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Diapause may promote coexistence of zooplankton competitors

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Production of diapausing eggs in many zooplankton species occurs after partial switching from parthenogenesis to sexual reproduction. Storage effect theory predicts the stable long-term coexistence of competitors investing in diapausing stages, but it does not address the effect of such investment on short-term coexistence. The freshwater rotifer *Brachionus calyciflorus* and the cladoceran *Moina micrura* were used in this study to test experimentally if diapause could promote coexistence of active individuals under inter-specific competition. Our results show that *B. calyciflorus* was more affected by inter-specific competition and exhibited a greater and earlier production of resting eggs compared with *M. micrura*. Costs of diapause investment in *Moina* were only observed in competition. This was translated into reduced probability of *B. calyciflorus* of being outcompeted by *M. micrura*. We show that investment in diapause might promote local and short-term coexistence of competing zooplankton species. Our study strongly suggests that the ecological effects of diapause encompass a wider range of temporal scales, from weekly to decadal, and levels of organization, from individuals to communities, than previously thought.

KEYWORDS: cladocerans; coexistence; crowding cues; rotifers; resting eggs

INTRODUCTION

Many zooplankton species develop diapause stages as a life history strategy, in which vital functions are suspended for a certain time period. The ecological and evolutionary significance of diapause rests in that this strategy allows harsh environmental conditions to be overcome, preventing population extinction (Fussmann *et al.,* 2007) and promoting dispersal and colonization of

new environments by passive transport of resting stages, mostly eggs (Hairston and Cáceres, 1996; Cáceres, 1997; Slusarczyk and Pietrzak, 2008). In addition, banks of resting eggs serve as a reservoir of genetic diversity, playing an important role in shaping the diversity and dynamics of zooplankton communities (Brendonck and De Meester, 2003).

The production of resting eggs in important zooplankton groups such as cladocerans and rotifers is most often a result of switching the reproductive mode from parthenogenesis to gametogenesis. Several environmental stimuli are known to trigger sexual reproduction in zooplankters, such as changes in photoperiod (Stross, 1969; Hairston et al., 1990), temperature (Ruttner-Kolisko, 1964), salinity (Hagiwara et al., 1988) and oxygen concentrations (Alekseev, 2007). Nevertheless, several studies have also demonstrated that the process leading to the production of diapausing eggs in rotifers, cladocerans and copepods could be mainly induced by dissolved chemicals exuded by conspecific (Ban and Minoda, 1994; Zadereev, 2003; Stelzer and Snell, 2006) or congeneric (Gilbert, 2002; Lurling et al., 2003; García-Roger et al., 2009) competitors.

Investment in switching to sexual reproduction and production of diapausing eggs would involve metabolic and demographic costs (Hairston et al., 1995; Cáceres and Hairston, 1998; Gilbert, 2004; Serra and Snell, 2009). Therefore, trade-offs may be found between production of diapausing eggs and other life history parameters, so that a population producing diapausing eggs could be affected in its parthenogenetic population growth (Serra and Carmona, 1993; Hairston and Cáceres, 1996; Serra and King, 1999; Gilbert, 2002). Consequently, this strategy may open an opportunity for other competing species whose population growth is not compromised by the production of diapausing eggs (Ciros-Pérez et al., 2002; Gilbert and Schröeder, 2004). Thus, it is expected that the inter-species competitive outcome could be affected by the production of diapausing eggs, affecting short-term species persistence and therefore local diversity.

Diapause has potentially strong implications for competitor coexistence, operating through two plausible mechanisms: (i) through so-called storage effect (Chesson, 1994; Chesson and Huntly, 1989, 1997), in which species avoid hazardous conditions generated in competitive environments, promoting stable long-term coexistence of competing species, i.e. over several growth seasons (Chesson and Huntly, 1989, 1997; Chesson, 1994, 2000), and (ii) through the mechanism proposed by Montero-Pau and Serra (Montero-Pau and Serra, 2011), in which production of diapause stages may slow down the proliferation of superior competitors and then affect their competitive capability on the basis of density-dependent induction of sex and diapause investment. Through a density-dependent investment in sex/diapause by competing species, an inferior competitor could invade a sex/diapause-investing resident population, which would help explain short-term species coexistence in facultative sexual species such as many planktonic organisms. Nevertheless, there is no empirical evidence of the effect of diapause investment by different species exposed to simultaneous inducing signals on short-term coexistence.

In this study, we evaluate experimentally the relevance of resting egg production, assessing diapausing egg production as a promoter of short-term active coexistence of competing zooplankton populations. For this purpose, we selected as a model two-herbivore zooplankton species, typical of subtropical fauna (Ruttner-Kolisko, 1974; Ramos-Jiliberto and Aránguiz-Acuña, 2007; Pagano, 2008), the rotifer Brachionus calyciflorus and the cladoceran Moina micrura. Both species produce resting eggs, and differ in body size and filtration rates on different food resources (Gilbert, 1988; MacIsaac and Gilbert, 1989; Agasild and Nõges, 2005), which suggests different competitive abilities in exploiting a single shared food resource, the microalga Pseudokirchneriella subcapitata. Here, we tested the hypothesis that a weaker competitor enhances its persistence probability when production of diapausing eggs is induced in competitors.

METHOD

Cultures of test species

The rotifer B. calyciflorus can be found throughout the year in lakes and ponds in Central Chile. On the other hand, the cladoceran M. micrura is a tropical species, which in temperate lakes of Central Chile exhibits a high growth potential at higher temperatures. Because of this, M. micrura is a characteristic summer species (Ramos-Jiliberto and Aránguiz-Acuña, 2007). Both species feed over a wide particle size range, although M. micrura has a lower specific feeding rate than B. caliciflorus (Pagano, 2008). Both herbivore species were isolated from temperate lakes of central Chile: Tranque Lo Orozco (33°22′S–71°41′W) and Carén Lagoon (33°25′S–70°51′W). A clonal lineage of each experimental species was established from a single parthenogenic female and maintained under standard laboratory conditions (pH 7.5 ± 0.1, temperature $20 \pm 1°C$, photoperiod 16:8, l:d) for 2 months prior to their experimental use, avoiding the presence of males in the cultures. For culture maintenance as well as for conducting the experiments, we used

COMBO medium (Kilham *et al.*, 1998). The stock cultures of herbivores were fed daily with the green alga *Pseudokirchneriella subcapitata* at a density of 10^6 cells mL^{-1}, which was cultured with Bold's basal medium (Borowitzka and Borowitzka, 1988). The algae were harvested during the exponential growth phase, and their concentration was measured by direct counting.

Induction of sexual reproduction by crowding cues

To assess the effect of population density on the production of mictic offspring in *B. calyciflorus* and production of males in *M. micrura*, herbivore-conditioned medium was prepared with *B. calyciflorus* at nominal densities of 0, 5, 8.125, 11.25, 17.5, 30 and 55 ind. mL^{-1} day^{-1}, and *M. micrura* at 0, 0.125, 0.25, 0.5, 1.5 and 3 ind. mL^{-1} day^{-1}. Every 24 h, herbivore individuals were filtered out, the conditioned medium was membrane-filtered (0.45 μm), diluted with COMBO medium to achieve the indicated density and the pH adjusted to 7.5 ± 0.1.

Life table experiments for *B. calyciflorus* and *M. micrura* were conducted with conspecific cues at the levels defined above, in order to obtain a daily record of reproduction and survival of parthenogenetic females exposed to different conspecific cue concentrations. Unfed neonates (<6 h old) hatched from mothers cultured at low population densities were cultured individually in conditioned medium with the alga *P. subcapitata* as food at densities of 5×10^5 and 10^6 cells mL^{-1}. Final concentrations of crowding cues were obtained considering the nominal concentration of the preconditioned medium and that provided by the experimental individuals in the plate. For *B. calyciflorus*, final densities of crowding cues were 0.05, 5.05, 8.125, 17.25, 22.5, 35 and 60 ind. mL^{-1}. High concentrations of crowding cues were obtained from conditioned medium with high conspecific densities cultured in multi-plates of 200 μL volume, while the control medium was obtained by inoculating one neonate in 20 mL of fresh medium in 50 mL glass vessels. For induction of *M. micrura*, the final range of crowding cues was 0.05, 0.13, 0.3, 0.55, 1.55 and 3.05 ind. mL^{-1}; 50 mL glass vessels with 20 mL of medium were used. In all experiments, treatment conditions were kept constant by removing all offspring and transferring the parental females to the corresponding medium with algae every other day. Neonates were collected and cultured in fresh medium until maturity, when the classification into mictic/amictic *B. calyciflorus* or female/male *M. micrura* was possible. Mictic daughters per parthenogenetic *B. calyciflorus* female and males per *M. micrura* were compared among treatments through one-way ANOVA and the Holm−Sidak test for multiple comparisons, after verifying parametric assumptions of normality (K−S test) and homocedasticity (Levene test). Because experiments were carried out with single individuals in flasks, and hence eggs had no opportunity to be fertilized, the relative abundance of sexual forms, mictic females in *B. calyciflorus* and males in *M. micrura*, were considered as proxies of production of diapausing eggs.

For each species at a given crowding level, the finite population growth rate was calculated from survivorship and maternity functions recorded in the life tables following the discrete Euler−Lotka equation:

$$1 = \sum_x \lambda^{-x} l_x m_x$$

where l_x and m_x are the survival and maternity functions, respectively. Confidence intervals of 95% for λ were obtained by bootstrapping through random sampling with replacement from the original life history data set.

Alternatively, age-specific vital rates were calculated considering only the sub-population of parthenogenetic females. Then a parthenogenetic population growth rate was calculated as before. The mictic ratio was calculated for *B. calyciflorus* as the proportion of mictic females over all females, and the male ratio was calculated for *M. micrura* as the proportion of males over all offspring. Correlations were calculated between parthenogenetic growth rates and *B. calyciflorus* mictic ratio and *M. micrura* male ratio.

Effect of resting egg production on competition

As an approach to identify the competitive performance of both herbivores, we evaluated their relative ability to exploit resources. Eighty adult *B. calyciflorus* and 20 adult *M. micrura* were inoculated separately in 40 mL COMBO medium at the following food densities: 6.25×10^4, 1.25×10^5, 2.5×10^5, 5×10^5, 10^6, 2×10^6, 4×10^6, 8×10^6, 16×10^6 and 32×10^6 cells mL^{-1}. The flasks were kept in darkness on an orbital shaker. Algal density was measured spectrophotometrically before and 12 h after the beginning of the experiment. The clearance rate (CR) and the ingestion rate (IR) were assessed as

$$CR = \frac{[Ln(A_0 - A_t)] \times V}{N \times \Delta t}$$

$$IR = CR \times A_0$$

where A_0 and A_t are algal density at the start and at the end of the experiment, respectively. Δt ingestion rates of each species over the resource gradient were adjusted to an Ivlev functional response model (Martínez, 2000; Mohr and Adrian, 2000). Differences in functional

response parameters: maximal ingestion rate (I_{max}) and consumption efficiency (b) between species were assessed using t-tests.

Population responses of both species, isolated and coexisting, to their own crowding cues were estimated through a factorial experiment of 18 treatments: three competition conditions (2 species alone + 1 together) × 3 food densities (5×10^5, 10^6 and 2×10^6 cells mL^{-1}) × 2 crowding cue concentrations (with/without), with four replicates of each treatment. In the treatment with both species together and with crowding cues, specific signals were added simultaneously every day to the beakers. Induction was avoided in treatments without crowding cues by randomly removing excess individuals above the specified crowding cue threshold for each species (see Results). In the treatments with species in competition and addition of crowding cues, signals from both species were introduced at the concentrations previously defined as effective. Each experimental trial was initiated with 50 randomly selected *B. calyciflorus* females and 5 *M. micrura* females in 100 mL of culture medium in a 250 mL glass container. The containers were continuously shaken in an orbital shaker at low speed (40 rpm).

Carbon content was estimated in a known number of individuals from both species, which allowed comparable density measured in biomass (in units of mg C) to be determined instead of numerical abundance. Adult female and male densities were measured daily, either by exhaustive counting or by counting three to four 10 mL samples from each population. Diapausing eggs were counted daily and removed from containers to avoid spontaneous hatching during the experiment. Individuals were transferred to new jars containing the appropriate test medium and food. Experiments were terminated after 20 days, when most experimental populations began to decline or reached a steady state.

To discount a cross-effect of the cues on diapause production, a life table experiment was developed with *B. calyciflorus* neonates exposed to a gradient of *M. micrura* cues and vice versa. The gradient of cue concentration and the corresponding methodology was the same as in life table experiments.

To assess the degree of alteration of diapause production as a consequence of competition, we calculated the ratio of diapause egg production, the number of resting eggs per female produced in coexistence with its competitor over the number of resting eggs per female produced in monoculture.

Finally, the competitive exclusion rate η was calculated from time series of population biomass in the competition experiments according to Grover (Grover, 1988, 1991). The response variable $\Upsilon(t) = \ln[\mathcal{N}_1(t)/\mathcal{N}_2(t)]$, where $\mathcal{N}_1$ and $\mathcal{N}_2$ are the population biomass at time t of

B. calyciflorus and *M. micrura*, respectively, was regressed against time. Exclusion rate η was calculated as $\eta = [\Upsilon(t_2) - \Upsilon(t_1)]/(t_2 - t_1)$, where Υ is the predicted value from the fitted linear or quadratic regression at reference times t_1 and t_2. Between t_1 and t_2 the competitor species coexist. Obtained values of exclusion rate were compared between treatments by means of two-way ANOVA, with crowding cue and resource level as factors. The Holm–Sidak *post hoc* test for multiple comparisons was performed whenever significant differences were found.

RESULTS

### Crowding level for induction of sexual reproduction

The fraction of mictic *B. calyciflorus* offspring was larger when exposed to crowding cues corresponding to a conspecific density greater than 30 ind. mL^{-1} at high resource level, and at 55 ind. mL^{-1} at low resource level. Male production of *M. micrura* increased with crowding cue concentrations of 0.5 ind. mL^{-1} and higher, at both tested resource levels. Our results did not show a clear association between induction of sexual reproduction and food level. *Moina micrura* required cue levels released by population densities an order of magnitude lower than *B. calyciflorus* to induce sexual reproduction (Fig. 1). For the subsequent experiments, levels of crowding cue of 55 ind. mL^{-1} for *B. calyciflorus* and 1.5 ind. mL^{-1} for *M. micrura* were used to induce sexual reproduction.

A significant inverse correlation between mictic ratio and parthenogenetic growth rate of *B. calyciflorus* revealed the cost of switching to sexual reproduction, in terms of population growth (Spearman coefficient = -0.657, $P < 0.001$). For *M. micrura*, no association was observed between parthenogenetic growth rate and male production.

Effect of resting egg production on competition

On the basis of measured CRs, we concluded that *M. micrura* exhibited a higher maximal ingestion rate as well as greater consumption efficiency than *B. calyciflorus* (Table I). This result suggests an advantage of *M. micrura* over *B. calyciflorus* in exploiting *P. subcapitata*.

Exposure of the principal species to heterospecific crowding cues did not affect production of mictic females in *B. calyciflorus*, relative to the control. On the other hand, *M. micrura* produced fewer males when exposed to the highest level of *B. calyciflorus* crowding cues (Fig. 2). Hence, heterospecific cues did not increase the production
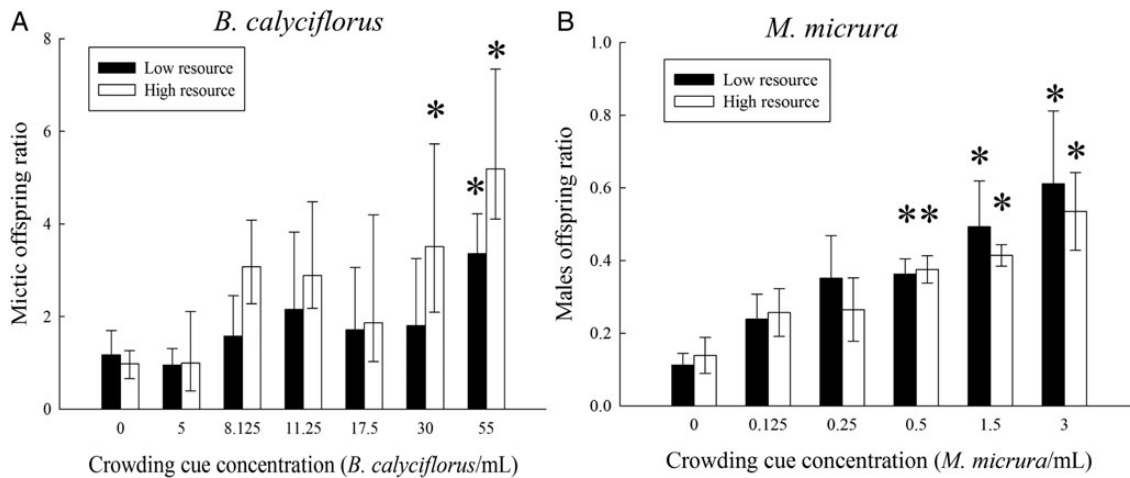
Fig. 1. The mictic offspring ratio of *B. calyciflorus* (**A**) and males offspring ratio of *M. micrura* (**B**) produced in a gradient of conspecific crowding cues (mean ± 95% CI, values obtained on the basis of five replicates). Asterisks show differences relative to control crowding cue level (ANOVA, $P = 0.001$, Holm–Sidak, $P < 0.05$).

Table I: Maximum ingestion rates (I$_{max}$, 10^5 cells herbivore^{-1} h^{-1}), and consumption efficiency (b, mL herbivore^{-1} h^{-1}) predicted from the Ivlev functional response model (estimate ± SD)

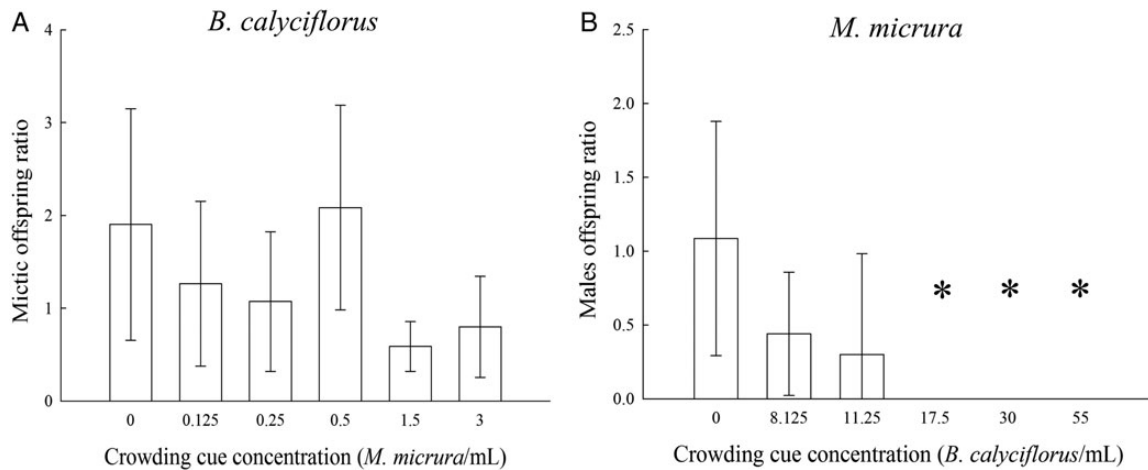|	*B. calyciflorus*	*M. micrura*
I_{max}*	0.84 ± 0.02	1.74 ± 0.07
b*	0.22 ± 0.07	0.89 ± 0.33

Asterisks denote significant differences between species (*t*-test, $P < 0.05$).

of sexual individuals in either species at the concentrations tested.

Species grown in isolation showed effects of crowding cue addition on biomass trajectories. The addition of crowding cues increased population oscillations of *B. calyciflorus*, which reached lower mean biomass relative to the no-addition condition (Table II). Interaction of crowding × food level was significant for *B. calyciflorus* average biomass over time (two-way ANOVA, $F = 6.948$, d.f. = 2, $P = 0.0058$), but not on the coefficient of variation (food level, $F = 10.093$, d.f. = 2, $P < 0.05$; crowding cue, $F = 8.78$, d.f. = 1, $P < 0.01$) (Fig. 3A and B). Conversely, *M. micrura* reached higher mean biomass values and oscillations were dampened with the addition of crowding cues (Table II). Interaction of crowding × food level was significant for *M. micrura* mean biomass (two-way ANOVA, $F = 13.007$, d.f. = 2, $P < 0.001$), but not for its coefficient of variation, where only the factor crowding cue was significant ($F = 15.49$, d.f. = 1, $P < 0.001$) (Fig. 3C and D).

In the experiment with the two species coexisting without the addition of crowding cues, the mean population biomass of *B. calyciflorus* gradually increased with resource density, while oscillations were independent of food level (Table II). On the other hand, *M. micrura* density values increased with food levels, although oscillations were independent of food level (Table II, Fig. 4A). From these results, it is possible to identify a negative effect of competition on average biomass at all resource levels for *B. calyciflorus*, which exhibited lower average biomass when coexisting with *M. micrura* (compare Figs 3A and 4A), although higher density peaks of *B. calyciflorus* at high food level were observed under competition. On the other hand, the presence of *B. calyciflorus* did not have competitive effects on biomass of *M. micrura*, conversely, biomass increase and oscillations were damped (Figs 3C and 4A). With conspecific crowding cues, the biomass of *B. calyciflorus* coexisting with *M. micrura*, gradually increased with resource density, and oscillations of biomass trajectory were independent of food levels (Table II). From these results, it was observed that *B. calyciflorus* had lower mean levels and more oscillating biomass when coexisting with *M. micrura* compared with growing alone (Figs 3B and 4B). On the other hand, negative effects of the presence of *B. calyciflorus* on *M. micrura* biomass emerge with crowding cue addition, with lower average values and higher coefficient of variation. Two-way ANOVA showed significant effects of crowding cues (d.f. = 1, $F = 21.33$, $P < 0.001$) and food levels (d.f. = 2, $F = 110.14$, $P < 0.000$) on the biomass of *B. calyciflorus* and significant effects of crowding cues on the oscillation amplitude of *B. calyciflorus* (d.f. = 1, $F = 115.61$, $P < 0.000$). On the other hand, the average biomass of

Fig. 2. *Brachionus calyciflorus* mictic offspring ratio (**A**) and *M. micrura* males offspring ratio (**B**) produced in a heterospecific crowding cue gradient (mean $\pm$ 95% CI, values obtained on the basis of five replicates). Asterisks show differences relative to control concentration (ANOVA, $P < 0.05$, Holm–Sidak, $P < 0.05$).

Table II: Mean biomass over time and coefficient of variation of biomass over time (estimate $\pm$ SD) of B. calyciflorus *and* M. micrura *under different experimental conditions: with or without presence of competitor, with or without addition of crowding cues, and at three food levels (low 5×10^5 cells mL^{-1}, medium 10^6 cells mL^{-1} and high 2×10^6 cells mL^{-1})*

Species	Competitor	Crowding cue	Food level	Biomass (mean $\pm$ SD)	CV (mean $\pm$ SD)
B. calyciflorus	Without	No	Low	104.84 ± 17.67	0.67 ± 0.084
			Medium	170.35 ± 3.93	0.55 ± 0.010
			High	261.78 ± 24.01	0.61 ± 0.030
		Yes	Low	59.84 ± 2.91	0.75 ± 0.05
			Medium	132.18 ± 11.95	0.63 ± 0.08
			High	175.71 ± 10.19	0.64 ± 0.04
	With	No	Low	53.45 ± 4.15	0.81 ± 0.04
			Medium	106.04 ± 6.06	0.89 ± 0.19
			High	166.37 ± 35.18	0.81 ± 0.13
		Yes	Low	28.52 ± 1.10	1.49 ± 0.07
			Medium	65.27 ± 5.65	1.33 ± 0.16
			High	144.23 ± 11.00	1.39 ± 0.09
M. micrura	Without	No	Low	15.30 ± 5.03	1.09 ± 0.14
			Medium	28.19 ± 4.78	1.05 ± 0.36
			High	37.47 ± 2.83	0.95 ± 0.087
		Yes	Low	30.07 ± 3.04	0.75 ± 0.098
			Medium	50.64 ± 8.52	0.768 ± 0.079
			High	77.06 ± 3.27	0.74 ± 0.038
	With	No	Low	26.44 ± 1.41	0.94 ± 0.03
			Medium	36.82 ± 3.18	0.91 ± 0.14
			High	54.26 ± 2.92	0.91 ± 0.02
		Yes	Low	15.64 ± 1.89	0.97 ± 0.16
			Medium	14.76 ± 4.40	1.03 ± 0.22
			High	22.96 ± 11.38	1.20 ± 0.22

M. micrura was affected by an interaction between crowding cues and food levels (d.f. $= 2$, $F = 6.72$, $P < 0.05$) and oscillation amplitude was affected by the presence of crowding cues (d.f. $= 1$, $F = 7.88$, $P < 0.05$). Likewise, *M. micrura* growing in the presence of *B. calyciflorus* had lower biomass at both medium and high resource levels (Figs 3 and 4B). Comparing competition scenarios with the addition of crowding cues, the mean biomass of both

competing species decreased and was more variable at each resource level compared with the treatments without the addition of crowding cues (Fig. 4A and B).

Resting egg only between isolated and coexisting populations showed that the diapause of *B. calyciflorus* was dependent on the competition condition, generating more diapausing eggs per female when competing at the lowest resource level. On the other hand, at medium and
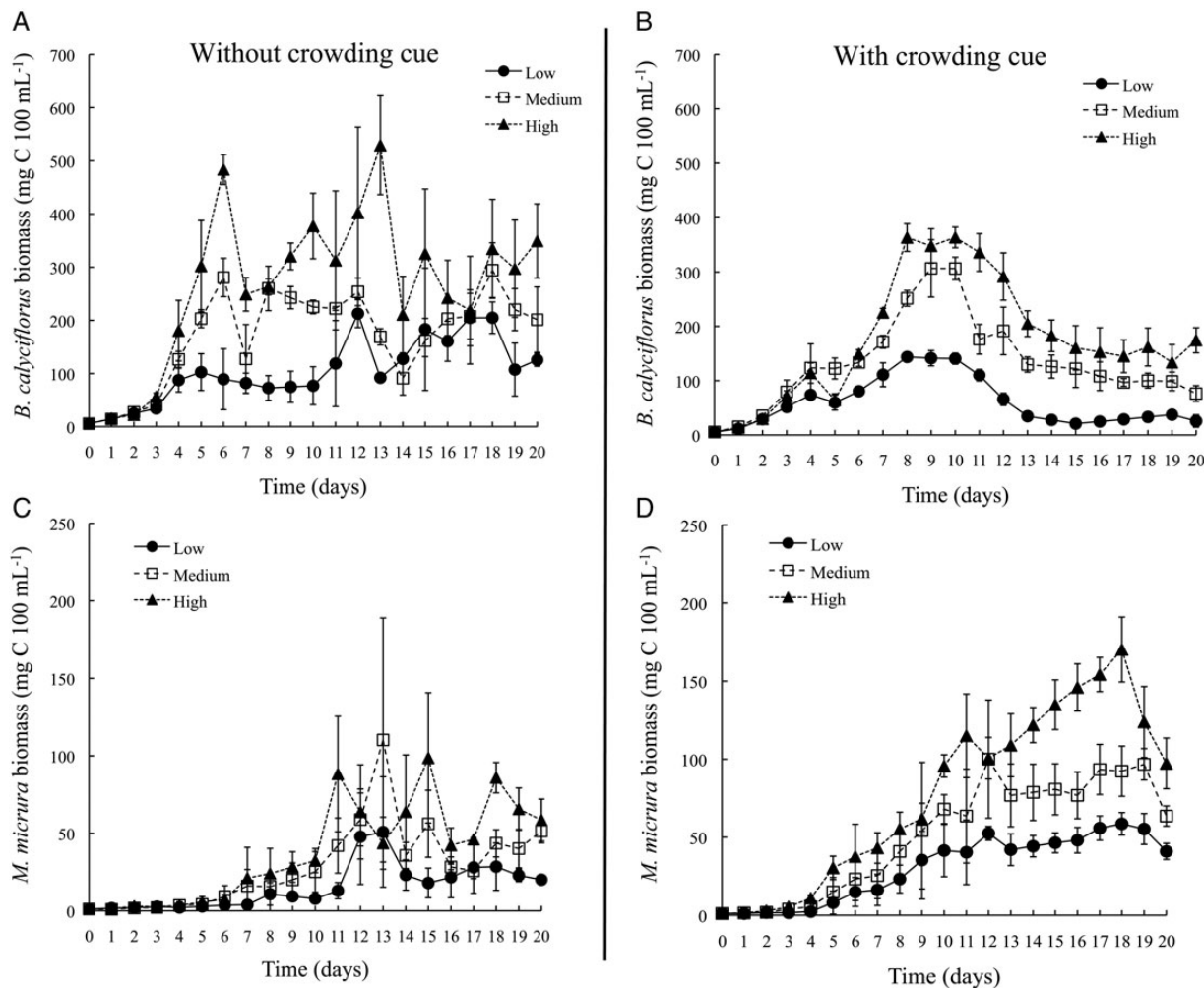
Fig. 3. Population biomass trajectories (mean $\pm$ 95% CI, values obtained on the basis of four replicates) of *Brachionus calyciflorus* (upper row) and *M. micrura* (lower row), without (**A** and **C**) and with (**B** and **D**) conspecific crowding cues through time, at food resource levels: low (5×10^5 cells mL^{-1}), medium (10^6 cells mL^{-1}) and high (2×10^6 cells mL^{-1}).

higher resource levels, *B. calyciflorus* produced fewer resting eggs under competition. Production of diapausing eggs of *M. micrura* did not differ between competition treatments, independent of the resource level (Fig. 5). Finally, exclusion rates of competitors were dependent on diapause induction by crowding cues. Without induction, *M. micrura* excluded *B. calyciflorus* at low and medium resource levels. In contrast, exclusion rates were null when production of resting eggs was induced (Fig. 6).

DISCUSSION

The results of our experiments show that the production of resting eggs by competing populations strongly affected the outcome of their short-term dynamics. For both competing species, addition of crowding cues was translated

into lower mean population density and more variable trajectories, of active individuals than when diapausing was not induced. Finally, in short-term competition *M. micrura* had higher probabilities to exclude *B. calyciflorus* at low and medium food levels; nevertheless, the probability of competitive exclusion was cancelled out by the addition of induction cues. This effect can be attributed to investment in mixis patterns exhibited by the competing species, which reduced the probability that *B. calyciflorus* was excluded by *M. micrura* and allowed it to persist despite its greater growth costs.

Previous records suggest high specificity of crowding stimulus for sexual reproduction in some *Daphnia* species and rotifers (Hobaek and Larsson, 1990; Gilbert, 2003). Crowding effects on sexual reproduction of *Daphnia* are attributable to unidentified chemicals produced by conspecific and congeners (Burns, 2000; Zadereev, 2003). In
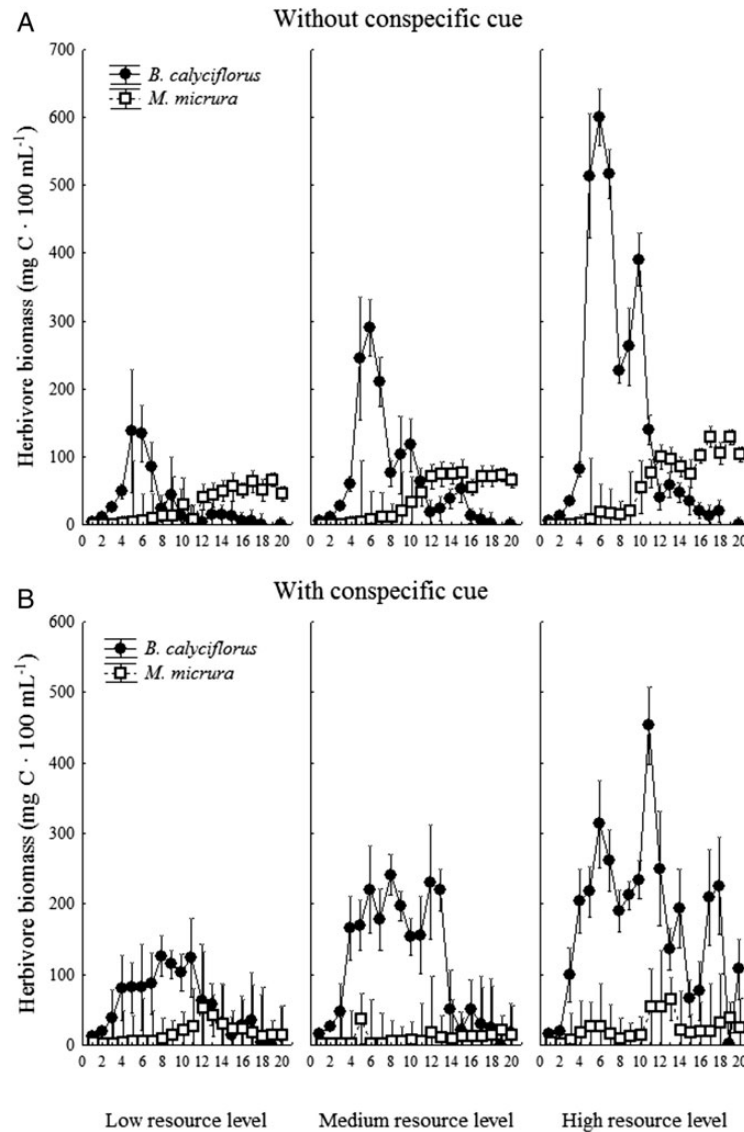
Fig. 4. Population biomass trajectories (mean ± 95% CI, values obtained on the basis of four replicates) of *B. calyciflorus* and *M. micrura* cultured together in media without conspecific crowding cue (**A**) and with conspecific crowding cue (**B**), in different levels of food resource: low (5 × 10^5 cell mL^{-1}), medium (10^6 cell mL^{-1}) and high (2 × 10^6 cell mL^{-1}).

our results, high concentration of *B. calyciflorus* cues inhibited appearance of males of *M. micrura*. To our knowledge, this is the first evidence that *Brachionus* crowding cues are perceived by *Moina*, and then, that recognition of non-congeneric competitor signals could have antagonistic effects on mixis. This inhibition of *M. micrura* males production could explain the equal diapause production by *Moina* when exposed to conspecific (a trigger for mixis) and heterospecific (inhibitor for mixis) cues, i.e. in competition experiments.

Sexual reproduction has been known to influence competitive outcomes through certain mechanisms such as density-dependent adjustment of sex ratio, sexual conflict between males and females or sexually transmitted diseases (Zhang and Hanski, 1998). Density-dependence of sexual populations would allow rare species to increase from low densities. This pattern is required, according to the invasibility criterion, for the coexistence of species in a community (Chesson, 2000). Montero-Pau and Serra (Montero-Pau and Serra, 2011) extended the coexistence mechanism proposed by Zhang and Hanski (Zhang and Hanski, 1998), incorporating density-dependent investment in sexual reproduction and diapause into a Lotka–Volterra competition model. Theoretically, if this
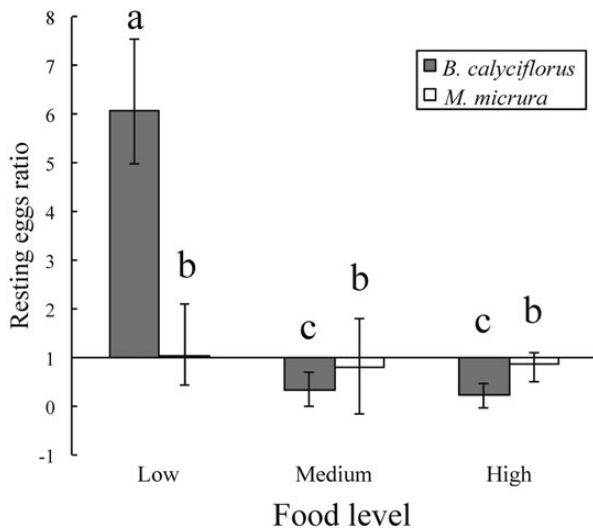
Fig. 5. Resting egg ratio between competing and alone condition, induced by adding conspecific crowding cue (estimate ± 95% CI, values obtained on the basis of four replicates). Significant differences between treatments are shown with different letters (ANOVA, $P < 0.05$).
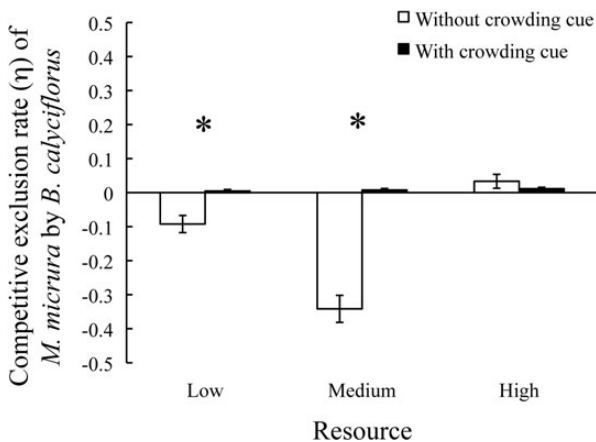
Fig. 6. Competitive exclusion rate η (estimate ± SE) in the competition experiment between *B. calyciflorus* and *M. micrura* at three levels of resource: low $(5 \times 10^5 \text{ cell mL}^{-1})$, medium $(10^6 \text{ cell mL}^{-1})$ and high $(2 \times 10^6 \text{ cell mL}^{-1})$. η values <0 represent competitive dominance of *M. micrura*, and η values >0 represent dominance of *B. calyciflorus*. Asterisks show significant differences between crowding cue presence (Holm−Sidak, $P < 0.001$).

density-dependent investment responds to species-specific stimuli, negative feedbacks could occur independently for each species, freeing up food resources that would be available for an inferior competitor, which would gain the opportunity to invade a resident population.

This effect cannot be explained without considering the costs of male production. Sexual reproduction incurs the so-called two-fold cost of males (Maynard-Smith, 1978), because a significant fraction of the offspring is typically composed of non-functional and short-lived males, which make no contribution to population growth. The cost of sex is common in cyclical parthenogens, to which must be added the need to locate mates (Cáceres and Tessier, 2004; Alver and Hagiwara, 2007). In addition, production of resting eggs involves the allocation of resources to the storage stage, typically as lipids in the form of oil droplets, to be used after hatching (Hairston *et al.*, 1995; Cáceres, 1997; Gilbert, 2004; Slusarczyk, 2009).

Our results can be explained in the light of the knowledge summarized above. We found a higher decrease in the average biomass of *B. calyciflorus* relative to its competitor *M. micrura* when diapause was induced, as expected from a higher cost of mixis. Investment in sex or diapause did not reveal cost for *M. micrura* in terms of reduced population growth when it was alone, but costs emerged when was competing. Density-dependent investment in sexual reproduction of *M. micrura* benefited *B. calyciflorus*, allowing coexistence. In our design, the mechanism proposed by Montero-Pau and Serra (Montero-Pau and Serra, 2011) was not proven, because both species were induced simultaneously and both started from low densities; therefore, the invisibility criterion was not assessed. Nevertheless, efficient exploitation of resources, and investment in diapause represented a greater cost to *Moina* in competition, which allowed persistence of the inferior competitor, *Brachionus*, which is in agreement with the predictions obtained by Montero-Pau and Serra (Montero-Pau and Serra, 2011).

Ciros-Pérez *et al.* (Ciros-Pérez *et al.*, 2002) is, to our knowledge, the only previous experimental study on zooplankters in which sexual reproduction has been linked with the outcome of competition. In agreement with our results, they showed that competitively inferior species invested more in sexual reproduction as a response to resource limitation. Nevertheless, and in contrast to our results, they found that this response could accelerate short-term competitive exclusion. This difference could be explained because they analyzed the competition between two closely related rotifer species, *B. rotundiformis* and *B. ibericus*, which are sibling species of the *B. plicatilis* complex. They exhibit great similarity in their morphology, and thus strong resource competition between them is expected (Ciros-Pérez *et al.*, 2001, 2002). Although the results of Montero-Pau and Serra (Montero-Pau and Serra, 2011) could explain coexistence of ecologically equivalent species such as cryptic species, the experimental results of Ciros-Pérez *et al.* (Ciros-Pérez *et al.*, 2001) and ours suggest that diapause could favor different exclusion patterns depending upon competition intensity. These hypotheses could be addressed in future extensions of this research.

Sexual reproduction leads to the production of resting stages, which have the potential to survive for decades or

centuries (Weider *et al.,* 1997; Cáceres and Hairston, 1998; García-Roger *et al.*, 2006), promoting long-term persistence of populations via overwintering and dispersal. Nevertheless, this long-term benefit has been believed to be compromised by the short-term coexistence of active individuals. Our results provide novel empirical evidence supporting that investment in sexual reproduction and diapause also promotes short-term coexistence of zooplankton species. Given that short-term coexistence of active animals allows massive production of resting stages, our study serves to link ecological processes occurring at different temporal and spatial scales under changing environmental conditions.

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