Toxic *Microcystis* (cyanobacteria) inhibit recruitment of the bloom-enhancing invasive bivalve *Limnoperna fortunei*

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SUMMARY

1. Toxic cyanobacterial blooms and biological invasions are major threats to freshwater systems worldwide. While usually dealt with independently, the two threats can interact to produce synergistic or antagonistic outcomes. The aim of this survey is to analyse interactions between the cyanobacterium *Microcystis* spp. and the Asian invasive mussel *Limnoperna fortunei*.

2. On the basis of 9 years of observational data in a large subtropical reservoir (Salto Grande, Argentina–Uruguay), we analyse causal relationships between recurring summer–early autumn blooms of *Microcystis* spp. and recruitment by *L. fortunei*. Reproduction of the mussel was interrupted during dry summers (January–April), coinciding with periods of peak *Microcystis* spp. growth and low water discharge (which favours build-up of algal biomass). On the other hand, wet summers with high discharge rates were characterised by low *Microcystis* spp. densities and high numbers of *L. fortunei* larvae in the water column.

3. Of the seven South American waterbodies investigated, Salto Grande was the only one with very marked cyanobacterial blooms and where larval numbers decrease to near zero during January–April; in all others, reproduction peaks in January–April.

4. The assumption that microcystin-producing algae are responsible for these troughs during periods when elsewhere larvae are very abundant was reinforced by experimental results indicating that microcystin-LR is highly toxic to the mussel's larvae, eliminating 58–100% of animals in 48 h at 10–20 μg L^{-1}.

5. Paradoxically, high concentrations of microcystin in water are probably partly due to *L. fortunei*'s own activity, which enhances growth of *Microcystis* spp. through modification of nutrient concentrations, selective grazing of solitary *Microcystis* spp. cells over colonial ones and production of chemical cues that trigger the formation of colonies.

6. These interactions have important implications for the management of biofouling of industrial raw cooling water facilities by the byssate mussels, as well as policies oriented at curtailing the spread of the invasive bivalve.

Keywords: bivalves, cyanobacteria, invasive species, larvae, *Limnoperna fortunei*, microcystin, *Microcystis* spp., reservoirs, toxic algal blooms, water quality

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1

Introduction

During recent decades, biological invasions by non-indigenous species have become one of the most widespread and challenging issues threatening habitats worldwide. Freshwater ecosystems are among the most severely affected by this problem (Dextrase & Madrak, 2006), and *Dreissena* species provide an outstanding northern hemisphere example. Their presence has profoundly influenced a wide range of properties, including water clarity, subsurface water temperature, nutrient concentrations and proportions, dissolved oxygen, phytoplankton and zooplankton abundance and composition, pelagic and benthic food webs, abundance and composition of benthic fauna (Karatayev *et al.*, 2007; Kelly, Herborg & MacIsaac, 2010).

Despite its remote geographical location, which results in low propagule pressure (i.e. the size and frequency of inoculations of aquatic invasive species; Cassey & Blackburn, 2005; Boltovskoy, Almada & Correa, 2011), Argentina is not immune from such introductions. About 1990, a freshwater byssate mussel, *Limnoperna fortunei*, was first recorded in the Río de la Plata estuary, Argentina (Pastorino *et al.*, 1993). This species was most probably introduced as a result of improper ballast water management by ships trading with South-East Asia, its native range. Twenty years later, this mussel had spread through five South American countries (Argentina, Uruguay, Brazil, Paraguay, Bolivia) to inhabit the entire Paraná-Uruguay basin at densities of up to $200\,000\ \mathrm{m^{-2}}$ or more (Boltovskoy *et al.*, 2006). Because of this fast colonisation (chiefly aided by the animal's wide environmental tolerance and its ability to travel upstream attached to ship's hulls), the number of transport hubs for further dispersion continues to grow exponentially. Several reports have suggested that *L. fortunei* can be expected to spread to Europe and North America in the near future (Ricciardi, 1998; Boltovskoy *et al.*, 2006; Oliveira, Hamilton & Jacobi, 2010), where it can be expected to successfully compete with *Dreissena polymorpha* due to its higher tolerance of adverse conditions (higher water temperature, higher pollution levels, lower calcium requirements; Karatayev *et al.*, 2007). Many of the known impacts of *L. fortunei* mimic those of *D. polymorpha*, but some differences have also been described. Among the latter, a strong facilitating effect of cyanobacterial blooms, which does not seem to depend on total phosphorus levels (as in *D. polymorpha*), warrants concern (Cataldo *et al.*, 2012b).

The increasing magnitude and recurrence of cyanobacterial blooms, due to river damming and growing nutrient input, are usually associated with production of the toxic peptide, microcystin, responsible for massive fish and bird mortalities, human and animal poisoning, liver cancer, disruption of pelagic food webs and plankton–benthos trophic coupling, changes in nutrient cycling and reductions in diversity (Paerl *et al.*, 2001). Traditionally, the impacts of cyanobacterial blooms and freshwater invasive mussels have been addressed separately. However, work in the mid-1990s showed that the alga and the mussel have complex interactions. Several studies showed that nutrients recycled by the mussel can significantly enhance cyanobacterial growth (Vanderploeg *et al.*, 2002; Knoll *et al.*, 2008; Sarnelle *et al.*, 2010; Cataldo *et al.*, 2012a,b). On the other hand, the effect of cyanobacterial toxins on filter-feeding invasive mussels has been less investigated, and the results are conflicting. While some authors concluded that zebra mussels fed *Microcystis* spp. show significantly reduced grazing and acute irritant responses (Juhel *et al.*, 2006a,b), others found no evidence of negative selectivity, to the point of suggesting that the mussel could effectively control cyanobacterial blooms (Dionisio Pires, Ibelings & van Donk, 2010).

The high visibility of *L. fortunei*, associated with its strong negative impact on industrial and power plants (where its growth in raw cooling water conduits leads to severe clogging, pressure loss and efficiency reduction problems, Perepelizin & Boltovskoy, 2011), has prompted many surveys, both in South America and in Japan (where it also invaded around 1990). One aspect receiving substantial attention has been the seasonal reproductive activity of *L. fortunei*. Surveys of the abundance of its planktonic larvae in the water column indicate that production of offspring is continuous between spring and autumn, peaking in summer and is interrupted by a 4–6 months resting period centred on winter (Rojas Molina & José de Paggi, 2008; Boltovskoy *et al.*, 2009b; Nakano, Kobayashi & Sakaguchi, 2010; Eilers, Oliveira & Roche, 2011).

In contrast to other studied waterbodies, the large subtropical reservoir Salto Grande has consistently exhibited a different pattern, characterised by marked drops in larval abundance in summer–early autumn. In this study, we report the results of 9 years of weekly monitoring in Salto Grande. Our aim was to assess causal relationships between recurring summer–early autumn blooms of *Microcystis* spp. and recruitment by *L. fortunei*. We also ran a laboratory experiment to determine the tolerance of mussel larvae to microcystin.

Methods

Regional setting

Data for this survey were collected in Salto Grande, a large (750 km^2, mean depth: 6.4 m, maximum depth: 35 m), eutrophic (mean total phosphorus: 40 µg L^{-1}, chlorophyll *a*: 14.8 µg L^{-1}; O'Farrell, Bordet & Chaparro, 2012) subtropical reservoir produced by damming of the Uruguay River in 1979 (Fig. 1). Surface water temperature varies seasonally between about 12 and 30 °C. Shallow coastal waters are usually warmer than those in the central channel, especially close to the dam. The reservoir is polymictic, with brief stratification gaps during low discharge periods. Average yearly water discharge is about 5000 m^3 s^{-1}, albeit with pronounced seasonal variations. The driest months are January–March (mean for 1950–2011: 3186 m^3 s^{-1}), while highest flows are in June–November (6176 m^3 s^{-1}) (Fig. 2). Over 95% of the input is provided by the Uruguay River. Mean water retention time is around 2 weeks (Chalar, 2006), but during low-water periods, this can increase to 9 weeks or more (O'Farrell *et al.*, 2012). Summer cyanobacterial blooms are a recurrent phenomenon in this waterbody (Beron, 1990; Chalar, 2006), especially in embayments along its western margin (O'Farrell *et al.*, 2012). Microcystin concentrations in the water have been assessed irregularly since 1999, yielding values of up to 200 µg L^{-1} or more (Chalar *et al.*, 2002; Giannuzzi *et al.*, 2011; Comisión Técnica Mixta Salto Grande, unpublished monitoring data). Downstream from the reservoir, in the Uruguay River and Río de la Plata estuary, toxicity values over three orders of magnitude higher than the World Health Organization limit for recreational water have been detected (Saizar *et al.*, 2010; Pirez *et al.*, 2013). These blooms have been associated with frequent massive fish mortalities, as well as episodes of gastroenteritis, allergic reactions and even acute poisoning in humans (Giannuzzi *et al.*, 2011).

Fig. 1 Map of Salto Grande reservoir and location of phytoplankton sampling sites (samples collected at each site in parentheses). Inset: map showing location of Salto Grande reservoir and of other long-term survey sites for *Limnoperna* larvae (triangles).

Microcystis spp

Phytoplankton data were obtained in the framework of the environmental monitoring programme carried out by the Joint Technical Commission of Salto Grande (CTM) and the Uruguay River Management Commission (CARU). Density estimates of *Microcystis* spp. are based on samples collected at 17 sites throughout the reservoir (Fig. 1) during spring–autumn of 2007–12 (9 January 2007–11 December 2012). Samples were collected weekly (not all sites were sampled every week; on average, each site was sampled 67 times; range: 35–99; SD: 18.7) at 20 cm depth, preserved with 1% Lugol's iodine solution and counted in two replicate sedimentation chambers under an inverted microscope. Dense phytoplankton scums were hot digested with sodium hydroxide to disintegrate mucilage-bound colonies (Reynolds & Jaworski, 1978) and isolated cells counted with a Neubauer hemocytometer (0.1 mm deep) under the light microscope. In total, 1148 phytoplankton samples were quantified. O'Farrell *et al.* (2012) published a general analysis of the recurrence of blooms in Salto Grande based partly on these plankton samples and ancillary environmental data (temperature, pH, conductivity, dissolved oxygen, transparency, nutrients, chlorophyll) collected in the course of the same sampling programme.
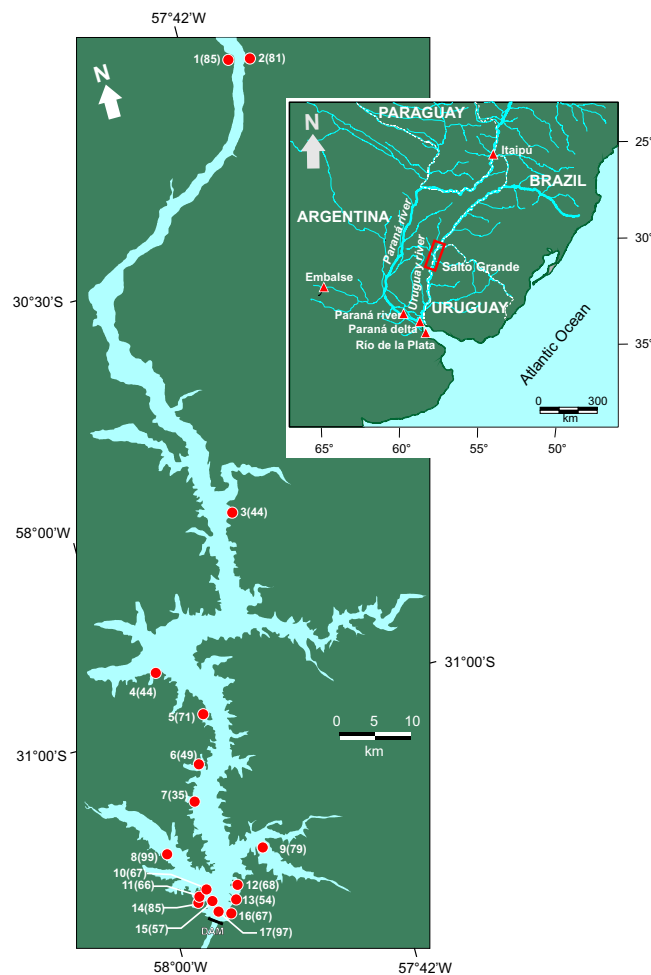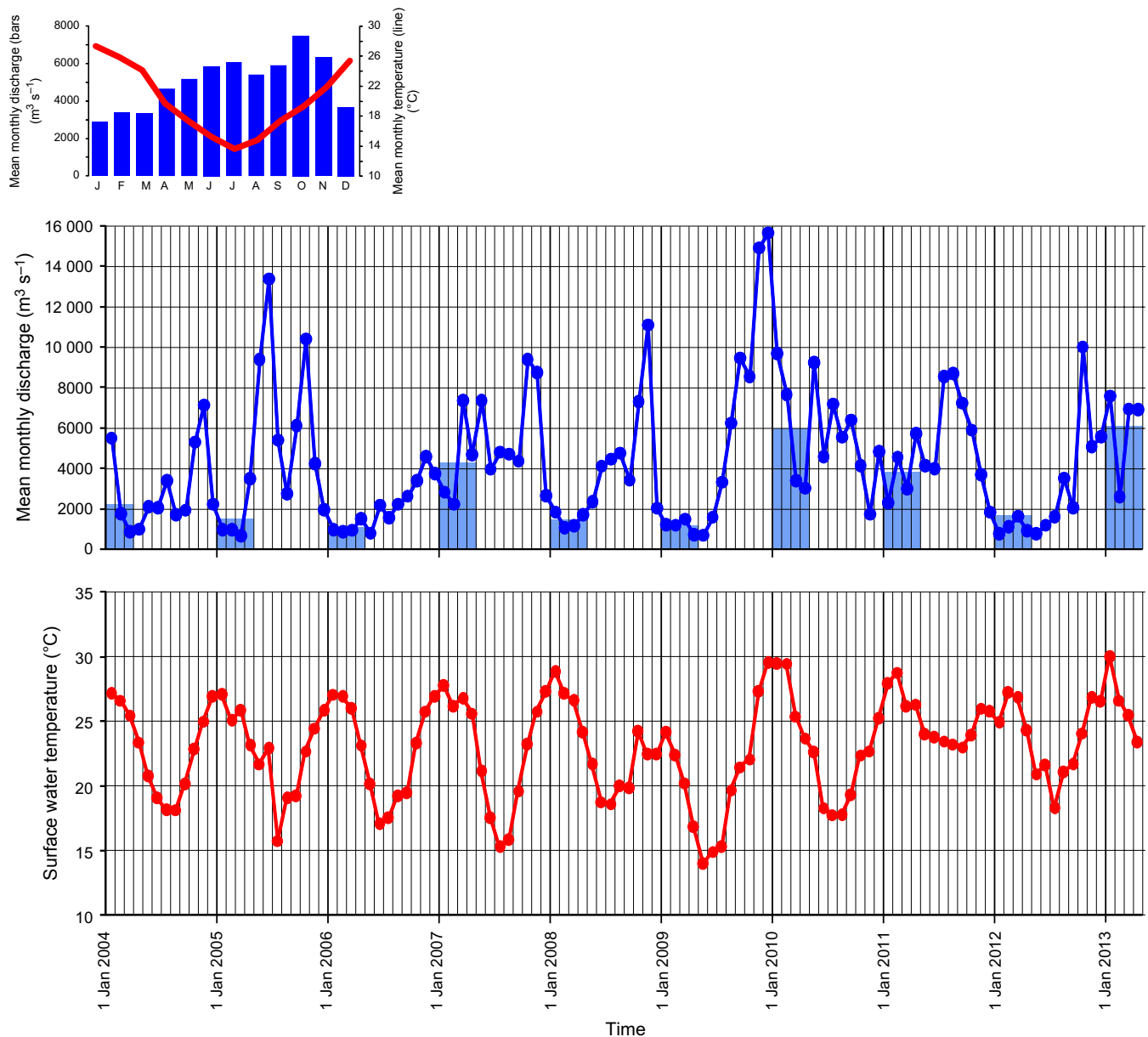
Fig. 2 Water discharge into Salto Grande reservoir from the Uruguay River (>95% of the water received by the reservoir). Grey bars denote means for January–April (upper panel), and water temperature in the main channel (3-point running mean, lower panel), between January 2004 and April 2013. Inset: historical (1950–2011) mean monthly discharge and water temperature.

Crustaceans and mussel larvae

Between 14 June 2004 and 28 January 2013, weekly plankton samples for abundance estimates of *L. fortunei* larvae, cladocerans and copepods were obtained at the dam of the hydroelectric power plant Salto Grande. Preliminary assessments of variability in larval abundance estimates based on samples taken at different sites in this reservoir (five different sampling sites) and in Itaipú reservoir (six sampling sites) indicated that between-site differences are statistically non-significant

(ANOVA, $P > 0.939$) and within the expected ranges of normal plankton patchiness. In total, 367 samples were collected and analysed with a mean sample-to-sample interval of 8.6 days. A single major gap in this series occurred in the winter of 2011 (2 May–5 October 2011); however, because larval densities during this time of the year are consistently very low (see below), and our analyses focus on recruitment during the summer months, we contend that these missing values did not affect our conclusions. Suspended particles were concentrated by filtering 2000 L through a 0.025-mm mesh plankton net,

preserved with 5% formaldehyde and stored in plastic jars. In the laboratory, samples were fractioned with a Folsom plankton sample splitter and zooplankton abundances were estimated under a binocular microscope counting between about 1 and 100% of the original sample (depending on the number of organisms retrieved). On average, 127 *L. fortunei* larvae per sample were counted (maximum: 1150, minimum: 0; estimated error: ±27%), 80 copepods (max.: 800, min.: 0; est. error: ±30%) and 28 cladocerans (max.: 360, min.: 0; est. error: ±43%). All samplings are the result of routine monitoring programmes by CTM-CARU. Hydrological data were provided by CTM.

Time series of larval abundance were also obtained in several other Argentinian and Brazilian freshwaterbodies, including both lotic (Río de la Plata estuary, Paraná river) and lentic environments (Itaipú, Embalse), spanning at least 12 months each (reported in Boltovskoy *et al.*, 2009a; see Fig. 1 for locations). Samples were obtained from cooling system lines of nuclear (Atucha, Embalse) and hydroelectric power plants (Itaipú), or with the aid of a centrifugal submersible pump (Río de la Plata, Delta), filtered through a 0.07-mm mesh plankton net and preserved with 5% formaldehyde. Further treatment and processing were similar to those indicated above for Salto Grande materials.

Exposure of mussel larvae to microcystin-LR

Tolerance of mussel larvae to microcystin was assessed through laboratory exposure experiments. Microcystin-LR (25 μg mL^{-1} in methanol) was acquired from Jena Bioscience GmbH (Jena, Germany) and stored at −20 °C until utilisation. *Limnoperna fortunei* larvae were collected with short plankton net tows in the Río de la Plata estuary, off Buenos Aires, in February 2013, and kept in aerated 10-L vessels with dechlorinated tap water until used (within 3 h of collection). Batches of 10 veliger larvae were gently transferred with a pipette to 10-mL experimental vials with a solution of microcystin in dechlorinated tap water at concentrations of 0, 0.5, 1, 2, 5, 10, 20 and 30 μg L^{-1} at 27 °C. Vials were checked at 2, 4, 6, 12, 24, 48, 72 and 96 h, withdrawing dead larvae. About 80% of the solution was replaced in all vials at 24-h intervals. All concentrations were tested in four replicates (total larvae exposed at each concentration: 40). To discount the potential effects of methanol (the solvent used in the microcystin concentrated solution) on larval mortality, we used two additional experimental vials with 10 larvae each where analytical grade methanol was added in the same proportion as that in

the highest microcystin concentration (30 μg L^{-1}). All other conditions were identical to those described previously.

Results

Hydrological regime

During January 2004–April 2013, water input to the reservoir varied between 434 and 29 730 m^{-3} s^{-1}. Seasonal cycles generally followed the historical trend, with high discharge rates during the winter to late spring (June–November) and drought periods in the summer–autumn (December–March; Fig. 2). However, in 2010, and again in 2013, this pattern changed markedly. The high water input starting around October 2009 persisted throughout most of 2010 (Fig. 2), a year characterised by the highest discharge values (37–451% higher than any other year in this period) and by the fact that the months of January–April accounted for the highest proportion of total discharge for the whole year (35%, as opposed to 7–27% for 2004–09 and 2011; Fig. 2). In 2012, the onset of the rainy season was delayed, and the very high discharge rates that started in October persisted until April 2013 (the last measurement available; Fig. 2). The summer–autumn of 2007 was also different from other years in that its dry season, which usually extends until mid to late April (Fig. 2), was punctuated by a wet pulse around mid-March (the discharge rate went from 2200 to 2800 m^3 s^{-1} in January and February to 7400 m^3 s^{-1} in March; Fig. 2).

Microcystis spp

Microcystis spp. densities varied widely, both spatially and temporally. Highest values occurred along the western, shallow beaches, tributary inlets and embayments (Stations 8, 10, 11, 14, 15 in Fig. 1) in January–April (Fig. 3). With the exception of 2010 (no data available for 2013), over 35% of the samples collected in these months yielded *Microcystis* spp. densities above 20 000 cells mL^{-1}, and about 10% above 100 000 cells mL^{-1} (values proposed by the World Health Organization as indicative respectively of moderate and high risk of acute health effects due to recreational exposure; Bartram *et al.*, 1999). The summer–autumn of 2007 was unusual with the cyanobacterial bloom declining earlier than usual, around the second week of March, coinciding with a strong discharge pulse (see above; Fig. 2). Unfortunately, this drop is poorly represented in our database because only 10 of 105 phytoplankton samples collected
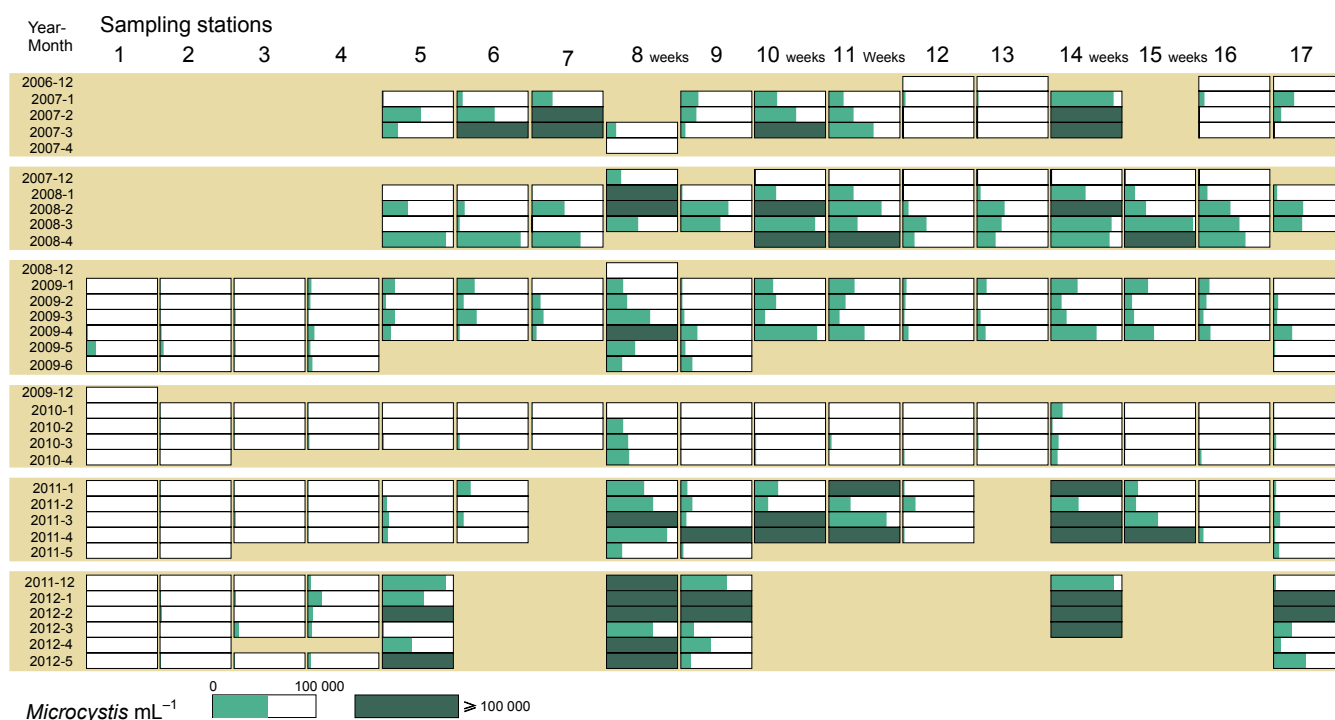
Fig. 3 *Microcystis* densities at the 17 sampling sites surveyed between December 2006 and May 2012 (each value is the average of up to five samples; see Fig. 1 for sampling locations). Stations denoted with 'W' are those along the western coast of the reservoir where highest *Microcystis* spp. densities occur.

in summer–autumn 2007 were obtained in late March, and only one in April. These few data points indicate that, on average, *Microcystis* spp. densities decreased from about 50 000 cells mL^{-1} (January–early March) to 14 000 cells mL^{-1} (in late March–April). In contrast to this pattern, 2010 was characterised by significantly lower densities of cyanobacteria (Kruskal–Wallis test, $P = 0.0001$): on average for the entire reservoir, in the summer–autumn of 2010, *Microcystis* spp. cells were 13–116 times less abundant than in any other summer (Fig. 3). Density estimates at the 17 sites throughout the reservoir (Fig. 1) indicate that the summer of 2010 (January–April) yielded the lowest (14 sites) or the second lowest (two sites) values for the 2007–12 period. In January–ucode>April 2010, *Microcystis* spp. densities never exceeded 65 000 cells mL^{-1}, and values above 20 000 were recorded in only 4% of the 196 samples.

Mussel larvae

During all the years monitored, *L. fortunei* larvae showed a strong winter (May–June to September–October) drop in density to levels below 100 m^{-3} (Fig. 4). Around September–October, densities recovered

to about 4000 ind. m^{-3}. During summer to early autumn (January–April), the pattern varied among years: in 2005, 2006, 2008, 2009, 2011 and 2012, abundances were very low, usually below 1000 ind. m^{-3}. In contrast, in January–April 2010 (mean: 12 413 ind. m^{-3}) and in January 2013 (mean: 23 413 ind. m^{-3}), values were very high (Fig. 4). In 2007 January–February, values were low as usual, but in March–April, there was a moderate peak. Interannual changes in larval densities were significantly correlated with the corresponding discharge values (Fig. 5).

Comparison of variations in the densities of larvae with those of *Microcystis* spp. cells for the summer–autumn of 2007–12 shows that during periods of strong cyanobacterial blooms, larvae were very scarce, whereas at times of low *Microcystis* spp., abundance *L. fortunei* recruitment was similar to those typical of the other waterbodies investigated (Figs 4 & 6) (r^2: 0.672, $P < 0.001$; excluding 2007 from the analysis the coupling is even stronger, r^2: 0.830, $P < 0.001$). A more detailed analysis, based on bi-weekly averages of *Microcystis* spp. and *L. fortunei* larvae densities, reinforces the above conclusion suggesting that abundance variations within each warm period mimic the interannual trend (Fig. 7).
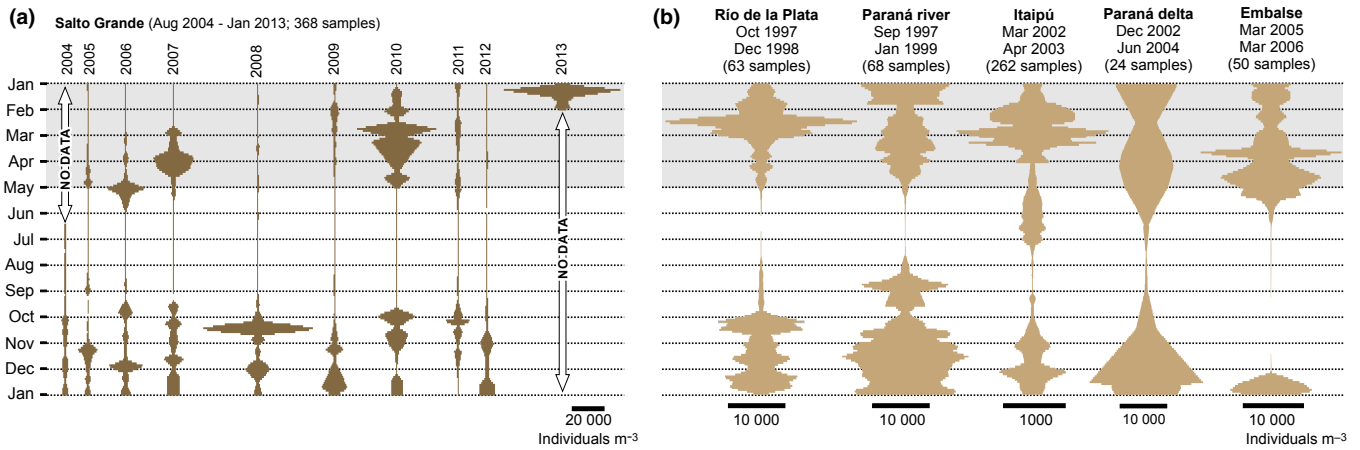
Fig. 4 (a) Densities of *Limnoperna fortunei* larvae in Salto Grande between 2004 and 2013, and (b) in other Argentine freshwater bodies at different times (profiles of daily values generated by lineal interpolation of sampling data, overlapping periods for consecutive years averaged). The period January–April (at top) is highlighted in grey.
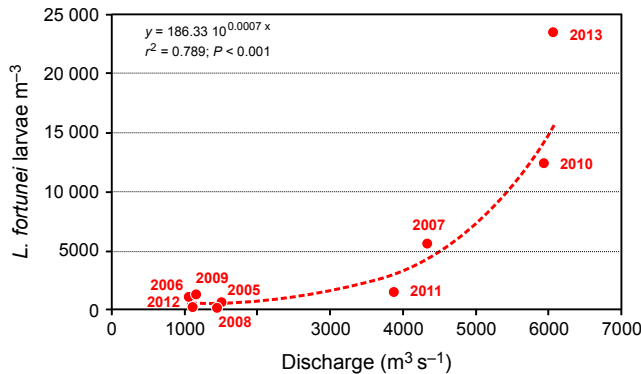
Fig. 5 Regression function between mean discharge rates and *Limnoperna fortunei* larvae for January–April of the years 2005–13 (2013 data point is for January only).
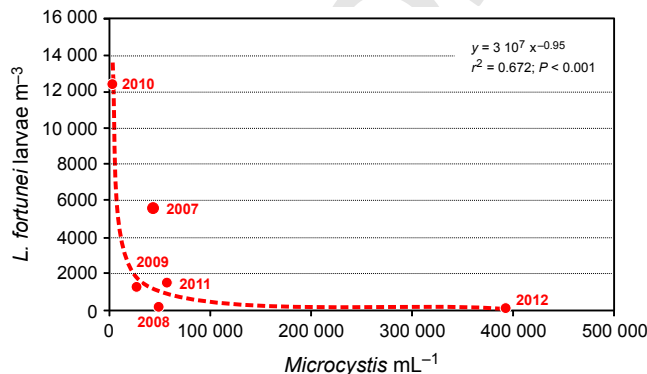

Fig. 6 Regression function between mean January–April abundances of *Microcystis* spp. cells and *Limnoperna fortunei* larvae for the years 2007–12.

Copepoda and Cladocera

Crustacean numbers were relatively high during most of the year, with the exception of the winter months (May–June to August–September), when they dropped noticeably, although, unlike *L. fortunei* larvae, rarely disappeared from the water column altogether (Fig. 8). In contrast to the larvae, during January–April crustacean densities were normally high, both in 2010 and the other years. A remarkable contrast with the mussel's larvae is that crustaceans were generally less abundant in January–April 2010 than in the same periods in 2005–09 and 2011–12 (Fig. 8), but these differences were not significant (Copepoda, ANOVA based on ln-transformed data $P = 0.0002$, orthogonal contrast for 2010, $P = 0.194$; Cladocera, Kruskal–Wallis test $P = 0.0058$, contrasts for 2010, $P = 0.351$).

Tolerance of mussel larvae to microcystin-LR

No mortality was observed in the controls, at 0.5 µg L^{-1}, or in exposures to methanol alone (Fig. 9). At microcystin-LR concentrations of 1–20 µg L^{-1}, mortality after 96 h was linearly related to the amount of microcystin in the solution, with the highest concentrations (20 and 30 µg L^{-1}) yielding 100% mortalities in 2 days (Fig. 9).

Discussion

Abundance cycles of *L. fortunei* larvae in the water column indicate that the mussel has an extended (7–10 months) reproductive period spanning from spring to autumn, and a single relaxation phase centred
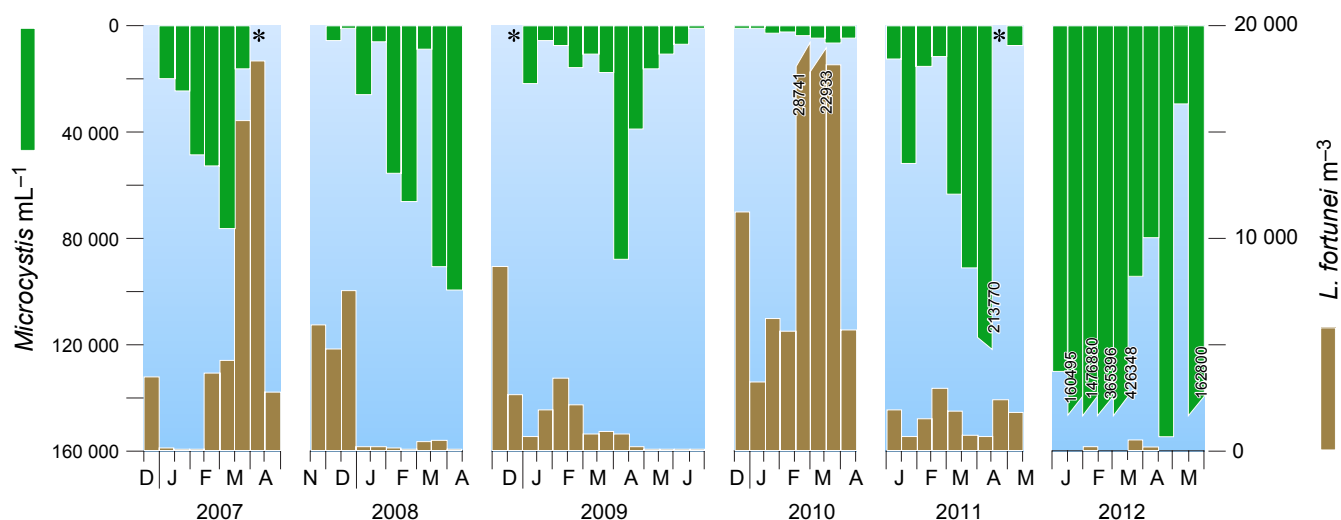
Fig. 7 *Microcystis* spp. and *Limnoperna fortunei* densities in the summer–autumn periods of 2007–12, as shown by averaged bi-weekly data.
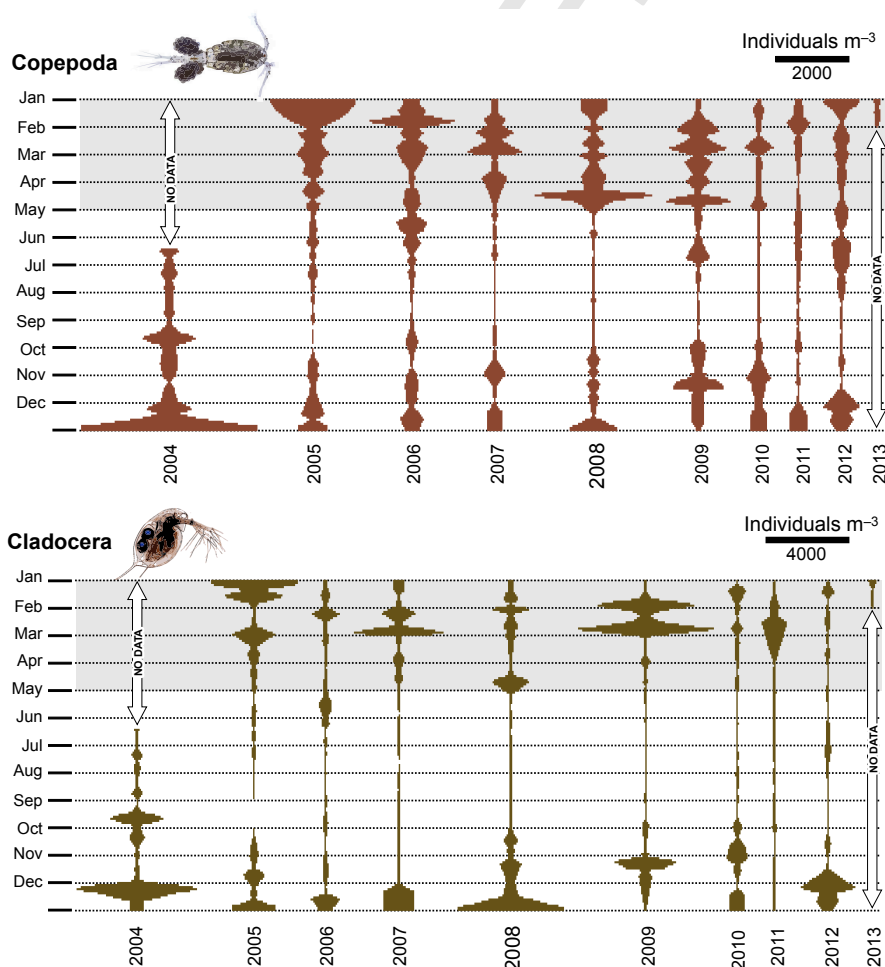

Fig. 8 Densities of Copepoda and Cladocera in Salto Grande between 2004 and 2013. The period January–April (at top) is highlighted in grey.
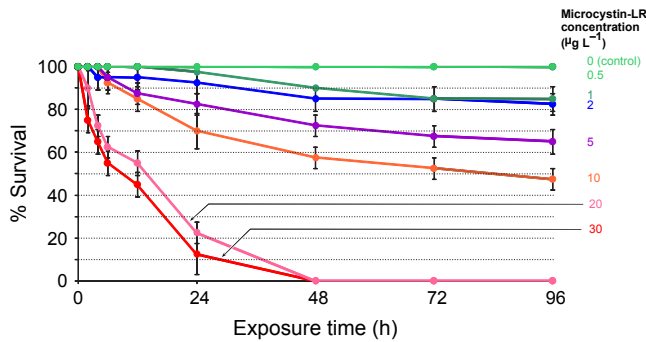
Fig. 9 Mortality rates of *Limnoperna fortunei* larvae exposed to different concentrations of microcystin-LR (each data point shows the average and the standard deviation of four replicates).

on the winter. This pattern is characteristic of all the sites surveyed in Argentina (Cataldo & Boltovskoy, 2000; Rojas Molina & José de Paggi, 2008; Boltovskoy *et al.*, 2009b) and elsewhere, including Brazil (Eilers *et al.*, 2011) and Japan (Nakano *et al.*, 2010), with the sole exception of Salto Grande reservoir. Here, in addition to the winter drop, there was a very marked decline in January–April. During the 9 years monitored in Salto Grande, the only summers with very high larval densities were 2010 and January of 2013 (in January–February

2007, larvae were scarce as usual, but peaked shortly in March–April). We contend that this diverging pattern reflects massive larval mortalities induced by the recurrent toxic cyanobacterial blooms facilitated by the low water discharge rates characteristic of the normally dry summer months (Fig. 10, left panel). During rainy summers, on the other hand, high flushing rates hinder cyanobacterial build-up and, hence, microcystin concentrations, thus allowing for normal larval survival (Fig. 10, right panel).

The waterbodies in which the reproduction of *L. fortunei* was investigated differ in several ways (temperature, food availability, predator diversity, water transparency, etc.), but the only one that clearly sets Salto Grande apart are its extremely strong summer–early autumn phytoplankton blooms (January–April), dominated by the cyanobacteria *Microcystis* spp. and, occasionally, *Dolichospermum* (=*Anabaena*) sp. (Beron, 1990; Chalar, 2006; O'Farrell *et al.*, 2012). Strong blooms were recorded throughout Salto Grande reservoir in January–February of 2007, and in January–April 2008, 2009, 2011 and 2012. In all these periods, *L. fortunei* larvae were very scarce in the water column. In 2007, larvae appeared in the water in late March–April, coinciding with an unusually early bloom decline, most probably caused by a peak in water discharge levels. In 2010, on the other hand,
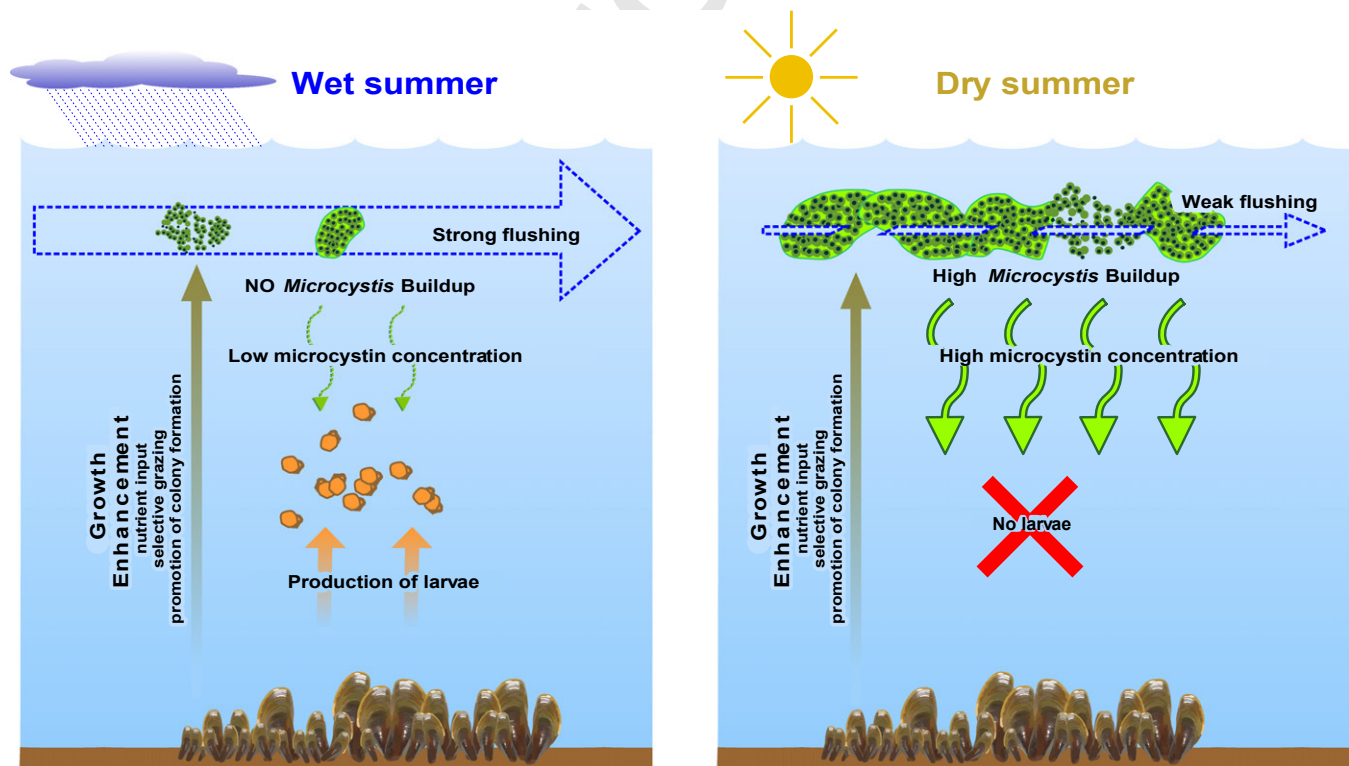

Fig. 10 Schematic diagram of proposed cause–effect relationships between *Microcystis* spp. blooms and *Limnoperna fortunei* reproduction.

recruitment was very high during the summer–autumn, as is normal for all other waterbodies investigated. Average densities of *Microcystis* spp. during this period (3390 cells mL^{-1}) were significantly lower than those during any other summer–autumn period (27 594 cells mL^{-1} in 2009 to 392 614 cells mL^{-1} in 2012). Larval densities were also very high in January 2013, coinciding with unusually high discharge rates (no phytoplankton data are available after 2012).

Our results suggest that these blooms of *Microcystis* spp. are responsible for the virtual absence of mussel larvae in the reservoir during the summer, which is supported by the significant correlation between mean numbers of *Microcystis* spp. cells and *L. fortunei* larvae for the five periods monitored. The assumption of this cause–effect relationship between abundance of *Microcystis* spp. and *L. fortunei* larvae is supported by the outcome of our experiments of the tolerance of larvae to algal toxins. At microcystin-LR concentrations of 10 µg L^{-1} or more, larval mortality was 57–100% after 2–4 days. These levels of dissolved microcystin in water are common during bloom periods in Salto Grande. The impact of the toxin may not be limited to survival of larvae, but also involve other aspects of mussel reproduction, such as gamete production and survival, fertilisation or hatching (Li *et al.*, 2008; Smith *et al.*, 2008; Lance *et al.*, 2011).

Larval densities recover after the bloom, which indicates that the adult population survives through these adverse periods (many adult bivalves, including *L. fortunei*, are quite tolerant to microcystin: von Rückert, Souza Campos & Rolla, 2004; Hwang *et al.*, 2010; White *et al.*, 2011; Gazulha *et al.*, 2012). However, it is conceivable that other physiological and/or behavioural traits indirectly associated with the production of larvae are hindered by the toxic. In the presence of toxic cyanobacterial strains, the filtering activity of the mussel is impaired significantly (Boltovskoy *et al.*, 2009a). These results agree with several reports where toxic cyanobacteria were found responsible for significantly lower grazing rates by zebra mussels and acute irritant responses (Juhel *et al.*, 2006a,b; but see also Dionisio Pires *et al.*, 2010).

Microcystis spp. blooms, in turn, depend primarily on the hydrological regime of the reservoir: normal (dry) summers are associated with high water retention times, especially in closed inlets and embayments where stagnant waters and high temperatures lead to the development of strong vertical stratification that favours *Microcystis* spp. build-up. Rainy summers, on the other hand, are associated with more active flushing and, therefore, less accumulation of phytoplankton cells (O'Farrell *et al.*, 2012; Rangel *et al.*, 2012). The summer of 2010, when *Microcystis* spp. densities were lowest, was characterised by extremely high water discharge rates between November 2009 and February 2010. In 2007, the situation was somewhat different, but also indicative of a strong discharge-*Microcystis* spp. coupling. In January–February, water input was low and *Microcystis* spp. densities were high. In mid-March, a rather strong discharge pulse took place and *Microcystis* spp. abundances dropped to values 1.3–23 times lower than before the pulse. For January 2013, which also had very high discharge rates, no phytoplankton data are available, but larval densities were very high, which suggests that *Microcystis* spp. was scarce. We conclude that because they hinder *Microcystis* spp. accumulation, discharge rates are positively associated with the abundance of *L. fortunei* larvae during the summer–autumn. Peaking in association with high discharge rates is an unusual behaviour for planktonic organisms, especially in subtropical floodplain waterbodies where high-water periods are associated with lower (rather than higher) planktonic abundances (José de Paggi & Paggi, 2007; Chaparro *et al.*, 2011), as observed for the crustaceans in our samples.

Paradoxically, the cyanobacterial blooms that inhibit *L. fortunei* reproduction are enhanced by the mussel itself. Experimental results of Cataldo *et al.* (2012b) showed that the presence of the mussel favours growth of *Microcystis* spp. through at least three mechanisms: (i) modification of nutrient concentrations and the N : P ratio, (ii) selective grazing of solitary *Microcystis* spp. cells over colonial ones and (iii) production of chemical cues that trigger the formation of colonies (Fig. 10). In a 35-day mesocosm experiment carried out in Salto Grande, Cataldo *et al.* (2012b) found in the presence of *L. fortunei* that there were very significant increases in overall *Microcystis* spp. biomass, in the proportion of *Microcystis* spp. cells included in colonies and in the size of these colonies. The association between the invasive mussel and cyanobacterial blooms is probably further reinforced by the fact that mussel beds are most dense and widespread in coastal, shallow areas (hard substrates necessary for the mussels' attachment are restricted to the shallow, coastal fringe, whereas deeper areas are covered with soft sediments, and therefore unfit for these sessile animals), where conditions are particularly favourable for the development of blooms (increased stagnancy, high water temperature, high external nutrient input).

As opposed to mussel larvae, crustaceans do not seem to be visibly affected by *Microcystis* spp.: the densities of

copepods and cladocerans were high during all the summers surveyed. During summers when *Microcystis* spp. was scarce (in January–February 2010 and, presumably, in January 2013), their numbers were somewhat lower (rather than higher) than when cyanobacteria were abundant; this difference most probably reflects the faster flushing and higher dilution associated with wet summers with scarce *Microcystis* spp. While microcystin-producing cyanobacteria are harmful to most organisms, their effects on zooplankton are controversial (Tillmanns *et al.*, 2008; Davis & Gobler, 2011). Several surveys have shown that freshwater crustacean zooplankton can tolerate rather high concentrations of microcystin (Ger, The & Goldman, 2009), or adapt to extended periods of *Microcystis* spp. exposure by increasing selective feeding on alternative food (Ger, Panosso & Lurling, 2011). Furthermore, experimental results indicate that small-sized zooplankton grazers are significantly more resistant to *Microcystis* spp. blooms than large ones (Guo & Xie, 2006), to the point that high microcystin concentrations in lakes are negatively correlated with *Daphnia* and calanoid copepods, but positively correlated with smaller phytoplankton feeders, such as cyclopoid copepods and *Bosmina* (Hansson *et al.*, 2007). Because our crustacean data do not include specific identifications, it is conceivable that in Salto Grande, dominant species differ between bloom and non-bloom periods.

While it may be tempting to speculate that by boosting *Microcystis* spp. the invasive mussel triggers self-limitation mechanisms aimed at shaping its own predator–prey dynamics, a key element needed to support this notion is missing: with the probable (albeit unlikely) exception of space (i.e. hard, colonisable substrates), resources – in particular food – are most probably not limiting in this waterbody (Sylvester *et al.*, 2005; Boltovskoy *et al.*, 2006, 2009b). Nevertheless, the unintended effects of this relationship are probably significant for *L. fortunei*. Our results show that annual reproductive output is 2–6 times higher during 2010, when reproduction is not hindered by cyanobacterial blooms, than in bloom years. Comparisons with other waterbodies also support this assumption: during the early years after Salto Grande was colonised (in 2000), mean annual densities of larvae increased gradually stabilising around a yearly mean of about 1900 larvae m^{-3} towards 2012 (excluding 2010). These annual means are about 20% lower than those recorded in all other Argentinian waterbodies surveyed (*c.* 3400–7000 larvae m^{-3}; excepting Itaipú, which was sampled shortly after *L. fortunei* started colonising the area, and therefore, adult population densities were still low). This suggests

that the impact of algal toxins on the population dynamics of the mussel is considerable.

Predation by fish and other aquatic animals, including invertebrates, has been identified as a major deterrent to the mussel's population growth and, probably, dispersal (Sylvester, Boltovskoy & Cataldo, 2007; Paolucci *et al.*, 2010; Torres, Giri & Williner, 2012). Our results suggest that *Microcystis* spp. blooms may also curtail its recruitment, which significantly affects strategies aimed at mitigating the growth of mussel beds in industrial raw water cooling systems, a major nuisance associated with the spread of *L. fortunei* (Morton, 1975; Goto, 2002; Cataldo, Boltovskoy & Pose, 2003; Perepelizin & Boltovskoy, 2011). Various cooling water treatment options have been developed, whose timing and recurrence depend on the mussel's reproductive cycle (Perepelizin & Boltovskoy, 2011). The fact that recruitment is interrupted for 2–4 months in waterbodies where toxic cyanobacteria allows for a reduction in the number of treatments per year, with a significant decrease in environmental impact and economic costs, especially when toxic chemicals are used.

Acknowledgments

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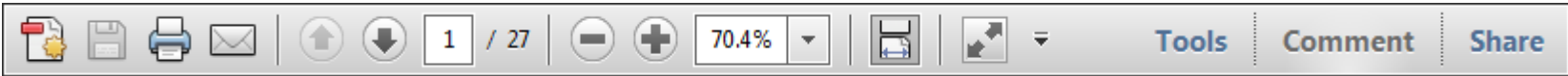
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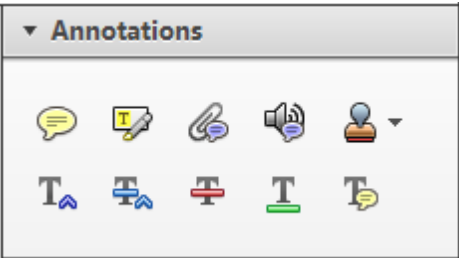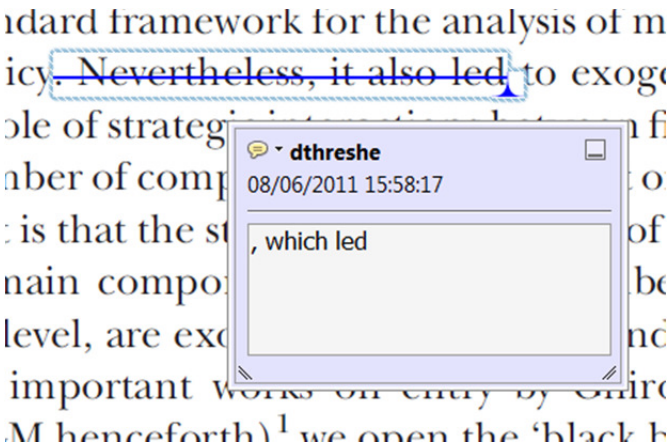
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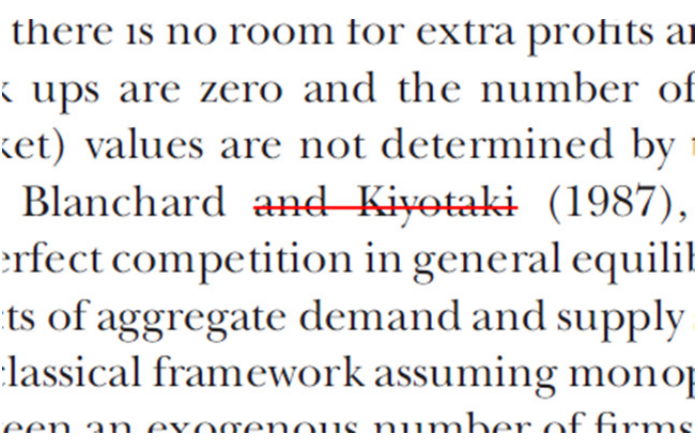- Type the replacement text into the blue box that appears.

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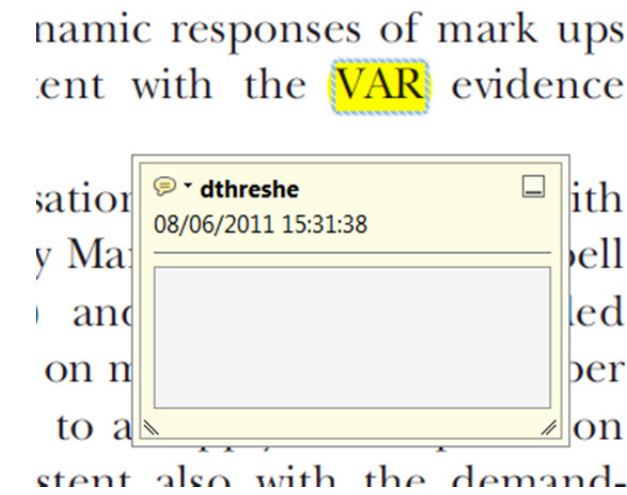lassical framework assuming mono
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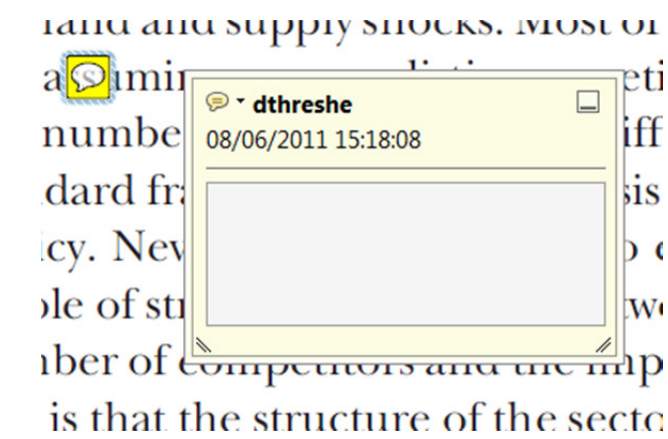namic responses of mark ups
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Marks a point in the proof where a comment needs to be highlighted.

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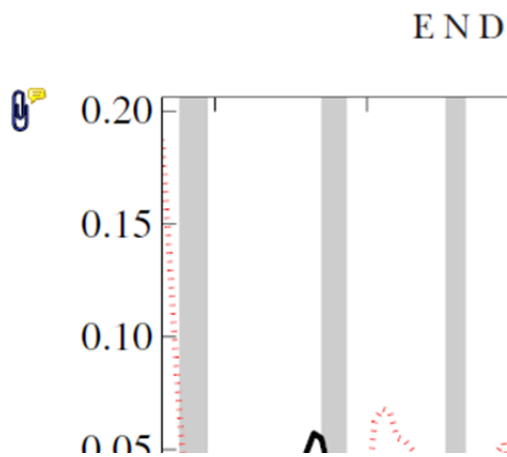- Type the comment into the yellow box that appears.

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USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

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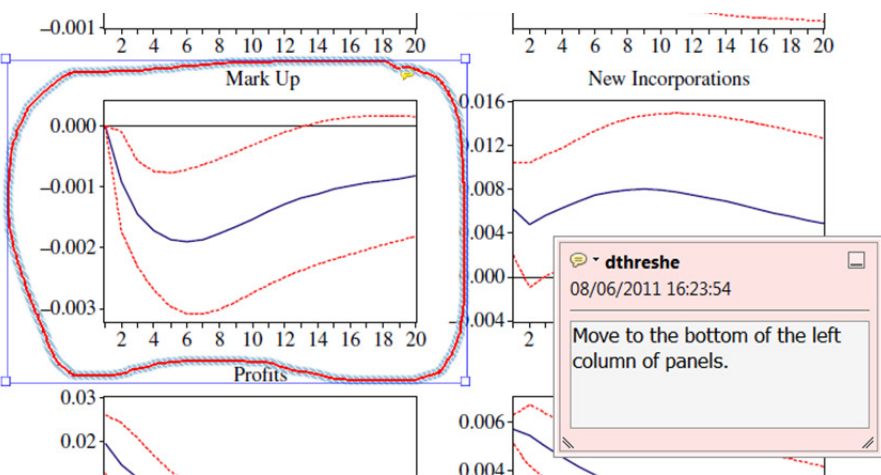Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..

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