Corbicula at its southernmost invasion front in Patagonia: unusual low density and asymmetric trait responses to varying environmental conditions

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ABSTRACT

Freshwater clams of the genus *Corbicula* are successful global invaders, but the processes that determine their geographic range limits remain poorly understood. Here we explored how this invasive clam responds to varying environmental pressures at the southernmost invasion front of its global distribution. We assessed population density, heavy metal content, body weight and shell growth of individuals collected from areas subjected to different farm drainage and runoff conditions in the Río Negro estuary, Patagonia, Argentina. We found that clams from a habitat directly exposed to this source of pollution exhibited higher accumulation of copper, lower body weight, but higher individual growth than conspecifics from a downstream habitat less exposed to pollution, which followed the opposite trend. This asymmetric pattern supports the idea of resource-allocation strategies to varying environmental pressures occurring even over a small geographical range. Density showed no spatial differences, yet it was unusually low in spite of *Corbicula* having been established in the area for more than 15 years, which is consistent with a negligible population growth rate at the invasion front. This is the first study to report such an extended lag period for this successful worldwide invader. We discuss the implications of low densities for life-history traits that are selected for optimizing population growth at the invasion front.

INTRODUCTION

Many species are shifting their ranges as a consequence of human-induced climate change and increasing global transport. The rate of range expansion of an invasive species is directly linked to its population growth, which depends on the time lags between initial colonization and the onset of the exponential increase in abundance (reviewed by Ricciardi, 2012). As colonization events are usually driven by a small number of individuals, front-edge (i.e. recently introduced) populations are typically of low density, below the carrying capacity (Cole, 1954; Sakai *et al.*, 2001). Low population densities can have a strong effect on life-history traits that are selected for optimizing population growth and survival to maturity of offspring (e.g. reduced age of maturity and reproduction, increased growth rate, increased energy allocated to reproduction; Stearns, 1976, 1977). An effective mechanism to increase population growth rate at the invasion front is to favour traits enhancing reproduction (MacArthur & Wilson, 1967; Pianka, 1970). For instance, individuals that grow faster will attain reproductive sizes earlier and bring down generation times, promoting a rapid build-up of population numbers (Cole, 1954; Lewontin, 1965; Roff,

1993) and accelerating primary invasion and secondary spread of invasive species (Skellam, 1951; Phillips *et al.*, 2006; Lockwood, Hoopes & Marchetti, 2007).

At the invasion front, individuals are forced to respond rapidly to novel stressful environmental (physical or biotic) conditions (Sakai *et al.*, 2001). Thus, organisms adapt to variable environmental conditions by finding an optimal balance between the different traits through the allocation of resources among competing physiological processes (e.g. maintenance, growth and reproduction; Congdon *et al.*, 2001). For instance, increased growth rate at the expense of decrease reproduction is well documented in different taxa as a strategy to maintain a high population growth rate in response to environmental stress (e.g. plants and barnacles: reviewed by Stearns, 1992; fish: Van Rooij *et al.*, 1995; nematodes: Alda Álvarez *et al.*, 2005). Effective allocation of resources (i.e. trade-offs) is critical for the establishment success and rates of spread of invasive populations exposed to stressful conditions at the invasion front of the distribution (Lockwood *et al.*, 2007; Burton, Phillips & Travis, 2010).

Bivalves of the genus *Corbicula* are native to Southeast Asia, Africa and Oceania, but they have colonized a large part of the

Americas and Europe (Mouthon, 2001; Schmidlin & Baur, 2007; Crespo *et al.*, 2015). The successful invasion of *Corbicula* clams has been attributed mainly to their rapid maturation, high fecundity and high dispersal (by gravid or potentially brooding adults; Prezant & Chalermwat, 1984), characteristics that make this bivalve one of the most significant groups of nonindigenous fauna in aquatic ecosystems (reviewed by Sousa, Antunes & Guilhermino, 2008). Invasive freshwater *Corbicula* populations belong to a small number of lineages composed of hermaphroditic individual clones, which reproduce through obligate androgenesis (an asexual mode of reproduction; Komaru, Kawagishi & Konishi, 1998; Pigneur *et al.*, 2012). This clonal mode of reproduction plays an important role in facilitating the success of introduced *Corbicula* and the re-establishment of populations after serious bottlenecks (Pigneur *et al.*, 2011, 2012). The current taxonomic status of the invasive *Corbicula* lineages in the Americas and Europe is still largely unresolved, despite several morphological and genetic studies (reviewed by Pigneur *et al.*, 2014). The most widespread and abundant lineage is the so-called Form A/R, widely known as *Corbicula fluminea* (see Pigneur *et al.*, 2014 for further details on systematics). In the present paper, we deal with this form and refer to it as *Corbicula* to avoid the current taxonomic debate.

In Argentina, *Corbicula* was first detected in 1979 in the brackish waters of the Río de la Plata estuary, but it might have been introduced between 1965 and 1975 (Ituarte, 1981). *Corbicula* is widely distributed across a diversity of aquatic habitats from northern to southern Argentina (Rumi *et al.*, 2008; Martín & Estebenet, 2011), the Río Negro basin being its southernmost reported location worldwide (Cazzaniga & Perez, 1999). Although this invasive bivalve has a wide distribution in Argentina (Fig. 1A), most of the biological and ecological studies in this country have been restricted to the Río de la Plata estuary (Ituarte, 1985) and the lower delta of the Paraná River (Boltovskoy *et al.*, 1997; Cataldo & Boltovskoy, 1999; Cataldo *et al.*, 2001). Knowledge of the biological traits of *Corbicula* at the invasion front is still limited, although this population has been established in the area for more than 15 years.

Here, we tested how *Corbicula* responds to varying environmental pressures, as a first step to understanding its biology at the front of the invasion. We assessed population density, heavy metal content, body weight and shell growth of clams collected from areas subjected to different farm drainage and runoff conditions in the Río Negro estuary. High concentrations of water pollutants and heavy metals have strong deleterious effects on aquatic organisms (Widdows & Donkin, 1992), in particular on filter-feeding animals such as *Corbicula* (Boltovskoy *et al.*, 1997; Baudrimont *et al.*, 1999; Cataldo & Boltovskoy, 1999; Cataldo *et al.*, 2001). Hence, clams that are more exposed to this source of pollution should exhibit higher accumulation of heavy metals, but a decreased response in weight and/or growth compared with clams less exposed. Likewise, density should be lower in areas next to farm drainage and runoff.

METHODS

Study area, sampling sites and field sampling procedure

Fieldwork was conducted in the intertidal area of the Río Negro estuary, Argentina. The estuary belongs to the Río Negro basin, which has the largest drainage basin ($140,000 \, km^2$) and water discharge ($600 \, m^3 \, s^{-1}$) in Argentinean Patagonia (Miglioranza *et al.*, 2013). This large river system provides water for urban and industrial consumption, recreation and irrigation for the main fruit production area of the country (Ondarza *et al.*, 2014).

From September 2012 to March 2013, we surveyed each month three intertidal sites *c.* 4.0 km apart in the estuary of the Río Negro close to Viedma city (population 54,779). The study area is located *c.* 30 km upstream from the estuary mouth. Site 1 (40°47′51.315″ S, 63°1′39.593″ W) is close to a major farm drainage and runoff; Site 2 (40°47′56.476″ S, 63°0′9.722″ W) is located *c.* 3.0 km downstream of Site 1 immediately upstream of Viedma; and Site 3 (40°48′7.382″ S, 63°59′35.321″ W) is located *c.* 4.0 km downstream of the major farm drainage in a recreational area and close to the urban rainwater drainage (Fig. 1B). Sampling sites were characterized by a pebbly substrate and individuals were sampled (5 samples per site and date) during low tide using circular quadrats 0.65 m in diameter (area $0.33 \, m^2$) excavated to a depth of 5 cm using a shovel, then sieved and washed from the sediment through a 1-mm mesh, and immediately transported to the laboratory in 0.5-l plastic bottles filled with *in situ* water.

Assessment of abiotic and biotic parameters in the sampling area

At each sampling site and sampling date, we measured water temperature and dissolved oxygen with a digital oximeter (ADWA AD610) and conductivity (a measure of water salinity) with a digital conductivity meter (Benchtop Meters 860031). We used a Secchi disk to measure water clarity and a digital pH-meter (ADWA 110) to measure pH. To estimate total organic carbon, total dissolved solids and photosynthetic pigment concentration (chlorophylls *a*, *b*, *c* and carotenoids), we took three samples of 0.5 l of water, transferred the water samples to dark plastic bottles and transported them to the laboratory in a cooler with ice to avoid chlorophyll photo-oxidation. The techniques, procedures and assessments of total organic carbon and pigment concentrations were carried out according to APHA (2005). We estimated Margalef's pigment ratio (i.e. the ratio of the optical density of the extract at 430 and 665 nm, D_{430}/D_{665}) to determine the qualitative aspect of photosynthetic pigments, which has been proposed as an index of the physiological state and structure of the phytoplankton community as a whole (see Margalef, 1983). The pigment ratio increases as the community becomes more mature, with a decreased rate of renewal, which is characteristic of eutrophic water bodies (Margalef, 1983).

Population density, size frequency distribution and cohort identification

We counted all sampled individuals and estimated population density. In addition, we measured their total shell length (L, anterior to posterior margins; 0.01 mm precision using ESSEX digital caliper) to calculate size-frequency distribution. To identify cohorts by means of length-frequency distributions (2-mm size-class interval), we applied Bhattacharya's method available in FISAT II software (v. 1.2.0, FAO-ICLARM Fish Assessment Tools; Gayanilo, Sparre & Pauly, 2002). To confirm each modal progression, we used the NORMSEP method also available in FISAT II (Pauly & Caddy, 1985). All individuals were stored at −18 °C for subsequent analyses of heavy metal content and body weight.

Heavy metal concentration in clam tissue

Based on size structure, three to four individuals from cohorts from each site and sampling date were selected to estimate heavy metal content for each subpopulation of *Corbicula*. Specifically, the group sizes (L) were 25.1 mm (±0.1 SD) (hereafter large-size group) and 18.5 mm (±0.2 SD) (hereafter intermediate-size group). Samples were thawed at room temperature and carefully washed with deionized water to remove sediment particles retained in the gills and the mantle cavity. For each site, sampling date and size group, soft tissues were removed from the shells, pooled and then homogenized using a stainless steel blender. We took aliquots of 1 to 1.5 g of the composite sample homogenates and split these into 12 test tubes. To digest soft tissue, we added 3 ml of concentrated
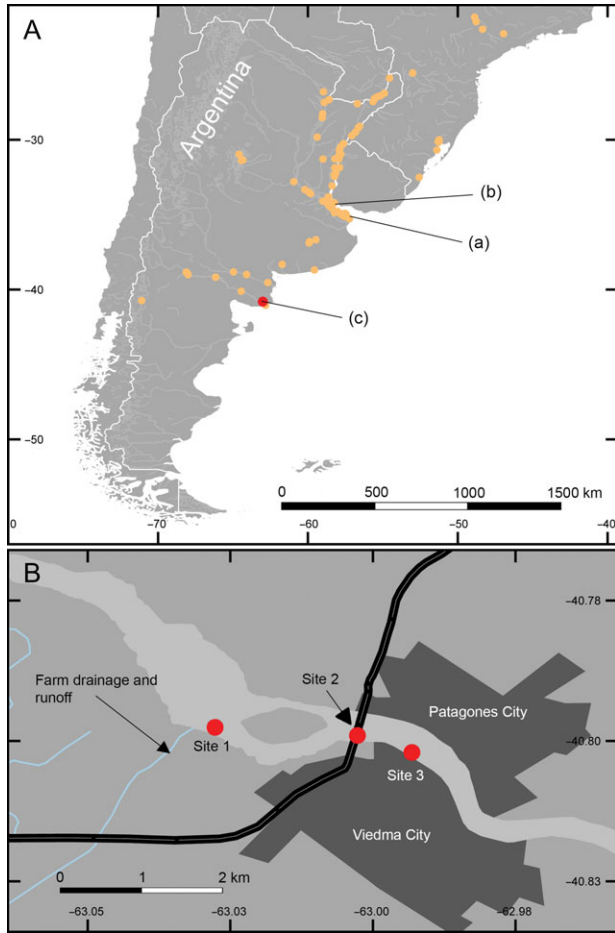
Figure 1. A. Distribution of *Corbicula* in Argentina. Occurrence data of *Corbicula* in Argentina from GBIF (GBIF.org, accessed 7 February 2018). Localities mentioned in text: (a) Punta Atalaya, Río de la Plata estuary (Ituarte, 1985); (b) lower delta Paraná River (Boltovskoy *et al.*, 1997; Cataldo & Boltovskoy, 1999; Cataldo *et al.*, 2001); (c) Río Negro estuary (this study). **B.** Sampling sites in the Río Negro estuary.

ultrapure HNO_3 (Merck[R]; Bilos, Colombo & Presa, 1998; Villar *et al.*, 1999). Tubes were kept at 100 °C for 4 h in borosilicate glass (Erlenmeyer) until total digestion of soft tissue. After cooling, we transferred the solutions to polyethylene tubes with 5 ml of 1% HNO_3. Standard curves were constructed using Merck-certified stock solutions. To overcome matrix interferences, all reagents used to treat the samples were added to the working standards in the same proportions. Every time a set of samples was digested, a procedure blank was made to correct sample readings. Copper and zinc were estimated by flame AAS on a Unicam 969-502155-v5.66 spectrophotometer. Detection limits (3 SD of blank values, with a probability of 99% of being different from the blank) were $0.02 \, \mu g \, ml^{-1}$ for copper and $0.01 \, \mu g \, ml^{-1}$ for zinc (APHA, 2005). The analytical quality was analysed considering oyster tissues as reference material (accession number: NIST SRM 1566b).

Body weight

To estimate and compare body weight between individuals from each site, we used a subsample of individuals ($n = 70$), representative of the population shell size-frequency distributions obtained previously for all individuals sampled on each date and site. We removed sediment and epibiota with a scouring pad before weighing and measuring the organic content of the clam as ash-free dry weight (AFDW, calculated as the difference between the dry

weight and the weight of the incombustible component of the shell; Vohmann *et al.*, 2009; Bonel & Lorda, 2015). To achieve this, we dried clams individually in porcelain crucibles for 48 h at 60 °C, weighed them with a digital scale (precision 0.1 mg), ashed the clams for 5 h in a muffle furnace at 500 °C and then reweighed them (Bonel & Lorda, 2015). In order to allow comparison of the weights of each subpopulation of *Corbicula*, the weight of a standard clam (20 mm in shell length) was calculated for each site using the length-weight regression equation $log_{10}AFDW = a + log_{10}L$ (where a is the theoretical weight of an individual of 1 unit of length).

Individual growth

To estimate and compare individual growth among study sites from the Río Negro estuary, we first identified age cohorts by means of the length-frequency distributions at each sampling date and site. Then, we fitted growth functions to cohorts that showed the highest percentage contribution (i.e. number of individuals) to total density in each site (for details see Supplementary Material S1). To do so, we used the von Bertalanffy growth function, as modified for seasonal oscillations by Pauly & Gaschutz (1979), Hoenig & Hanumara (1982) and Somers (1988):

$$L_t = L_{\infty}(1 - exp^{-K[(t-t_0)+T_1-T_2]}),$$

$$T_1 = Csin(2\pi(t-t_s))/2\pi,$$

and

$$T_2 = Csin(2\pi(t_0-t_s))/2\pi,$$

where L_t is the predicted length at age t, L_{∞} is the asymptotic length, K is the growth constant of dimension time (yr^{-1} in most seasonally oscillating growth curves, expressing the rate at which L_{∞} is approached), t_0 is the theoretical 'age' of a clam at length zero, C expresses the relative amplitude of the seasonal oscillation (varying between 0 and 1; 0 indicating lack of summer–winter differences in growth; this was constrained to be less than or equal to 1 during model fitting) and t_s is the starting point of the oscillation. For visualization, we defined the period when growth is slowest as a fraction of the year, called the Winter Point ($WP = t_s + 0.5$).

The parameters of the function were estimated by the modelling method available in JMP v. 9.0 (SAS Institute). Preliminary results of the growth function failed to converge and estimate the asymptotic L (L_{∞}). Therefore, we used the maximum L (*Lmax*) observed at each site to calculate the asymptotic L following the equation of Taylor (1958):

$$Lmax/0.95 = L_{\infty}$$

These values were fixed when we performed a second fit.

Because the negative correlation between growth parameters (K and L_{∞}) prevents making comparisons based on individual parameters (Pauly, 1979; Vakily, 1992; Ramón, Fernández & Galimany, 2007), we used the growth-performance index (*GPI*), which reflects the growth rate of a given organism of unit length. In other words, *GPI* can be viewed as the (theoretical) value of K that would occur in organisms with a L_{∞} value of 1 unit of length (Munro & Pauly, 1983); it was defined by Pauly & Munro (1984) as:

$$GPI = 2log_{10}L_{\infty} + log_{10}K$$

Thus, we calculated the *GPI* for cohorts from each study site based on growth parameters (L_{∞} and K) obtained after fitting the

growth function using the *Growth Performance Indices* application in FISAT II (Gayanilo *et al.*, 2002).

Statistical analyses

We analysed biotic and abiotic variables, heavy metal concentration and variables measured on *Corbicula* using linear mixed models (LMMs), assuming a Gaussian error distribution where Sites (1, 2 and 3) were considered as fixed effect and sampling dates (Months) as a random effect. Note that we previously tested for temporal autocorrelation and found that sampling dates were not autocorrelated (Supplementary Material S1). However, we considered it appropriate to add time as a random factor in all models, because it can explain a significant portion of the variance in measured variables. The estimates for *Corbicula* were $\log_{10}$ transformed following Box-Cox transformation. Body weight analysis included L as a covariate and estimates were statistically corrected for variation that could be explained by the covariate (we also tested its interaction with Sites). Statistical significance of the fixed effect was obtained from model comparisons using likelihood-ratio tests. Random effect was tested using chi-square likelihood-ratio tests with the corrections indicated by Zuur *et al.* (2009: 123–125). All analyses were performed with R v.3.3.3 packages *lme4* (Bates *et al.*, 2014), *nlme* (Pinheiro *et al.*, 2017), *car* (Fox & Weisberg, 2011), *effects* (Fox, 2003) and *MASS* (Venables & Ripley, 2002). Post-hoc tests were performed when the Site or Size effects were significant, using Holm-Bonferroni correction for multiple testing to compare their effects. We used independent *z*-tests to compare mean growth performance indices between subpopulations. Values are given as means ± SE unless otherwise stated.

RESULTS

Descriptive statistics and results of the linear models on all variables measured are reported in Tables 1 and 2. Further descriptive statistics on size-frequency distribution, population density and individual growth parameters are reported in detail in Supplementary Material Tables S1 to S3.

Abiotic and biotic parameters

Water temperature was comparable among sites ($P = 0.974$) and, on average, it was $21.7 \pm 0.8\,°C$. The dissolved oxygen concentration was high overall ($9.2 \pm 0.2\,mg\,l^{-1}$) and showed no spatial differences ($P = 0.265$). The three sites were characterized by slightly alkaline water (pH = 7.4 ± 0.1) and the pH showed no significant difference among sites ($P = 0.820$). The study area was characterized by low conductivity, as expected for the large input of freshwater, but it varied significantly among sites ($P < 0.001$) and post-hoc comparisons showed that it was higher at Site 1 ($398 \pm 27\,\mu S\,m^{-1}$) than at Site 2 ($268 \pm 14\,\mu S\,m^{-1}$) and Site 3 ($255 \pm 10\,\mu S\,m^{-1}$), whereas Site 2 and 3 did not differ. Correspondingly, total dissolved solids differed among sites ($P = 0.014$), being higher at Site 1 ($316 \pm 29\,mg\,l^{-1}$) with respect to sites 2 and 3 (249 ± 24 and $255 \pm 10\,mg\,l^{-1}$, respectively), but these two sites showed no differences. The water clarity showed significant spatial differences ($P = 0.026$) and was lower at Sites 1 and 2 ($0.64 \pm 0.05\,m$ and $0.62 \pm 0.08\,m$, respectively) with respect to Site 3 ($0.74 \pm 0.09\,m$). On average, total organic carbon was $58 \pm 11\,mg\,l^{-1}$ and it did not differ among sites ($P = 0.130$). Photosynthetic pigments showed no spatial differences (Chl *a*: $P = 0.110$; Chl *b*: $P = 0.228$; Chl *c*: $P = 0.247$). Chlorophylls *a*, *b* and *c* showed, on average, a concentration of

Table 1. Descriptive statistics and results of linear mixed models on biotic and abiotic variables measured in Río Negro estuary, Argentina.

Variables measured	Mean ± SE	Sites	Months	n	Site comparison
Water temperature (°C)	21.7 ± 0.8 (14.0–26.8)	$\chi^2_2 = 0.04$	variance = 18.01	24	—
		$P = 0.974$	$P < 0.001$		
Dissolved oxygen (mg l^{-1})	9.2 ± 0.2 (8.1–10.5)	$\chi^2_2 = 2.66$	variance = 0.27	15	—
		$P = 0.265$	$P < 0.001$		
pH	7.4 ± 0.1 (6.7–8.0)	$\chi^2_2 = 0.40$	variance = 0.20	27	—
		$P = 0.820$	$P < 0.001$		
Conductivity (µS cm^{-1})	S1: 398 ± 27 (317–533)	$\chi^2_2 = 30.69$	variance = 1457	27	S1>S2***
	S2: 268 ± 14 (186–324)	$P < 0.001$	$P = 0.013$		S1>S3***
	S3: 255 ± 10 (219–311)				S2~S3
Total dissolved solids (mg l^{-1})	S1: 316 ± 29 (237–397)	$\chi^2_2 = 8.51$	variance = 1656	15	S1>S2***
	S2: 249 ± 24 (199–330)	$P = 0.014$	$P = 0.039$		S1>S3***
	S3: 255 ± 10 (181–295)				S2~S3
Water clarity (cm)	S1: 64 ± 5 (40–85)	$\chi^2_2 = 7.33$	variance = 374	25	S1~S2
	S2: 62 ± 8 (35–100)	$P = 0.026$	$P < 0.001$		S1<S3*
	S3: 74 ± 9 (40–100)				S2<S3*
Total organic carbon (mg l^{-1})	58 ± 11 (20–95)	$\chi^2_2 = 4.09$	variance = 287	12	—
		$P = 0.130$	$P < 0.001$		
Chlorophyll *a* (mg m^{-3})	3.0 ± 0.3 (0.5–4.9)	$\chi^2_2 = 4.42$	variance = 1.02	17	—
		$P = 0.110$	$P = 0.005$		
Chlorophyll *b* (mg m^{-3})	0.9 ± 0.1 (0. –2.0)	$\chi^2_2 = 2.95$	variance = 0.29	17	—
		$P = 0.228$	$P < 0.001$		
Chlorophyll *c* (mg m^{-3})	1.3 ± 0.2 (0.4–2.9)	$\chi^2_2 = 2.80$	variance = 0.59	17	—
		$P = 0.247$	$P < 0.001$		
Margalef pigment ratio	3.5 ± 0.1 (2.8–4.4)	$\chi^2_2 = 2.48$	variance = 0.12	17	—
		$P = 0.290$	$P < 0.001$		

Minimal and maximal values are indicated in parentheses. Significant values are indicated in bold. All analyses assumed Gaussian error distributions. *Sites* (S1, S2 and S3) were considered as fixed effect and *Months* (sampling dates) as random effect; *n* is the number of observations. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ after Holm-Bonferroni correction for multiple testing.

Table 2. Results of linear mixed models of variables measured on *Corbicula* from Río Negro estuary, Argentina.

Variables measured	Sites	Size group	Sites: Size group	Shell length	Sites: Shell length	Months	n
				Fixed effects		Random effect	
Population density	$\chi^2_2 = 2.75$					variance = 0	105
	$P = 0.25$					$P = 0.5$	
Heavy metal content (copper)	$\chi^2_2 = 12.98$	$\chi^2_1 = 48.00$	$\chi^2_2 = 1.04$			variance = 0.001	36
	$P = 0.002$	$P < 0.001$	$P = 0.596$			$P = 0.111$	
Heavy metal content (zinc)	$\chi^2_2 = 6.37$	$\chi^2_1 = 25.21$	$\chi^2_2 = 0.80$			variance = 0.0002	36
	$P = 0.041$	$P < 0.001$	$P = 0.671$			$P = 0.354$	
Body weight	$\chi^2_2 = 49.22$			$\chi^2_1 = 3320.1$	$\chi^2_2 = 2.51$	variance = 0.01	1,421
	$P < 0.001$			$P < 0.001$	$P = 0.285$	$P < 0.001$	

Significant values of each effect indicated in bold. All analyses assumed Gaussian error distributions. Size group indicates the most representative group shell-length sizes (large group mean $\pm$ SD: 25.1 $\pm$ 0.1 mm; intermediate group: 18.5 $\pm$ 0.2 mm) selected to estimate heavy metal content. Abbreviation: n, number of observations.
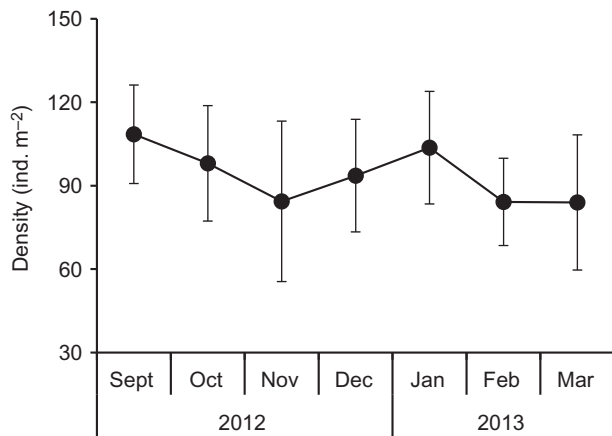
Figure 2. Average density of *Corbicula* in Río Negro estuary, Argentina. Bars represent $\pm$ 1 SE.

3.0 $\pm$ 0.3, 0.9 $\pm$ 0.1 and 1.3 $\pm$ 0.2 mg m^{-3}, respectively. The Margalef pigment ratio did not differ among sites ($P = 0.290$) and showed an overall value of 3.5 $\pm$ 0.1, suggesting that the phytoplankton community had an intermediate rate of algal renewal.

Population density, size frequency distribution and cohort identification

We found no significant differences in density among study sites (Table 2). On average, population density at the sampling area was 94 $\pm$ 10 ind. m^{-2} (Table S1). Throughout the period studied, we detected two density peaks occurring in September (108 $\pm$ 17 ind. m^{-2}) and January (104 $\pm$ 8 ind. m^{-2}; Fig. 2), which were consistent with two independent recruitment events that occurred in early spring and early summer mostly in sites 2 and 3; Site 1 only exhibited one recruitment event in mid-summer (Fig. S1). Size-frequency distributions were defined based on the length of 3,272 individuals (Site 1: $n = 1,137$; Site 2: $n = 983$; Site 3: $n = 1,152$) out of which we identified seven cohorts for Site 1 and six cohorts for sites 2 and 3 (Table S2, Fig. S1).

Heavy metal concentration in clam tissue

We found spatial differences in metal concentration and a significant effect of group size, but no interaction among factors (Table 2). Thus, mean estimates for both metals were corrected for size group, though results did not change when considering uncorrected values. Individuals from the large size group had higher levels of copper (4.07 $\pm$ 0.20 µg g^{-1}) than those from the intermediate size group (2.47 $\pm$ 0.11 µg g^{-1}; Table 2, Fig. 3A)

whereas zinc concentration showed the opposite pattern (Large: 9.82 $\pm$ 0.36 µg g^{-1}, Intermediate: 13.01 $\pm$ 0.40 µg g^{-1}; Table 2, Fig. 3A). On average, individuals from Sites 1 and 2 had higher copper levels (3.46 $\pm$ 0.08 and 3.44 $\pm$ 0.06 µg g^{-1}, respectively) than those from Site 3 (2.91 $\pm$ 0.07 µg g^{-1}; Table 2, Fig. 3B). Likewise, we found marginally significant differences in zinc levels among individuals from each site (Table 2). Post-hoc comparisons showed that individuals from Site 1 had higher zinc concentration (11.80 $\pm$ 0.04 µg g^{-1}) than those from Site 2 (10.24 $\pm$ 0.05 µg g^{-1}), but showed no differences from Site 3 (11.15 $\pm$ 0.04 µg g^{-1}); Sites 2 and 3 were not different either (Fig. 3B).

Body weight and individual shell growth

We measured AFDW of 487 individuals from Site 1, 445 from Site 2 and 489 from Site 3. We found spatial differences and a significant effect of the covariable (L), but no interaction among factors (Table 2). Weight estimates were corrected for L, though results remained the same when considering uncorrected estimates. Clams from Site 3 were 23 and 22% heavier (by *c.* 35 mg) than those from Sites 1 and 2, respectively, whereas we found no differences between Sites 1 and 2 (Fig. 4).

The maximum observed L values were 34.45 mm (Site 1), 31.25 mm (Site 2) and 32.77 mm (Site 3). The corresponding L_∞ values for each study site were 36.26, 32.89 and 34.89 mm, respectively (Supplementary Material Table S3). We fitted the growth function to cohorts that showed the highest percentage of contribution to total density in each site (Site 1: cohorts 3, 4 and 6; Site 2: cohorts 2 and 3; Site 3: cohorts 2 and 3; Supplementary Material Fig. S2). We found that individuals from Site 1 showed a higher shell growth rate ($GPI = 3.07 \pm 0.05$) than those from Site 3 ($GPI = 2.68 \pm 0.16$; $z = 2.50$, $P = 0.006$). Likewise, clams from Site 2 showed a higher growth rate (3.08 $\pm$ 0.06) than those from Site 3 ($z = 2.42$, $P = 0.008$), whereas sites 1 and 2 showed no difference in growth performance ($z = -0.13$, $P = 0.448$; Fig. 4). By plotting weight and growth estimates together from *Corbicula* from the three study sites, we observed that shifting from Site 1 (adjacent to a major farm drainage and runoff) to Site 3 (located 4 km downstream), clams decreased in growth rate but increased in weight, and *vice versa* (Fig. 5).

DISCUSSION

This study provides the first comparative analysis of how the invasive clam *Corbicula* responded to dissimilar environmental pressures at the southernmost edge of its global range distribution, the Río Negro estuary. Our most striking result is an asymmetrical response: clams from a habitat adjacent to a farm drainage and
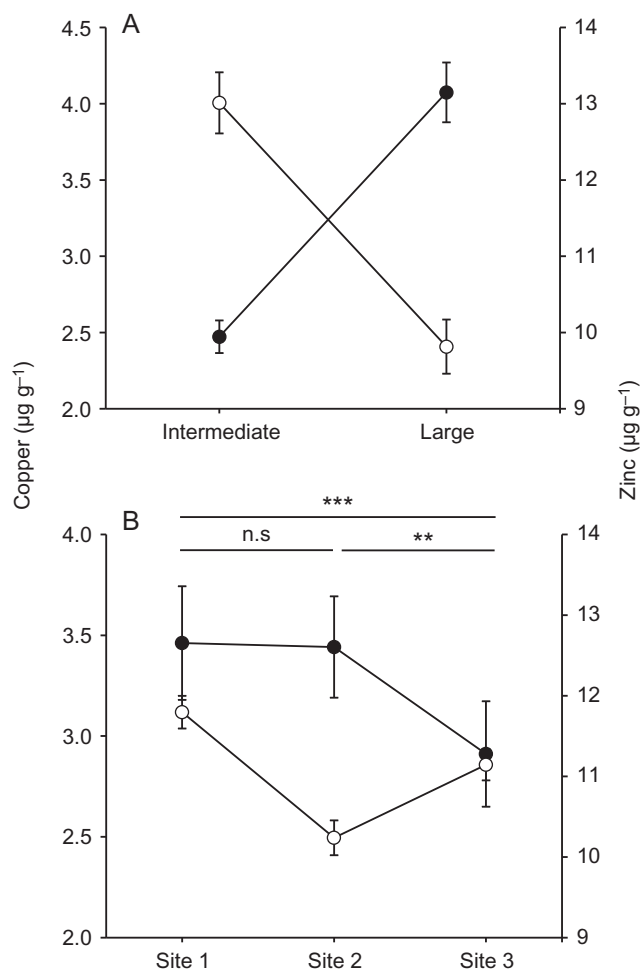
Figure 3. Mean values of copper (black circles) and zinc (white circles) concentrations in tissue of *Corbicula* for each size group (**A**) and study site (**B**) in Río Negro estuary, Argentina. Values for each study site were back-transformed and statistically corrected for significant variation explained by the two most representative size-groups (Large size: mean shell length L $\pm$ SD = 25.7 $\pm$ 0.1 mm; Intermediate size: L = 18.5 mm $\pm$ 0.2). Site and Size effect were compared using post-hoc tests with Holm-Bonferroni correction for multiple-testing (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Bars represent $\pm$ 1 SE.

runoff showed higher accumulation of copper and lower body weight, but higher individual shell growth rate as compared with those from a downstream less impacted habitat, which followed the opposite trend. Another unexpected result is the extremely low abundance of this leading-edge *Corbicula* population in spite of it having been established in the area for more than 15 years. Below, we discuss these main findings in the context of resource-allocation trade-offs and extended lag-time in population growth of invasive species.

Biotic and abiotic characterization of the study area

Because of the intense anthropogenic activities along the basin, the Río Negro estuary—and adjacent waters—receives variable loads of untreated sewage discharge, farm drainage and runoff, heavy metals and organic pollutants, which likely affect aquatic biota (Isla *et al.*, 2010; Ondarza *et al.*, 2012, 2014; Miglioranza *et al.*, 2013; Kopprio *et al.*, 2015). We accordingly found that the water conductivity and total dissolved solids were higher, but water transparency lower, at the site adjacent to the major farm drainage and runoff (Site 1). However, we found no evidence

suggesting that other abiotic variables, as well as biotic ones such as total organic carbon and chlorophylls (considered as a proxy for phytoplankton, i.e. food availability) differed among sites. In addition, the Margalef pigment ratio showed no spatial differences but indicates that, on average, phytoplankton community had an intermediate rate of algal renewal, which is not characteristic of eutrophic water bodies (Margalef, 1983). Collectively, these findings suggest that Site 1 showed no differences in terms of phytoplankton availability or eutrophication with respect to other sites, whereas differences in conductivity, total dissolved solids and transparency were observed and were most likely caused by intense input from farm drainage and runoff at that site. Likewise, a previous study (Abrameto *et al.*, 2012) reported higher heavy metal concentrations in the sediments at Site 1—exceeding the values considered safe for aquatic life (e.g, copper: $50 \, \mu g \, g^{-1}$ and zinc: $34 \, \mu g \, g^{-1}$)—than at Site 3 (located *c.* 4.0 km downstream of the major farm drainage; copper: $6 \, \mu g \, g^{-1}$ and zinc: $30 \, \mu g \, g^{-1}$).

Heavy metal concentration in clam tissue

Corbicula clams have higher filtration and assimilation rates than many other bivalve species (McMahon, 2002; Sousa *et al.*, 2008), leading to higher accumulation of pollutants and heavy metals in their valves and tissues (Boltovskoy *et al.*, 1997; Bilos *et al.*, 1998; Cataldo *et al.*, 2001). Heavy metal concentrations reported for bed sediments in the Río Negro study area, in particular zinc and copper, are associated with the extensive use of pesticides in fruit plantations (Gaiero *et al.*, 2002). In agreement with spatial differences found in sediments (Abrameto *et al.*, 2012), clams from Site 1 accumulated higher concentrations of copper than those from Site 3, whereas zinc was higher in Site 1 than Site 2, but showed no differences with individuals from Site 3. Moreover, we found that clams from the large-size group showed copper levels 1.6 times higher compared with those from the intermediate-size group which, in contrast, had zinc content 1.3 times higher than large-size one. The observed pattern is consistent with previous studies that showed that copper concentration increases with shell size of clams, whereas zinc shows an opposite trend (Belanger *et al.*, 1990; Cataldo *et al.*, 2001; Bilos *et al.*, 1998, 2009). Both copper and zinc are essential elements involved in several enzymatic systems (Bilos *et al.*, 1998) and this inverted relationship between size and metal concentration suggest different needs related to age. Hence, our findings conform to the idea that *Corbicula* passively accumulates copper with age, but strongly regulates zinc levels through detoxification mechanisms (Bilos *et al.*, 1998).

Asymmetric response of body weight and shell growth of Corbicula at the invasion front

High concentrations of water pollutants and heavy metals can play a major role as stressors for aquatic animals and can have deleterious effects on population biology and ecology of species (Widdows & Donkin, 1992). Severe detrimental effects on both somatic and shell growth of *Corbicula* have been observed at higher levels of habitat pollution (Boltovskoy *et al.*, 1997; Baudrimont *et al.*, 1999; Cataldo & Boltovskoy, 1999; Cataldo *et al.*, 2001). In this context, we would have expected to observe a decrease in body weight and in shell growth rate in *Corbicula* from polluted sites. As predicted, we observed that individuals from the habitat adjacent to the major farm drainage and runoff (Site 1) showed a lower body weight but, unexpectedly, they had a higher individual shell growth rate than to those located downstream (Site 3), and *vice versa* (Figs 4, 5).

One possible explanation could be that differences in shell growth and body mass were due to food differences among sites. If this was true, there should be more food availability due to nutrient enrichment from runoff and eutrophication at Site 1 (close to the farm drainage) and it would have been expected to find clams with higher body mass at that site. However, this was
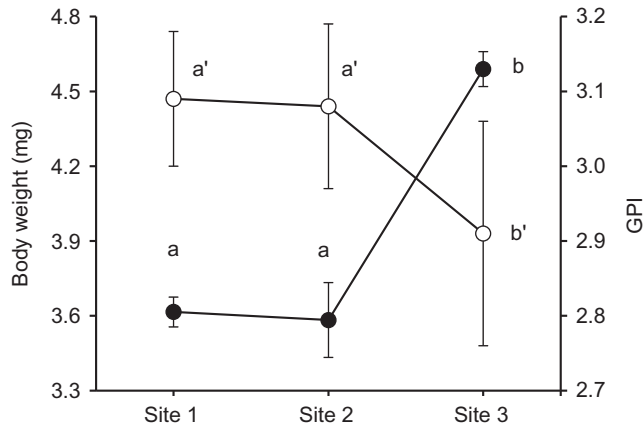
Figure 4. Asymmetric responses of weight and individual shell growth rate of *Corbicula* in Río Negro estuary, Argentina. Black circles indicate mean estimates of body weight (AFDW). White circles indicate shell growth rates (*GPI*) estimated from growth parameters ($L\infty$ and K) after fitting seasonally oscillating von Bertalanffy growth function to the most representative cohorts (Site 1: cohorts 3, 4 and 6; Site 2: cohorts 2 and 3; Site 3: cohorts 2 and 3; see Supplementary Material S1, Tables S2 and S3, for details). Weight estimates were back-transformed and statistically corrected for variation explained by covariate (shell length L). Bars represent 1 ± SE.
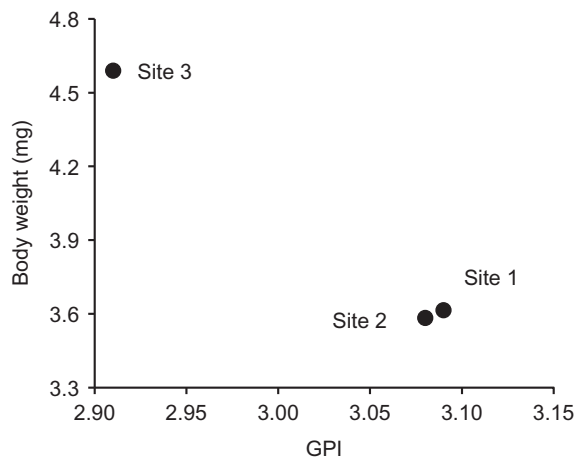

Figure 5. Differences in growth (*GPI*) and body weight (AFDW) of *Corbicula* in Río Negro estuary, Argentina. Individuals from Site 1 (adjacent to a major farm drainage and runoff) showed lower growth rate but greater body weight than at Site 3 (located *c.* 4 km downstream). Weight estimates are the same as those in Fig. 4.

not the case, but quite the opposite: clams from that site showed the lowest body mass but the highest shell growth. Nevertheless, it does seem reasonable to infer that the observed differences in body weight and shell growth are related in some way to the exposure to the farm drainage and runoff. In the following, we explore a resource-allocation trade-off scenario as a possible mechanism to explain the asymmetrical pattern of these two traits.

A resource-allocation trade-off hypothesis for the asymmetric trait responses

Ecological trade-offs favour the increase in one trait at the expense of another, if this increase yields fitness benefits exceeding the costs of the reduction of the second trait (Stearns, 1992). In iteroparous animals such as *Corbicula*, resource-allocation trade-offs are expressed throughout the organism's life-history (e.g. between

current and future reproduction; Kozlowski & Uchmanski, 1987; Stearns, 1992). In the case of *Corbicula* facing environmental pollution, it would be ineffective for clams with diminished physiological condition to increase current somatic investment (considered here as a proxy of present reproductive capacity; Bayne & Worrall, 1980) if this compromises long-term survival and reproduction. This hypothesis is supported by the fact that the site adjacent to the farm drainage and runoff (Site 1) showed only one recruitment event, whereas the downstream sites (Sites 2 and 3) exhibited two (Supplementary Material Fig. S1). Fecundity and survival are both associated with size (McMahon, 2002; Aira *et al.*, 2007; Creighton, Heflin, & Belk, 2009) and, hence, reallocating resources from body weight into shell growth increases the chances that individuals survive to adulthood and produce more offspring. Indeed, clams with bigger shells have higher internal shell volumes, which allows for brooding a larger number of larvae relative to smaller clams (Byrne *et al.*, 2000; Mouthon & Parghentanian, 2004). Thus, this asymmetric pattern of *Corbicula*'s body weight and shell growth is in line with energy reallocation strategies (Stearns, 1992), probably driven by varying levels of environmental pollution in the Río Negro estuary. Further experimental work is needed to test whether this compensatory response is explained by a resource-allocation trade-off.

Low population density of Corbicula at the invasion front

We found no differences in density among sites in spite of the striking differences in heavy metal concentration and clam body weight. Because sea water can enter the estuary up to 66 km upstream from the mouth (Piccolo & Perillo, 1997), the tidal effect may act as a propagule homogenizing factor, entraining non-swimming juveniles (GISD, 2005) and likely cancelling recruitment differences between sites. In addition, larval survival and recruitment of this predominately freshwater species might be affected by daily fluctuations of salinity (Morton, 1982), although direct evidence on how this affect juveniles is still missing (Sousa, Antunes & Guilhermino, 2006; Franco *et al.*, 2012). Notably, overall *Corbicula* density in the Río Negro basin is extremely low (<170 ind. m^{-2}, see Molina *et al.*, 2015; present study) compared with mean abundances reported for other habitats in Argentina, which are ten to sixteen times higher (e.g. Boltovskoy *et al.*, 1997; Cataldo & Boltovskoy, 1999; Cataldo *et al.*, 2001). This low density cannot be explained by the effect of daily fluctuations of salinity as other sites located much further upstream (>150 km) show mean density values lower than 50 ind. m^{-2} (e.g. Molina *et al.*, 2015). One possible explanation for the extreme low density could be that *Corbicula* abundances are below equilibrium level in all sites and not (or less) related to environmental suitability. This is supported by the fact that overall condition index value (CI: dry tissue weight divided by standard shell length, 20 mm) in the Río Negro is more than twice as high (CI = 6.6 mg mm^{-1}) as estimates from a population established in a more strongly polluted environment (San Antonio River: CI = 2.9 ± 0.3 SD mg mm^{-1}, density = 1,604 ± 409 SD ind. m^{-2}; Cataldo *et al.*, 2001).

Interestingly, although individuals at Site 1 appeared to be negatively affected by farm drainage and runoff, most biotic and abiotic conditions measured in the study area and the high CI of clams suggest that environmental conditions were less detrimental than those in other invaded areas. So, we infer that the effect of environmental conditions on the biology of *Corbicula* clams was less significant than that of other ecological factors. The low abundances observed herein are consistent with the idea that densities in the Río Negro estuary are currently controlled by demographic forces associated with an early invasion stage. Therefore, we focus the next part of the discussion on ecological factors rather environmental ones.

Extended lag times of Corbicula at the invasion front

Lag phases (i.e. negligible or lack of population growth) followed by swift density increases are common in newly introduced

populations, in particular for *Corbicula* (Cohen *et al.*, 1984). In Argentina, for instance, a recently established *Corbicula* population at Punta Atalaya, Río de La Plata estuary (3 years since first introduction; Ituarte, 1985) showed a population density two to three times higher (339 ± 84 ind. m^{-2}) than that reported in this study for the Río Negro estuary. On the other hand, the abundance of a long-established *Corbicula* population in the Paraná River (*c.* 25 years) was, on average, sixteen times higher than that of the Río Negro population (Cataldo & Boltovskoy, 1999). The abovementioned increase in population size is expected according to the theory of biological invasions (Sakai *et al.*, 2001; Ricciardi, 2012). Time, however, does not appear to be the sole determinant of the invasion stage in the case of the Río Negro population studied here, which presents extremely low densities. Considering this feature, this population could be viewed as an incipient leading-edge population facing difficulties typical of initial invasion stages, yet it has been present in the area for more than 15 years (first reported by Cazzaniga & Perez, 1999). In our view, the extremely low density of this front population suggests that the time taken for an invasive population to overcome initial low densities and become fully established is not only species- but also context-dependent. Clearly, more work is required to investigate why this population shows an unusually low density and such an extended delay in the expansion phase at the southernmost invasion front.

The Río Negro leading-edge Corbicula *population: an open question*

The extreme low density of the Río Negro population is particularly intriguing, because the mating system and reproductive mode of *Corbicula* should facilitate the success of introduced clams or the re-establishment of populations after massive bottlenecks. *Corbicula* is an hermaphrodite capable of asexually reproducing (obligate androgenesis) by both cross- and self-fertilization (Dillon, 2004; Pigneur *et al.*, 2011). Clonal reproduction and self-fertilization provide a colonization advantage as a single individual may found a population if the conditions are suitable (Pigneur *et al.*, 2014). Because colonization events are usually driven by a small number of individuals (Sakai *et al.*, 2001), it is therefore likely that the founding event at the Río Negro estuary consisted on a small sized inoculum. It is unlikely, however, that these clonal individuals reproduce predominantly by selfing, because we should otherwise have seen an increase in density with time since establishment, which is not the case. We therefore hypothesize that individuals from the Río Negro population should reproduce mainly by cross-fertilization. This would imply that a small-sized inoculum during the founding event might have resulted in a severe Allee effect, decreasing low reproductive success due to low likelihood of individual and gamete matching (Davis *et al.*, 2004; Davis, 2005). This might explain the delay in the abundance build-up and the extended lag period of this leading-edge *Corbicula* population. If so, this effect is likely to persist through time, since migration of individuals might be limited or nil due to the geographic isolation of this population from others in Argentina (Fig. 1A). Hence, we adhere to the idea that initial success (or lack of it) may be a poor indicator of an invasive species' ultimate success and ecological significance (Siemann & Rogers, 2001). Further studies should focus on determining the predominant mating system of *Corbicula* in order to understand particular cases more fully, such as the one presented in this study.

What are the consequences of low population density on life-history traits?

Low densities below the carrying capacity are a typical feature of newly established populations (Sakai *et al.*, 2001). An effective mechanism to increase population growth rate at the invasion front is to favour traits enhancing reproduction (MacArthur & Wilson, 1967; Pianka, 1970). Early reproduction (as a result of higher individual growth rates) brings down generation times, promoting a rapid build-up of population numbers (Cole, 1954; Lewontin, 1965; Roff, 1993; Phillips, 2009), accelerating primary invasion and secondary spread of invasive species (Skellam, 1951; Phillips *et al.*, 2006; Lockwood *et al.*, 2007). For example, this has been well documented for the invasive cane toad *Bufo marinus*, individuals from frontal populations growing faster than those from denser, long-established populations (Phillips, 2009). The question arises whether *Corbicula* clams at the invasion front grow faster than conspecifics from core populations. For heuristic purposes, we re-fitted the growth function (following the approach mentioned in Methods, above) to length-frequency data available for the only two well-studied *Corbicula* populations in Argentina. One is a low-density frontal population at Punta Atalaya in the Río de la Plata estuary ($GPI = 2.87 \pm 0.02$; Fig. 1A; Ituarte, 1985) and the other is a high-density core population from the Paraná River ($GPI = 2.73$; Fig. 1A; Cataldo & Boltovskoy, 1999). We found that *Corbicula* from both these frontal populations grow, on average, 5 to 7% faster than conspecifics from the Paraná River. This implies that clams at the corresponding fronts will reach reproductive size earlier (*c.* 10 mm L; see Aldridge & McMahon, 1978; McMahon, 2002), or at a younger age (i.e. *c.* 4–5 months old) than individuals from the Paraná River, which take twice as long to reach that size. This supports the idea that life-history traits enhancing individual reproduction are favoured and consequently promote increase of population growth rate at the expanding front. We acknowledge that this is far from being conclusive and needs to be considered with caution. It suggests, however, that life-history traits might change across the invasive range of *Corbicula* and merits further study.

Conclusion

To sum up, the asymmetric pattern between body mass and shell growth may reflect a compensatory response by *Corbicula* clams to varying environmental pressures occurring even across a very small (4 km) geographical range. This asymmetric response is consistent with the high physiological tolerance of *Corbicula* which, combined with unusual life-history traits, favours its invasion success in different ecosystems, ranging from pristine to polluted ones. The low density found in the Río Negro is characteristic of an initial invasion stage typically below the carrying capacity at the invasion front, yet the clam has been present in the area for more than 15 years. This implies that this population is experiencing an extended lag-phase, which is unusual for this worldwide successful invader. This finding, together with faster growth observed for clams at the invasion front, support the idea that early reproduction is an effective mechanism to promote a rapid build-up of population size at the expanding edge. Taken together, our results provide new information on how *Corbicula* clams respond to varying environmental, ecological and selective pressures at the invasion front, which is essential to better comprehend the processes that determine their range limits.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Molluscan Studies* online.

ACKNOWLEDGEMENTS

REFERENCES

ABRAMETO, M., BARRIO, D., BANZATO, L., FELLENZ, N., GIBELI, T. & MOLINA, L. 2012. Libro de resúmenes. In: *II Reunión Argentina de Geoquímica de la Superficie: Compilado de trabajos extendidos* (J.E. Marcovecchio & H.R. Freije, eds), 295. EdiUns. Universidad Nacional del Sur, Bahía Blanca, Argentina.

AIRA, M., DOMÍNGUEZ, J., MONROY, F. & VELANDO, A. 2007. Stress promotes changes in resource allocation to growth and reproduction in a simultaneous hermaphrodite with indeterminate growth. *Biological Journal of the Linnean Society*, **91**: 593–600.

ALDA ÁLVAREZ, O., JAGER, T., KOOIJMAN, S.A.L.M. & KAMMENGA, J.E. 2005. Responses to stress of *Caenorhabditis elegans* populations with different reproductive strategies. *Functional Ecology*, **19**: 656–664.

ALDRIDGE, D.W. & MCMAHON, R.F. 1978. Growth, fecundity and bioenergetics in a natural population of the Asiatic freshwater clam, *Corbicula manilensis* Philippi, from North Central Texas. *Journal of Molluscan Studies*, **44**: 49–70.

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, WATER ENVIRONMENTAL FEDERATION (APHA). 2005. *Standard methods for the examination of water and wastewater*. American Public Health Association. Washington, DC.

BATES, D., MAECHLER, M., BOLKER, B. & WALKER, S. 2014. Introduction to statistical analysis using R. *R package version*, 1–23.

BAUDRIMONT, M., ANDRÈS, S., METIVAUD, J., LAPAQUELLERIE, Y., RIBEYRE, F., MAILLET, N., LATOUCHE, C. & BOUDOU, A. 1999. Field transplantation of the freshwater bivalve *Corbicula fluminea* along a polymetallic contamination gradient (River Lot, France): II. Metallothionein response to metal exposure. *Environmental Toxicology and Chemistry*, **18**: 2472–2477.

BAYNE, B.L. & WORRALL, C.M. 1980. Growth and production of mussels *Mytilus edulis* from two populations. *Marine Ecology Progress Series*, **3**: 317–328.

BELANGER, S.E., FARRIS, J.L., CHERRY, D.S. & CAIRNS, J.J. 1990. Validation of *Corbicula fluminea* growth reductions induced by copper in artificial streams and river systems. *Canadian Journal of Fisheries and Aquatic Sciences*, **47**: 904–914.

BILOS, C., COLOMBO, J.C. & PRESA, M.J. 1998. Trace metals in suspended particles, sediments and Asiatic clams (*Corbicula fluminea*) of the Río de la Plata Estuary, Argentina. *Environmental Pollution*, **99**: 1–11.

BILOS, C., COLOMBO, J.C., SKORUPKA, C.N., DEMICHELIS, S.O. & TATONE, L.M. 2009. Size-related trace metal bioaccumulation in Asiatic clams (*Corbicula fluminea*) from the Rio de la Plata estuary, Argentina. *International Journal of Environment and Health*, **3**: 390–409.

BOLTOVSKOY, D., CORREA, N., CATALDO, D., STRIPEIKIS, J. & TUDINO, M. 1997. Environmental stress on *Corbicula fluminea* (Bivalvia) in the Paraná River delta (Argentina): complex pollution-related disruption of population structures. *Archiv für Hydrobiologie*, **138**: 483–507.

BONEL, N. & LORDA, J. 2015. Growth and body weight variability of the invasive mussel *Limnoperna fortunei* (Mytilidae) across habitat and season. *Malacologia*, **58**: 129–145.

BURTON, O.J., PHILLIPS, B.L. & TRAVIS, J.M.J. 2010. Trade-offs and the evolution of life-histories during range expansion. *Ecology Letters*, **13**: 1210–1220.

BYRNE, M., PHELPS, H., CHURCH, T., ADAIR, V., SELVAKUMARASWAMY, P., & POTTS, J. 2000. Reproduction and development of the freshwater clam Corbicula australis in southeast Australia. *Hydrobiologia*, **418**: 185–197.

CATALDO, D. & BOLTOVSKOY, D. 1999. Population dynamics of Corbicula fluminea (Bivalvia) in the Paraná River Delta (Argentina). *Hydrobiologia*, **380**: 153–163.

CATALDO, D.H., BOLTOVSKOY, D., STRIPEIKIS, J. & POSE, M. 2001. Condition index and growth rates of field caged *Corbicula fluminea* (Bivalvia) as biomarkers of pollution gradients in the Paraná river delta (Argentina). *Aquatic Ecosystem Health and Management*, **4**: 187–201.

CAZZANIGA, N.J. & PEREZ, C. 1999. Asiatic clam, *Corbicula fluminea*, in northwestern Patagonia (Argentina). *Journal of Freshwater Ecology*, **14**: 551–552.

COHEN, R.R.H., DRESLER, P.V., PHILLIPS, E.J.P. & CORY, R.L. 1984. The effect of the Asiatic clam, *Corbicula fluminea*, on phytoplankton of the Potomac River, Maryland. *Limnology and Oceanography*, **29**: 170–180.

COLE, L.C. 1954. The population consequences of life history phenomena. *Quarterly Review of Biology*, **29**: 103–137.

CONGDON, J.D., DUNHAM, A.E., HOPKINS, W.A., ROWE, C.L. & HINTON, T.G. 2001. Resource allocation-based life histories: a conceptual basis for studies of ecological toxicology. *Environmental Toxicology and Chemistry*, **20**: 1698–1703.

CREIGHTON, J.C., HEFLIN, N.D. & BELK, M.C. 2009. Cost of reproduction, resource quality, and terminal investment in a burying beetle. *American Naturalist*, **174**: 673–684.

CRESPO, D., DOLBETH, M., LESTON, S., SOUSA, R. & PARDAL, M.Â. 2015. Distribution of *Corbicula fluminea* (Müller, 1774) in the invaded range: a geographic approach with notes on species traits variability. *Biological Invasions*, **17**: 2087–2101.

DAVIS, H.G. 2005. *r*-Selected traits in an invasive population. *Evolutionary Ecology*, **19**: 255–274.

DAVIS, H.G., TAYLOR, C.M., CIVILLE, J.C. & STRONG, D.R. 2004. An Allee effect at the front of a plant invasion: *Spartina* in a Pacific estuary. *Journal of Ecology*, **92**: 321–327.

DILLON, R.T. 2004. *The ecology of freshwater molluscs*. Cambridge University Press, Cambridge.

FOX, J. 2003. Effect displays in R for Generalised Linear Models. *Journal of Statistical Software*, **8**: 1–27.

FOX, J. & WEISBERG, S. 2011. *An {R} companion to applied regression. CRAN Repository*. Sage, Thousand Oaks, CA.

FRANCO, J.N., CEIA, F.R., PATRÍCIO, J., MODESTO, V., THOMPSON, J., MARQUES, J.C. & NETO, J.M. 2012. Population dynamics of *Corbicula fluminea* (Müller, 1774) in mesohaline and oligohaline habitats: invasion success in a southern Europe estuary. *Estuarine, Coastal and Shelf Science*, **112**: 31–39.

GAIERO, D.M., PROBST, J.L., DEPETRIS, P.J., LELYTER, L. & KEMPE, S. 2002. Riverine transfer of heavy metals from Patagonia to the southwestern Atlantic Ocean. *Regional Environment Change*, **3**: 51–64.

GAYANILO, F.C., SPARRE, P. & PAULY, D. 2002. *FAO-ICLARM Stock assessment tools (FiSAT). Software version 1.2.0.* FAO, Rome.

GLOBAL INVASIVE SPECIES DATABASE (GISD). 2005. *Corbicula fluminea*. http://www.iucngisd.org/gisd/speciesname/Corbicula+fluminea.

HOENIG, J.M. & HANUMARA, R.C. 1982. *A statistical study of a seasonal growth model for fishes*. Technical Report, Department of Computer Science, University of Rhode Island, Narragansett, RI.

ISLA, F., MIGLIORANZA, K., ONDARZA, P., SHIMABUKURO, V., ESPINOSA, M., LONDOÑO, M.Q., FERRANTE, A. & MORENO, V. 2010. Sediment and pollutant distribution along the Negro River: Patagonia, Argentina. *International Journal of River Basin Management*, **8**: 319–330.

ITUARTE, C.F. 1981. Primera noticia acerca de la introduccion de pelecípodos asiáticos en el área rioplatense. *Neotrópica*, **27**: 79–82.

ITUARTE, C.F. 1985. Growth dynamics in a natural population of *Corbicula fluminea* (Bivalvia Sphaeriacea) at Punta Atalaya, Rio de La Plata, Argentina. *Studies on Neotropical Fauna and Environment*, **20**: 217–225.

KOMARU, A., KAWAGISHI, T. & KONISHI, K. 1998. Cytological evidence of spontaneous androgenesis in the freshwater clam *Corbicula leana* Prime. *Development, Genes and Evolution*, **208**: 46–50.

KOPPRIO, G.A., BIANCALANA, F., FRICKE, A., GARZÓN CARDONA, J.E., MARTÍNEZ, A. & LARA, R.J. 2015. Global change effects on biogeochemical processes of Argentinian estuaries: an overview of vulnerabilities and ecohydrological adaptive outlooks. *Marine Pollution Bulletin*, **91**: 554–562.

KOZLOWSKI, J. & UCHMANSKI, J. 1987. Optimal individual growth and reproduction in perennial species with indterminate growth. *Evolutionary Ecology*, **1**: 214–230.

LEWONTIN, R.C. 1965. Selection for colonizing ability. In: *Genetics of colonizing species* (H. Baker & G. Stebbins, eds), pp. 79–94. Academic Press, New York.

LOCKWOOD, J., HOOPES, M. & MARCHETTI, M. 2007. *Invasion ecology*. Blackwell, London.

MACARTHUR, R.H. & WILSON, E.O. 1967. *The theory of island biogeography*. Princeton University Press, Princeton, NJ.

MARGALEF, R. 1983. *Limnología*. Ediciones Omega, S.A., Barcelona.

MARTÍN, P.R. & ESTEBENET, A.L. 2011. Spread of the Asiatic Clam *Corbicula fluminea* in southern Pampas and northern Patagonia, Argentina. *Journal of Freshwater Ecology*, **17**: 331–333.

MCMAHON, R.F. 2002. Evolutionary and physiological adaptations of aquatic invasive animals: *r* selection versus resistance. *Canadian Journal of Fisheries and Aquatic Sciences*, **59**: 1235–1244.

MIGLIORANZA, K.S.B., GONZALEZ, M., ONDARZA, P.M., SHIMABUKURO, V.M., ISLA, F.I., FILLMANN, G., AIZPÚN, J.E. & MORENO, V.J. 2013. Assessment of Argentinean Patagonia pollution: PBDEs, OCPs and PCBs in different matrices from the Río Negro basin. *Science of the Total Environment*, **452–453**: 275–285.

MOLINA, L.M., PEREYRA, P.J., CARRIZO, N.G.M. & ABRAMETO, M.A. 2015. Here come the clam: southernmost record worldwide of the Asian clam *Corbicula fluminea* (Patagonia, Argentina). *Russian Journal of Biological Invasions*, **6**: 129–134.

MORTON, B. 1982. Some aspects of the population-structure and sexual strategy of *Corbicula-cf-fluminalis* (Bivalvia, Corbiculacea) from the Pearl River, People's Republic of China. *Journal of Molluscan Studies*, **48**: 1–23.

MOUTHON, J. 2001. Life cycle and population dynamics of the Asian clam *Corbicula fluminea* (Bivalvia: Corbiculidae) in the Rhone River at Creys-Malville (France). *Archiv für Hydrobiologie*, **151**: 571–589.

MOUTHON, J. & PARGHENTANIAN, T. 2004. Comparison of the life cycle and population dynamics of two *Corbicula* species, *C. fluminea* and *C. fluminalis* (Bivalvia: Corbiculidae) in two French canals. *Archiv für Hydrobiologie*, **161**: 267–287.

MUNRO, J.L. & PAULY, D. 1983. A simple method for comparing the growth of fishes and invertebrates. *Fishbyte*, **1**: 5–6.

ONDARZA, P.M., GONZALEZ, M., FILLMANN, G. & MIGLIORANZA, K.S.B. 2012. Increasing levels of persistent organic pollutants in rainbow trout (*Oncorhynchus mykiss*) following a mega-flooding episode in the Negro River basin, Argentinean Patagonia. *Science of the Total Environment*, **419**: 233–239.

ONDARZA, P.M., GONZALEZ, M., FILLMANN, G. & MIGLIORANZA, K.S.B. 2014. PBDEs, PCBs and organochlorine pesticides distribution in edible fish from Negro River basin, Argentinean Patagonia. *Chemosphere*, **94**: 135–142.

PAULY, D. 1979. Gill size and temperature as governing factors in fish growth: a generalization of von Bertalanffy´s growth formula. *Berichte aus dem Institut für Meereskunde Kiel*, **63**: 45–64.

PAULY, D. & CADDY, J.F. 1985. *A modification of Bhattacharya's method for the analysis of mixtures of normal distributions*. Food and Agricultural Organization of the United Nations, Fisheries Circular no.781.

PAULY, D. & GASCHUTZ, G. 1979. A simple method for fitting oscillating length growth data, with a program for pocket calculators. *Intenational Council for the Exploration of the Sea, Council Meeting. G: 24. Demersal Fish Committee*. ICES.

PAULY, D. & MUNRO, J.L. 1984. Once more on the comparison of growth in fish and invertebrates. *Fishbyte*, **2**: 21.

PHILLIPS, B.L. 2009. The evolution of growth rates on an expanding range edge. *Biology Letters*, **5**: 802–804.

PHILLIPS, B.L., BROWN, G.P., WEBB, J.K. & SHINE, R. 2006. Invasion and the evolution of speed in toads. *Nature*, **439**: 803.

PIANKA, E.R. 1970. On *r*- and *K*-selection. *American Naturalist*, **104**: 592–597.

PICCOLO, M.C. & PERILLO, G.M.E. 1997. Geomorfología e hidrografía de los estuarios. In: *El Mar Argentino y sus recursos pesqueros* (Boschi E.E., ed.), pp. 133–161. Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Mar del Plata, Argentina.

PIGNEUR, L.M., ETOUNDI, E., ALDRIDGE, D.C., MARESCAUX, J., YASUDA, N. & VAN DONINCK, K. 2014. Genetic uniformity and long-distance clonal dispersal in the invasive androgenetic *Corbicula* clams. *Molecular Ecology*, **23**: 5102–5116.

PIGNEUR, L.-M., HEDTKE, S.M., ETOUNDI, E. & DONINCK, K., V.A.N. 2012. Androgenesis: a review through the study of the selfish shellfish *Corbicula* spp. *Heredity*, **108**: 581–591.

PIGNEUR, L.-M., MARESCAUX, J., ROLAND, K., ETOUNDI, E., DESCY, J.-P. & VAN DONINCK, K. 2011. Phylogeny and androgenesis in the invasive *Corbicula clams* (Bivalvia, Corbiculidae) in Western Europe. *BMC Evolutionary Biology*, **11**: 147.

PINHEIRO, J., BATES, D., DEBROY, S. & SARKAR, D., R CORE TEAM. 2017. nlme: Linear and nonlinear mixed effects models. R package version 3.1–131.1, http://CRAN. R-project. org/package=nlme.

PREZANT, R.S. & CHALERMWAT, K. 1984. Flotation of the bivalve *Corbicula fluminea* as a means of dispersal. *Science*, **225**: 1491–1493.

RAMÓN, M., FERNÁNDEZ, M. & GALIMANY, E. 2007. Development of mussel (*Mytilus galloprovincialis*) seed from two different origins in a semi-enclosed Mediterranean Bay (N.E. Spain). *Aquaculture*, **264**: 148–159.

RICCIARDI, A. 2012. Invasive species. In: *Ecological systems: selected entries from the Encyclopedia of sustainability science and technology* (R. Leemans, ed.), pp. 161–178. Springer-Verlag, New York.

ROFF D.A. (ed.) 1993. *Evolution of life histories: theory and analysis*. Springer Science & Business Media, USA.

RUMI, A., GUTIÉRREZ GREGORIC, D.E., NÚÑEZ, V. & DARRIGRAN, G.A. 2008. Malacología Latinoamericana. Moluscos de agua dulce de Argentina. *Revista de Biología Tropical*, **56**: 77–111.

SAKAI, A.K., ALLENDORF, F.W., HOLT, J.S., LODGE, D.M., MOLOFSKY, J., KIMBERLY, A., BAUGHMAN, S., CABIN, R.J., COHEN, J.E., ELLSTRAND, N.C., DAVID, E., NEIL, P.O., PARKER, I.M., THOMPSON, J.N., WELLER, S.G. & NEIL, O. 2001. The population biology of invasive species. *Annual Review of Ecology and Systematics*, **32**: 305–332.

SCHMIDLIN, S. & BAUR, B. 2007. Distribution and substrate preference of the invasive clam *Corbicula fluminea* in the river Rhine in the region of Basel (Switzerland, Germany, France). *Aquatic Sciences*, **69**: 153–161.

SIEMANN, E. & ROGERS, W.E. 2001. Genetic differences in growth of an invasive tree species. *Ecology Letters*, **4**: 514–518.

SKELLAM, J.G. 1951. Random dispersal in theoretical populations. *Biometrika*, **38**: 196–218.

SOMERS, I.F. 1988. On a seasonally oscillating growth function. *Fishbyte*, **6**: 8–11.

SOUSA, R., ANTUNES, C. & GUILHERMINO, L. 2006. Factors influencing the occurrence and distribution of *Corbicula fluminea* (Müller, 1774) in the River Lima estuary. *Annales de Limnologie—International Journal of Limnology*, **42**: 165–171.

SOUSA, R., ANTUNES, C. & GUILHERMINO, L. 2008. Ecology of the invasive Asian clam *Corbicula fluminea* (Müller, 1774) in aquatic ecosystems: an overview. *Annales de Limnologie—International Journal of Limnology*, **44**: 85–94.

STEARNS, S.C. 1976. Life-history tactics: a review of the ideas. *Quarterly Review of Biology*, **51**: 3–47.

STEARNS, S.C. 1977. The evolution of life history traits: a critique of the theory and a review of the data. *Annual Review of Ecology and Systematics*, **8**: 145–171.

STEARNS, S.C. 1992. *The evolution of life histories*. Oxford University Press, Oxford.

TAYLOR, C.C. 1958. Cod growth and temperature. *Journal du Conseil*, **23**: 366–370.

VAKILY, J.M. 1992. *Determination and comparison of bivalve growth, with emphasis on Thailand and other tropical areas*. WorldFish, Manila.

VAN ROOIJ, J.M., BRUGGERMANN, J.H., VIDELER, J.J. & BREEMAN, A.M. 1995. Plastic growth of the herbivorous reef fish *Sparisoma viride*: field evidence for a trade-off between growth and reproduction. *Marine Ecology Progress Series*, **122**: 93–106.

VENABLES, W.N. & RIPLEY, B.D. 2002. *Modern applied statistics with S*. Springer, New York.

VILLAR, C., STRIPEIKIS, J., HUICQUE, L.D., TUDINO, M., TROCCOLI, O. & BONETTO, C. 1999. Cd, Cu and Zn concentrations in sediments and the invasive bivalves *Limnoperna fortunei* and *Corbicula fluminea* at the Río de la Plata basin, Argentina. *Hydrobiologia*, **416**: 41–49.

VOHMANN, A., BORCHERDING, J., KURECK, A., BIJ DE VAATE, A., ARNDT, H. & WEITERE, M. 2009. Strong body mass decrease of the invasive clam *Corbicula fluminea* during summer. *Biological Invasions*, **12**: 53–64.

WIDDOWS, J. & DONKIN, P. 1992. Mussels and environmental contaminants: bioaccumulation and physiological aspects. In: *The mussel Mytilus: ecology, physiology, genetics and culture* (E. Gosling, ed.), pp. 383–424. Elsevier, Amsterdam.

ZUUR, A.F., IENO, E.N., WALKER, N.J., SAVELIEV, A.A. & SMITH, G.M. 2009. *Mixed effects models and extensions in ecology with R*. Springer Science and Business Media, New York.