW g L^{-1}).

Treatment 3: received live *E. densa.*

Treatment 4: it consisted of *Egeria*-conditioned medium (in distilled water but supplemented with appropriate quantities of Bold's basal chemicals just before the start of the experiment). The biomass of *Egeria* used in the treatments corresponded to that we found in the field (1.373±1.21 DW g L^{-1}).

All the treatments received continuous fluorescent illumination (4300 lux 399 footcandle) and aeration. Following initiation of the algal growth experiment detailed previously, we daily quantified the density of *Scenedesmus* in the culture jars for 8 days, and later the algae were separately harvested. Algae harvested from the four treatments were used for further experiments with *Simocephalus* as mentioned below.

Life table demography of *Simocephalus* spp.

Four different treatments for each of the three species of *Simocephalus* were simultaneously setup. For each cladoceran species, the experimental design consisted of treatments 5-8.

Treatment 5: Controls (EPA medium+*S. acutus* cultured on Bold's medium (EPA+SceC), Treatment 6: EPA medium+*S. acutus* grown on macrophyte-allelochemicals (EPA+SceA), Treatment 7: conditioned- medium with allelochemicals+*S. acutus* grown on Bold's medium (CM+SceC); Treatment 8: conditioned medium with allelochemicals+ *S. acutus* grown on macrophyte-allelochemicals (CM+SceA). Each treatment contained four replicates.

The life table demography experiments were conducted at one food level (0.5×10^6 cells mL^{-1} of *S. acutus*=13 µg DW mL^{-1} per day (Mayeli *et al*., 2004). We used transparent jars of 100 mL capacity containing 50 mL test medium. Each jar received 10 neonates (<24 h age) of one of the three species of *Simocephalus*. The cohorts were individually introduced into the test jars using Pasteur pipette under a stereoscopic microscope at 20x. Later, the test jars were placed on a horizontal shaker under continuous but diffused fluorescent illumination set at 22±1°C. Following initiation of life table experiment, we daily quantified the number survived from the original cohort and the number of neonates produced, if any. Dead adults and neonates were removed and the surviving individuals of the original cohort were transferred to fresh jars of corresponding treatment. The survivorship and fecundity data of *Simocephalus* were used to calculate the following variables: i) average lifespan; ii) life expectancy; iii) gross reproductive rate, net reproductive rate; iv) generation time; and v) rate of population increase *per* day following Krebs (1985):

Life expectancy: $e_x = \frac{T_x}{n_x}$

Gross reproductive rate: $= \sum_0^\infty m_x$

Net reproductive rate: $R_o = \sum_0^\infty l_x \cdot m_x$

Generation time: $T = \frac{\sum l_x \cdot m_x \cdot x}{R_o}$

Rate of population increase, Euler-Lotka equation (solved iteratively)

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

where, T_x=number of individuals per day, n_x=number of living individuals at the initiation, l_x=the probability of an individual surviving to an age class x, m_x=the age specific fecundity, R_o=the average number of offspring per female, and r=growth rate of the population.

Statistical analysis

Data from *Scenedesmus acutus* specific growth rate and the demography of zooplankton were statistically analyzed using analysis of variance (ANOVA) (Sokal and Rohlf, 2000) after satisfying the assumption of normality. *Post-hoc* (Tukey test) analysis was used for multiple comparisons utilizing the software Sigma Plot ver. 11.

RESULTS

Scenedesmus acutus grew better in the presence of allelochemicals in all the treatments, followed by the controls (Fig. 1). However, the growth rate of the alga was lowest in the treatment involving live *Egeria*. The maximum algal abundance (22.3×10^6 cells mL^{-1}) was obtained in the presence of allelochemicals. *Egeria* in both natural and artificial forms reduced the algal specific growth rates (µ) compared with treatments with allelochemicals and controls (Fig. 2). Statistically, there were significant differences among the treatments ($P<0.001$, one-way ANOVA).

The survivorship curves of the three *Simocephalus spp*. considerably differed depending on the treatment type and species (Fig. 3). The survivorship was longer in treatments containing macrophyte-conditioned medium (Treatments 7 and 8). In addition, the cladocerans in treatment CM+SecA, *i.e.* conditioned-medium and algae cul-

tured on macrophyte-allelochemicals, had also a longer survivorship. The neonates of *S. exspinosus* in controls (EPA+SecC) experienced some mortality during the first three weeks but thereafter, it was heavier. However, for *S. mixtus* the survivorship improved during the first two weeks in the treatment with allelochemicals from the macrophyte. *Simocephalus serrulatus* showed relatively little mortality during the first 10 days regardless of the treatment type. On the other hand, *S. mixtus* continued to die, albeit at a lower rate, starting from the first week in all treatments.

Data on the age-specific fecundity of the three tested cladoceran species are presented in Fig. 4. There were distinct patterns in the offspring production by the three species: regardless of treatment, *S. serrulatus* had higher rate of offspring production after reaching mid-age while *S. exspinosus* and *S. mixtus* showed continuous reproduction starting from first week. In general, macrophytes-allelochemicals treatment enhanced the age-specific reproductive output for all the three *Simocephalus* species compared to controls. However, the offspring production in the three *Simocephalus* species differed depending on the treatments. Only *S. exspinosus*, but not the other two species of *Simocephalus*, showed higher fecundity in treatments containing both CM+SceA (direct+indirect effects) compared to that in CM+SceC (direct effect).

Data on the selected life history variables of the three *Simocephalus* spp. subjected to different treatments are presented in Tab. 1. The average lifespan of the three cladocerans species ranged from 17 to 46 days depending on the species and the treatment. Generally, *S. serrulatus* had a lower duration of life (17-24 d) than the other two *Simocephalus* spp. (range, 30-46 d). For the three species, lifespan, compared with controls, significantly increased in treatments containing macrophyte conditioned-medium with allelochemicals+algae grown on the plant-allelochemicals ($P<0.05$, F-test; two way ANOVA; Tab. 2). The rate of offspring production, both gross and net reproductive rates, was strikingly higher than in controls; the cladocerans on an average increased by about 350% for these parameters previously mentioned in treatment containing CM+SceA. This highly stimulatory effect was also evident in the generation time which was also enhanced by about 50%. The rate of population increase (0.23 to 0.38 per day for all the species) was, however, not significantly ($P>0.05$) affected by the growth medium and the food source.

DISCUSSION

Egeria densa, in both natural and artificial forms, reduced the algal specific growth rates (μ) of *Scenedesmus* compared with controls or the treatments containing allelochemicals. The effect of the macrophyte on plankton is variable and in some cases phytoplankton growth is inhibited but in others stimulated (Erhard and Gross, 2006). On the other hand, Vanderstukken *et al*. (2011) have observed that the presence of *Egeria densa* has an adverse impact on the growth of *Scenedesmus*. Van Donk and van de Bund (2002) have also reported a significant reduction in the abundance of *Scenedesmus acutus* grown in the

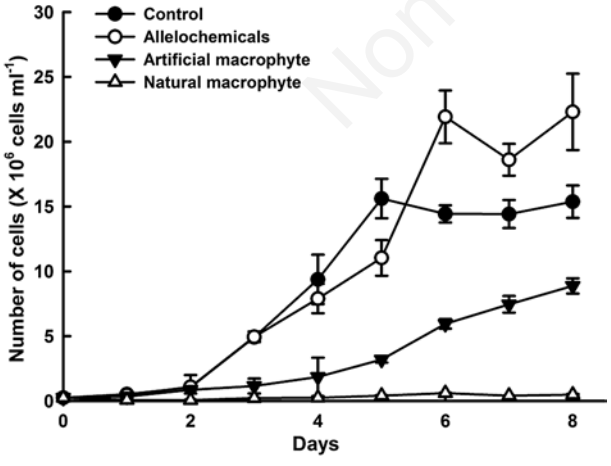

Fig. 1. Growth curves of the alga *Scenedesmus acutus* subjected to different treatments: with and without the allelochemicals and the presence or absence of the macrophyte *Egeria densa*. Shown are the mean values±SD based on four replicates.

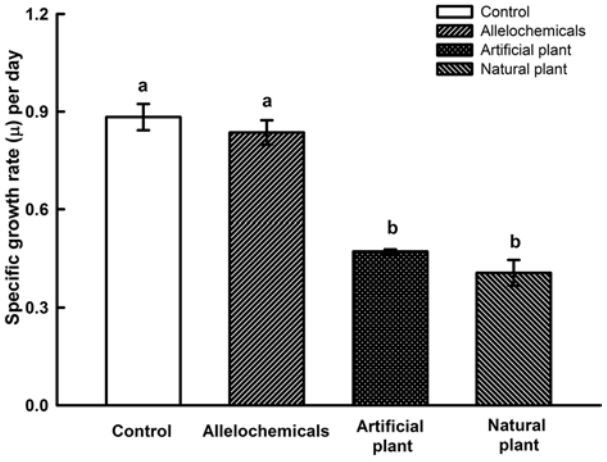

Fig. 2. Specific growth rate of the alga (*Scenedesmus acutus*) subjected to different treatments: with and without the allelochemicals and the presence or absence of the macrophyte *Egeria densa*. Shown are the mean values ± SD based on four replicates. Data bars carrying similar alphabet are not statistically significant ($P>0.05$, Tukey test).

presence of *Chara aspera* under laboratory conditions. The macrophytes affect algae through physical interference in capturing light, compete for nutrients in the medium and produce allelopathic substances (Gross, 2003). In order to assess the effect of *Egeria densa* on *Scenedesmus acutus,* we conducted the *Scenedesmus* growth experiments under optimal conditions of light, temperature and nutrients and using a defined medium so that the differences found among the treatments could reflect the impact of macrophytes or macrophyte-derived allelochemicals. Thus, the differences in the specific growth rates (μ) of *S. acutus* in treatments containing the macrophytes (both natural and artificial) were evidently lower due to the physical structure that limited the algal growth as also observed by Lurling *et al*. (2006). *Scenedesmus* grown together with natural *Egeria* showed the lowest specific growth rate (0.40) compared with controls (0.83). This is because the live macrophyte caused both, physical interference in light availability, allelopathic influence and possibly physiological nutrient limitation to the alga.

In natural waterbodies macrophytes also inhibit phytoplankton growth by diminishing turbulence in water column, which increases the rate of algal sedimentation (Scheffer, 2004; Vanderstukken *et al*., 2014). We solved this problem by continuous aeration in all treatments. Macrophytes also decrease light availability by shading the phytoplankton (Mulderij *et al*., 2007; Hilt and Gross, 2008) which was observed in both the treatments containing natural and artificial plants compared with controls, *i.e.* treatment with no plants. Though the jars with algal cultures were continuously aerated, the shading by the macrophytes further decreased light availability and hence algal growth was reduced. Nutrient limitation of phytoplankton due to competition with macrophytes is still another possibility (we did not measure the nutrient levels in the test jars periodically), but provided adequate nutrient levels in the medium (0.37 g.L^{-1} of $NaNO_3$, 0.11 g.L^{-}

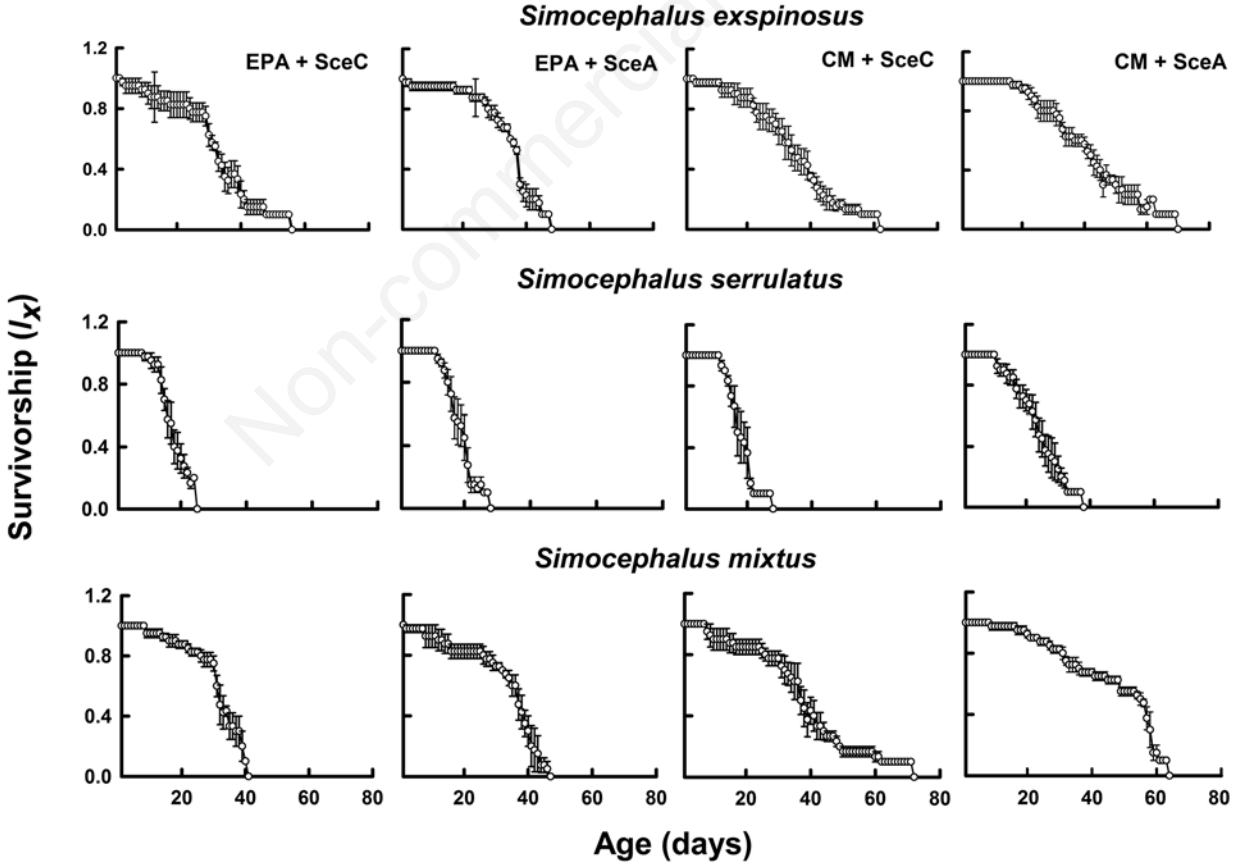

Fig. 3. Age-specific survivorship (l_x) curves of *S. exspinosus*, *S. serrulatus* and *S. mixtus* cultured in relation to different treatments: EPA+SceC (EPA medium+*S. acutus* cultured on Bold's medium), EPA+SceA (EPA medium+*S. acutus* grown on macrophyte-allelochemicals), CM+SceC (conditioned- medium+*S. acutus* grown on Bold's medium) and CM+SceA (conditioned medium+*S. acutus* grown on macrophyte allelochemicals). Shown are mean ±SD based on four replicates (cohorts).

^{1} of K_2HPO_4 and 0.262 g.L^{-1} of KH_2PO_4) to prevent nutrient limitation, if any. Allelopathic effects of macrophytes may also operate simultaneously with their other adverse effects from macrophytes, *e.g.* increasing light limitation for algae, in the algal culture systems (Gopal and Goel, 1993; Vanderstukken *et al*., 2011). Conditioned-medium enhanced phytoplankton growth leading to *Scenedesmus* to reach densities maxima. Our observations support Erhard and Gross (2006) who have demonstrated that allelochemicals from *Elodea* have inhibitory effects on some phytoplankton species but stimulated *Scenedesmus* growth. Our observations show that the highest specific growth of *Scenedesmus* was recorded in cultures exposed to *Egeria*-conditioned medium but without the physical presence of the macrophytes.

Zooplankton species also show variable responses to the presence of macrophytes (Dawidowicz and Ozimek, 2013; Gutierrez and Paggi, 2014). In general, studies concerning allelopathic effects of macrophytes on zooplankton are relatively few compared with macrophyte-phytoplankton interactions (van Donk and van de Bund, 2002). Our results showed that the allelochemicals indirectly (through *Scenedesmus* grown on *Egeria*-conditioned-medium) and directly (through the medium with allelochemicals in the test jars), affected the life history variables of *Simocephalus* spp., and the impact was high through both these routes. Age-specific survivorship curves reveal the pattern of mortality of a cohort population as they age. In laboratory populations, survivorship curves are usually rectangular where *Simocephalus* population experiences little mortality during the early stages and thereafter experiences heavy mortality due to physiological senescence (Krebs, 1985). This typical trend was evident only for *S. exspinosus* and *S. mixtus*. However, for *S. serrulatus*, after two weeks, the population began to experience heavy mortality in all treatments. These suggest that though the three cladoceran species belong to the same genus, they differ in their optimal conditions. Species of *Simocephalus* in general have long lifespan (30-70 days: Malhotra and Langer, 1991), a fact we also observed (average lifespan up to 45 days).

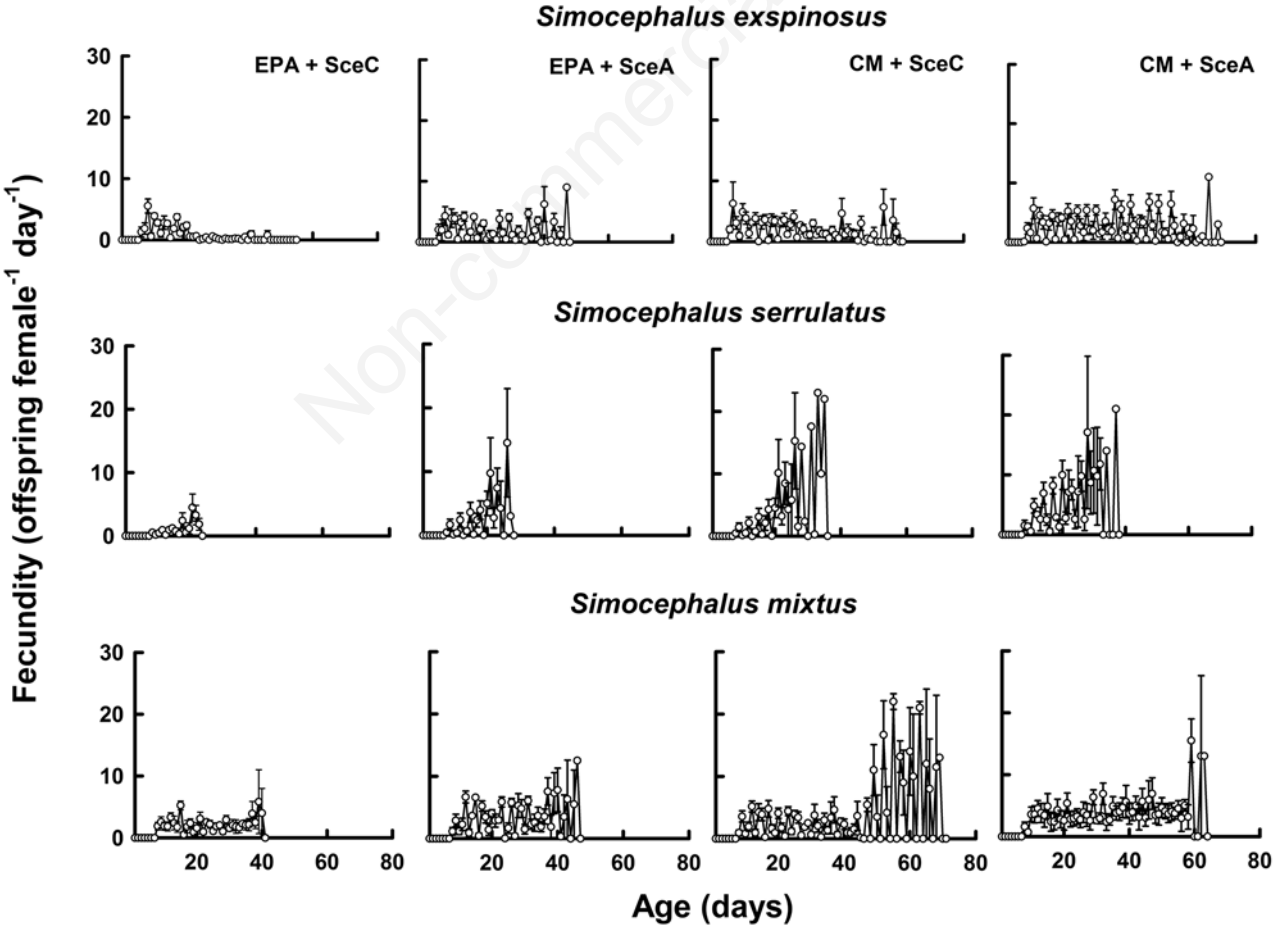

Fig. 4. Age-specific fecundity (m_x) curves of *S. exspinosus*, *S. serrulatus* and *S. mixtus* cultured in relation to different treatments.

Age-specific reproductive output of cladocerans varies depending on the species and the test conditions (Sarma *et al*., 2005). Since *Simocephalus* begins to increase their body size even after achieving age at first reproduction, the number of neonates developing in the brood chamber increases with age (Dumont and Negrea, 2002). This results in higher offspring production during the mid-reproductive age and thereafter it decreases due to physiological aging of the reproducing individuals (Dodson and Frey, 2001). This typical offspring production is also evident in this work on *S. serrulatus* and *S. mixtus*. However, this pattern varied in *S. exspinosus* suggesting that offspring production in this species is more or less continuous with similar number of neonates per clutch over a period of time as was also observed in *S. vetulus* by Fernández *et al*. (2014).

Studies on temperate pelagic cladocerans such as *Daphnia cucullata* and *Daphnia longispina* indicate that these taxa show horizontal migration towards macrophytes (*Potamogeton lucens*, *Elodea canadensis*, *Equisetum fluviatile*, *etc*.) during the day to evade fish-predation (Wojtal *et al*., 2003; Iglesias *et al*., 2007). Allelochemicals from *Elodea, Nitella* and *Myriophyllum* have been reported to repel some cladoceran species including *Daphina* or reduce their reproductive output (Pennak, 1973; Burks *et al*., 2000). However, in our study we observed the presence of *Egeria* allelochemicals enhanced the duration of lifespan and generation time of *Simocephalus* spp. as well as elevated their gross and net reproductive rates. This stimulatory effect of allelochemicals on zooplankton may be explained by hormesis (Duke, 2011). It seemed that hormesis occurs at relatively low stress levels as a measure of overcompensation through enhanced reproductive output (Gama-Flores *et al*., 2007). Some heavy metals and various chemical substances, including pesticides and plant allelochemicals, enhance the offspring production in zooplankton by elevating the reproductive rates (Calabrese, 2004). It is interesting to note that a great diversity of periphytic zooplankton including rotifers and cladocerans specialize in living in macrophyte-dominated environments indicates that an adverse allelopathic effect does not per se occur (Burks *et al*., 2000). In this context the suggestion of Burns and Dodds (1999) that the allelopathic interactions between macrophytes and zooplankton be studied more thoroughly is relevant, especially for periphytic species in tropical and sub-tropical environments.

CONCLUSIONS

The inhibition of the abundance and specific growth rates (μ) of *Scenedesmus acutus* by *Egeria densa* was due to its physical structure as well as its biological activity while the presence of its allelochemicals resulted in slightly higher algal densities. *E. densa* had a significant effect directly by enhancing survivorship as well as raising the age-specific reproductive output for the all three

Tab. 1. Selected life history variables of the three species of *Simocephalus* cultured under different test conditions: EPA+SceC (EPA medium+*S. acutus* cultured on Bold's medium), EPA+SceA (EPA medium+*S. acutus* grown on macrophyte-allelochemicals), CM+SceC (conditioned- medium+*S. acutus* grown on Bold's medium) and CM+SceA (conditioned medium+*S. acutus* grown on macrophyte allelochemicals). For each cladoceran species, data carrying similar alphabet are not significant (P>0.05, Tukey test).

Treatments	Species / life history variable			
		S. exspinosus	*S. serrulatus*	*S. mixtus*
EPA+SceC	Average lifespan (days)	30.25±0.72^{a}	17.10±0.93^{a}	30.05±0.54^{a}
EPA+SceA		33.67±1.11^{b}	18.20±0.97^{a}	32.4±1.71^{ab}
CM+SceC		34.25±1.91^{abc}	23.65±3.04^{a}	36.12±2.86^{ab}
CM+SceA		40.22±1.93^{c}	23.15±1.54^{a}	45.57±0.72^{b}
EPA+SceC	Gross reproductive rate (offspring female^{-1})	39.32±1.99^{a}	17.05±1.93^{a}	67.22±4.65^{a}
EPA+SceA		79.70±5.75^{b}	48.05±6.24^{ab}	126.53±4.31^{ab}
CM+SceC		100.21±3.85^{bc}	129.15±39.56^{b}	193.83±42.10^{b}
CM+SceA		145.78±12.17^{d}	108.83±13.74^{ab}	211.17±11.35^{b}
EPA+SceC	Net reproductive rate (offspring female^{-1}) (survival-weighted)	32.62±2.07^{a}	7.95±1.94^{a}	47.97±0.85^{a}
EPA+SceA		56.5±1.76^{b}	19.10±6.86^{ab}	83.2±5.21^{b}
CM+SceC		66.50±3.91^{bc}	49.17±19.81^{b}	77.1±8.98^{bc}
CM+SceA		99.07±6.01^{d}	58.45±8.94^{ab}	144.45±2.53^{d}
EPA+SceC	Generation time (days)	13.97±0.66^{a}	15.14±0.23^{a}	19.04±0.74^{a}
EPA+SceA		19.17±0.41^{b}	16.58±0.23^{a}	22.87±0.48^{ab}
CM+SceC		19.53±0.51^{bc}	20.37±0.23^{b}	27.91±2.85^{ab}
CM+SceA		24.77±1.53^{d}	17.73±0.29^{a}	30.78±0.24^{b}
EPA+SceC	Rate of population increase (r) day^{-1}	0.33±0.01^{a}	0.28±0.06^{a}	0.29±0.003^{a}
EPA+SceA		0.34±0.01^{a}	0.23±0.07^{a}	0.30±0.01^{a}
CM+SceC		0.38±0.01^{a}	0.23±0.06^{a}	0.29±0.01^{a}
CM+SceA		0.36±0.01^{a}	0.29±0.04^{a}	0.31±0.01^{a}

Tab. 2. Results of the two-way analysis of variance conducted on the average lifespan, gross reproductive rate, net reproductive rate, generation time and rate of population increased of *Simocephalus exspinosus*, *S. serrulatus* and *S. mixtus* under direct (allelochemicals from the plant) and indirect (effect through the alga) effects of *Egeria densa*.

Source of variation	df	Sum of square	Mean square	F-ratio
Average lifespan				
S. exspinosus				
Direct (A)	1	111.3	111.3	12.07**
Indirect (B)	1	88.36	88.36	9.58**
Interaction of A X B	1	6.5	6.5	0.7 ns
Error	12	110.57	9.21	
S. serrulatus				
Direct (A)	1	132.25	132.25	9.8**
Indirect (B)	1	0.36	0.36	0.02 ns
Interaction of A X B	1	2.56	2..56	0.19 ns
Error	12	161.8	13.48	
S. mixtus				
Direct (A)	1	370.56	370.56	30.0***
Indirect (B)	1	139.24	139.24	11.61**
Interaction of A X B	1	50.41	50.41	4.2 ns
Error	12	143.86	11.98	
Gross reproductive rate				
S. exspinosus				
Direct (A)	1	16121.59	16121.59	80.56***
Indirect (B)	1	7387.13	7387.13	36.91***
Interaction of A X B	1	26.87	26.87	0.71 ns
Error	12	2401.43	200.11	
S. serrulatus				
Direct (A)	1	44186.96	44186.96	155.82***
Indirect (B)	1	710.11	710.11	2.5 ns
Interaction of A X B	1	7858.4	7858.4	27.71***
Error	12	3402.86	283.57	
S. mixtus				
Direct (A)	1	44625.89	44625.89	22.98***
Indirect (B)	1	5875.61	5875.61	3.02 ns
Interaction of A X B	1	1761.48	1761.48	0.9 ns
Error	12	23300.78	1941.73	
Net reproductive rate				
S. exspinosus				
Direct (A)	1	5844.6	5844.6	99.28***
Indirect (B)	1	3186.6	3186.6	54.13***
Interaction of A X B	1	75.69	75.69	0.27 ns
Error	12	706.39	58.86	
S. serrulatus				
Direct (A)	1	6492.33	6492.33	12.4**
Indirect (B)	1	417.18	417.18	0.79 ns
Interaction of A X B	1	3.51	3.51	0.006
Error	12	6280.78	523.39	
S. mixtus				
Direct (A)	1	8167.64	8167.64	70.97***
Indirect (B)	1	10521.63	10521.63	91.42***
Interaction of A X B	1	1032.01	1032.01	8.96*
Error	12	1381.01	115.08	
Generation time				
S. exspinosus				
Direct (A)	1	124.44	124.44	38.63***
Indirect (B)	1	108.95	108.95	33.83***
Interaction of A X B	1	0.001	0.001	0.0005 ns
Error	12	38.64	3.22	
S. serrulatus				
Direct (A)	1	75.6	75.6	38.77***
Indirect (B)	1	12.36	12.36	6.34*
Interaction of A X B	1	40.96	40.96	21.007***
Error	12	23.4	1.95	
S. mixtus				
Direct (A)	1	281.47	281.47	31.3***
Indirect (B)	1	44.76	44.76	4.97*
Interaction of A X B	1	0.9	0.9	0.1 ns
Error	12	107.89	8.99	

To be continued on next page

Table 2. Continued from previous page.

Source of variation	df	Sum of square	Mean square	F-ratio
Rate of population increase				
S. exspinosus	1	0.004	0.004	5.51*
Direct (A)	1	0.000005	0.000005	0.007 ns
Indirect (B)	1	0.001	0.001	1.44 ns
Interaction of A X B	12	0.009	0.0007	
Error				
S. serrulatus				
Direct (A)	1	0.002	0.002	0.59 ns
Indirect (B)	1	0.004	0.004	0.89 ns
Interaction of A X B	1	0.004	0.004	0.94 ns
Error	12	0.058	0.004	
S. mixtus				
Direct (A)	1	0.00005	0.00005	0.18 ns
Indirect (B)	1	0.0006	0.0006	2.29 ns
Interaction of A X B	1	0.0003	0.0003	1.25 ns
Error	12	0.003	0.0002	

*df, degrees of freedom; ns, not significant; *P> 0.05; **P< 0.01; ***P< 0.001.*

Simocephalus spp. This implies the existence of allelopathic interactions between *E. densa* and *Simocephalus*. It is, however, necessary to analyze the allelopathy between macrophytes and zooplankton under natural conditions (*e.g*., through mesocosms) which are more complex than the somewhat simplified laboratory tests. We suggest that future studies on *Egeria* interactions with zooplankton need to focus on other zooplankton groups including rotifers and copepods in order to understand the effect of allelochemicals from macrophytes on zooplankton.

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