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PRIMARY RESEARCH PAPER

Physiological and morphological assessments suggest opposite structural allocation strategies between closely related invasive clams

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Abstract Respiration is a central physiological function related to the rates of energy acquisition, transformation, and use by organisms, while allometric relationships provide insight into patterns of allocation of that energy. To assess life strategy differences between closely related invasive species, we measured oxygen consumption rates, tissue mass, shell mass, and gill area of *Corbicula fluminea* and *Corbicula largillierti* clams. We compared patterns of metabolic scaling between the two species using model II regressions. Although oxygen consumption rates were strongly associated with gill area in both species, *C. fluminea* had consistently higher metabolic rates, shell thickness, and shell mass, but a lower tissue-to-shell mass ratio, than *C. largillierti*. These differences were more marked among small individuals. Our results suggest opposite structural allocation strategies between the two species. *Corbicula fluminea* invests more energy in the development of thicker shells in early life stages, which can be subsidized by higher metabolic rates than *C. largillierti*. By contrast, *C. largillierti* allocates more energy to tissue mass production. These differences may play a role in explaining contrasting competitive and colonization abilities and geographical distribution patterns between both species in invaded areas.

Keywords *Corbicula* · Physiology · Allometry · Morphology · Introduced species

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Introduction

Metabolic rates reflect organisms' ability to uptake and convert energy into materials that support biological processes and structures (Hochachka & Somero, 2002). In aerobic organisms, metabolic rates can be estimated by aerobic respiration (R), related to body mass (M) by the power function $R = aM^b$; where a is the scaling (or allometric) coefficient (or the antilog of the intercept in a log–log plot; Glazier, 2010) and b is the scaling (or allometric) exponent (Glazier, 2014; Hirst et al., 2014). These scaling parameters show inter- and intra-specific variability based on external (e.g., ecological) and internal (e.g., anatomical) factors affecting organisms' metabolism (Glazier, 2005, 2010). Both high and low metabolic levels have been associated with competitive advantages by different authors ('increased-intake' and 'compensation' hypotheses, respectively; Steyermark, 2002; Lagos et al., 2017). Thus, as physiological differences can result in contrasting degrees of biological performance, establishment success, and dominance (Karatayev et al., 2011; Fly & Hilbish, 2013), the experimental determination of metabolic scaling parameters constitutes a powerful tool for biological comparisons among species (Lagos et al., 2017; Wesselmann et al., 2020).

Organisms' energy budgets must be split among survival, growth, and reproduction (Gadgil & Bossert, 1970; Stearns, 1976). In bivalves, lifetime patterns of energy allocation are directly linked to individuals' shell-tissue growth ratio (i.e., structural allocation) and ontogenetic period (McMahon & Bogan, 2001; Labecka & Czarnoleski, 2019). The shell represents a considerable part of the total energy investment in growth in these organisms (Wilbur & Saleuddin, 1983). Thus, it can be assumed that thin-shelled species devote less energy to shell production than thick-shelled counterparts. This frees resources to fast tissue growth and reproduction but does so at the expense of increased mortality to predation, desiccation, and dislodgement from the substrate (McMahon & Bogan, 2001). Allometric scaling (i.e., proportional change in body size) can reveal patterns of structural allocation in these organisms (Dudycha & Lynch, 2005; Labecka & Czarnoleski, 2019), such that meaningful comparisons among species should include physiological and allometric assessments (Killen et al., 2016).

Invasive clams of the genus *Corbicula* were first detected in Argentina in the Río de la Plata estuary in 1979 (Ituarte, 1981). *Corbicula largillierti* (Philippi, 1844) arrived first and dispersed throughout the basin, but subsequent colonization by its *C. fluminea* (O.F. Müller, 1774) congener was followed by its replacement in many areas (Darrigran, 2002; Reshaid et al., 2017). Presently, *C. largillierti* persists in low-order rivers and lakes in northern and central Argentina, where *C. fluminea* is rare (Fig. 1A; Rumi et al., 2008; Pereira et al., 2014). It has been proposed that competition between the two species and different adaptive traits underlie this disjunct distribution pattern (Darrigran, 2002; Torre & Reyna, 2013; Pereira et al., 2014). For example, organisms with a low metabolism are often good early colonizers, while those with a high metabolism prevail in competitive environments in the long run (Pettersen et al., 2020). Previous studies have reported metabolic rates seemingly different for both species, ranging 9.38–87.5 µmol O_2 $g_{TDW}{}^{-1}$ h^{-1} for *C. fluminea* and 2.35–30.09 µmol O_2 $g_{TDW}{}^{-1}$ h^{-1} in the case of *C. largillierti* (Table 1). Yet, the use of different methods and experimental conditions hamper comparison between the two species.

Other differences between the species potentially affecting relative fitness and dominance patterns, include the relationships between metabolic rate and gill area and the shell-tissue structural allocation ratio. Given that the gills are the primary site of gas exchange in bivalves (Jørgensen, 1990), a relationship between gill area and metabolic activity is possible. Likewise, a relationship between life strategies and how organisms allocate energy could be expected. For instances, the shorter life expectancy of *C. largillierti* might be related to a lower shell investment than that made by *C. fluminea*, since those resources are likely wasted; therefore, their allocation to tissue growth and reproduction might be a better investment in a short-lived organism (Heino & Kaitala, 1996, 1999; Anthony et al., 2002). Unfortunately, in the absence of direct comparisons concerning these and other biological traits relevant to relative fitness between *C. largillierti* and *C. fluminea*, the mechanisms underlying dominance and substitution between the two species remain unexplained.

The main objective of this work is to directly compare physiological and morphological features between *C. largillierti* and *C. fluminea*. We tested three hypotheses. First, that invasive *Corbicula* spp.

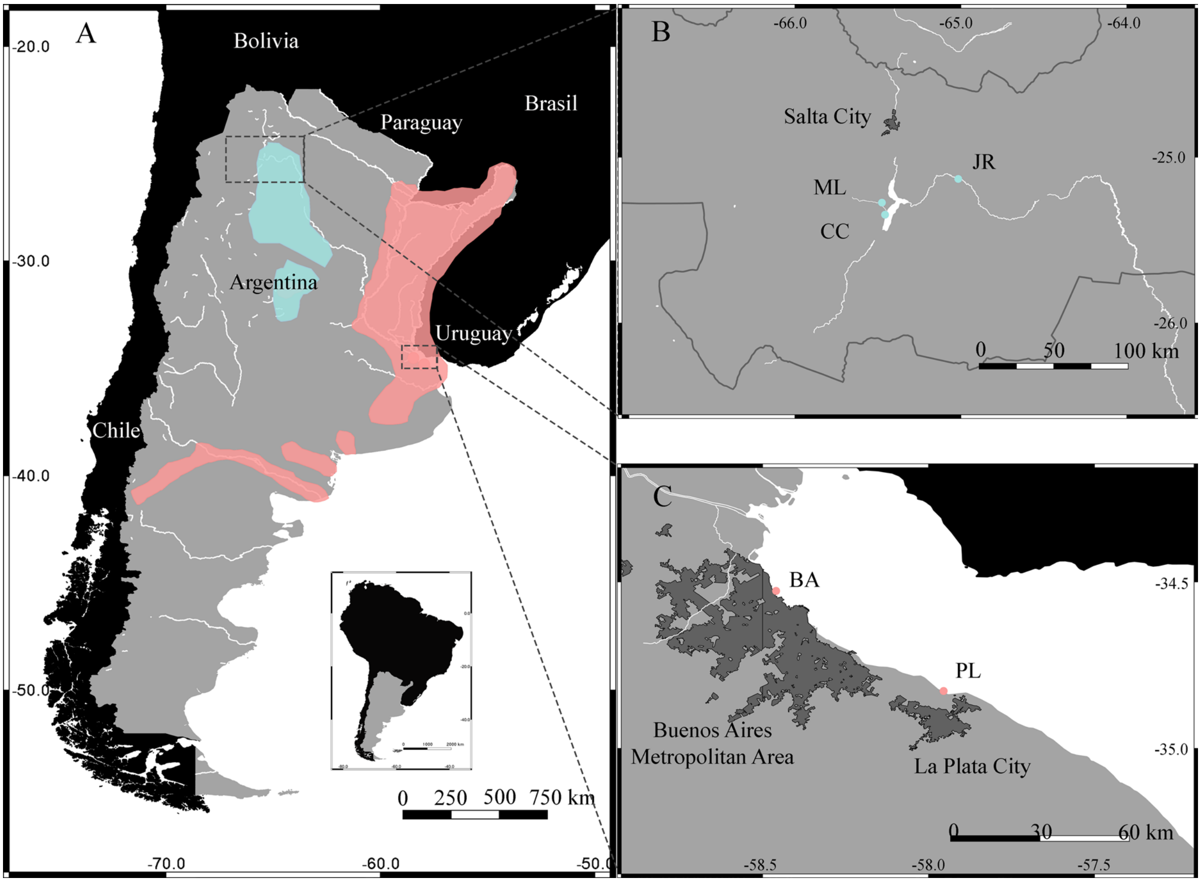

Fig. 1 **A** Distribution ranges of *Corbicula fluminea* (red), *C. largillierti* (blue) obtained from malacological collections of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" and the Museo de La Plata and own surveys, and regional location of the sampling areas of five *Corbicula* populations studied in Argentina between November 2016 and January 2017. **B** Sampling sites for *C. largillierti* in the Salta province: *CC* Cabra Corral Reservoir, *ML* agricultural irrigation channel in the town of Coronel Moldes, *JR* Juramento River. **C** Sampling sites for *C. fluminea* in the Río de la Plata estuary: *PL* Punta Lara, *BA* Buenos Aires

in Argentina have different oxygen consumption and standard metabolic rates. We expect that *C. largillierti*, the colonizer taxon, will show a lower metabolic level relative to body mass (scaling coefficient *a*) than *C. fluminea*, the competitive taxon. An associated prediction is that the slope of this relationship (scaling exponent *b*) will be higher for *C. largillierti* than *C. fluminea*, given the negative correlation between the two scaling parameters (Glazier, 2010). Second, that the tissue mass scaling of the gill area parallels the scaling of standard metabolic rates in both species. And third, that the two species differ in their structural allocation of tissue vs. shell mass with a lower shell investment and a higher tissue investment in *C. largillierti* than in *C. fluminea*. In the present study, we conduct laboratory measurements that allow direct comparison between both species, to test these hypotheses, and propose probable relationships between morphological and respiratory traits, relative competitive abilities, and patterns of geographical distributions of invasive *Corbicula* spp. in Argentina.

Methods

Specimen sampling and maintenance

During November–December 2016, individuals of *C. largillierti* were collected from three sites in the Upper Juramento basin in northern Argentina

Table 1 Comparison of standard metabolic rates (SMR) measured in this study with those reported in the literature for *C. fluminea* and *C. largillierti*

References	Lineage	Experimental setting	Temperature (°C)	Size (Length, mm)	*N*	SMR (µmol O_2 g_{TDW}^{-1} h^{-1})
Tran et al. (2000)	*C. fluminea*	Open-flow	15	30.2 ± 0.7	12	53.13–59.13
Belanger (1991)	*C. fluminea*	Closed chamber (4 clams per chamber)	25	15–15.5	4	87.5
Xiao et al. (2014)	*C. fluminea*	Closed chamber (10 clams per chamber)	4	19.31 ± 1.14	3	9.38
			11		3	18.75
			18		3	28.13
			25		3	46.88
			32		3	50
Ortman & Grieshaber (2003)	*C. fluminea*	Open-flow	15	10–33	18	19.42
McMahon (1979)	*C. fluminea*	Closed chamber	10	28.5–36.4	3	17.86
			20		3	58.04
			30		3	44.64
Rodriguez et al. (2020)	*C. largillierti*	Closed chamber	20	16.15–29.21	40	12.84 ± 6.43
Liquin et al. (2021)	*C. fluminea*	Closed chamber	12	23.68 ± 5.88	99	16.0 ± 5.27
This study	*C. fluminea*	Closed chamber	20	17.07 ± 3.68	59	134.0 ± 59.9
This study	*C. largillierti*	Closed chamber	20	17.00 ± 2.54	84	115.8 ± 44.9

The type of experimental setting, experimental temperature, average size of the clams, and number of replicates (*N*) used in each study are shown. Size and SMR values are means ± SD or ranges, where available. All experimental settings comprised one clam per respirometric chamber unless indicated

(Salta province): an agricultural irrigation channel in the town of Coronel Moldes (25° 16′25.4″ S 65° 28′31.2″ W), the Cabra Corral Reservoir (25°20′47.4"S 65°27′24.4"W), and the Juramento River (25° 07′50.7″ S 65° 00′56.0″ W) (Fig. 1A, B); at these sites the sampling water temperatures values were 23.2, 21.8, and 22.4°C, respectively. In January 2017, *C. fluminea* were collected from two sites in the Río de la Plata estuary (Buenos Aires province) at the shores of the cities of Punta Lara (34° 49′40.0″ S 57° 57′16.7″ W) and Buenos Aires (34° 31′37.7″ S 58° 27′33.8″ W) (Fig. 1A, C), with sampling water temperatures of 21.1 and 22.8°C, respectively. Individuals were collected manually or using a shovel to wash sediments through a sieve (1 mm pore) from the nearshore sandy-rocky beaches. Where the bottom depth precluded manual collection, clams were collected using a Petersen-type grab sampler (bottom depth 3–5 m). Ca. 30 individuals of *Corbicula* were sampled from each population. Both species were identified by shell features (external coloration, general shell shape, prominence of the umbo and the posterior margin, and number and deep of shell sulcations) according to Ituarte (1994), Mansur and Pereira (2006), and Reyna et al. (2013). The bivalves were transported within 3 h to the laboratory, placed into small holding aquaria (20 l), supplied with dechlorinated tap water, and fed ad libitum on a green algae monoculture diet. Aquarium water was exchanged weekly, and water temperature adjusted daily at a rate of 1°C day^{-1} until reaching the experimental temperature (20°C, see below). Individual clams to be used in the metabolism experiments were selected randomly among clams between 11 and 24 mm in shell length, kept unfed for a minimum of 24 h, and maintained 48 h at the experimental temperature before the onset of the experiments (Liquin et al., 2021).

Metabolic measurements

Oxygen consumption rates (OCR) were measured on individual specimens (30 clams by site, three times each clam to take into account possible intrinsic variations of each individual) in 20-ml, acrylic respirometric chambers containing air-saturated water and

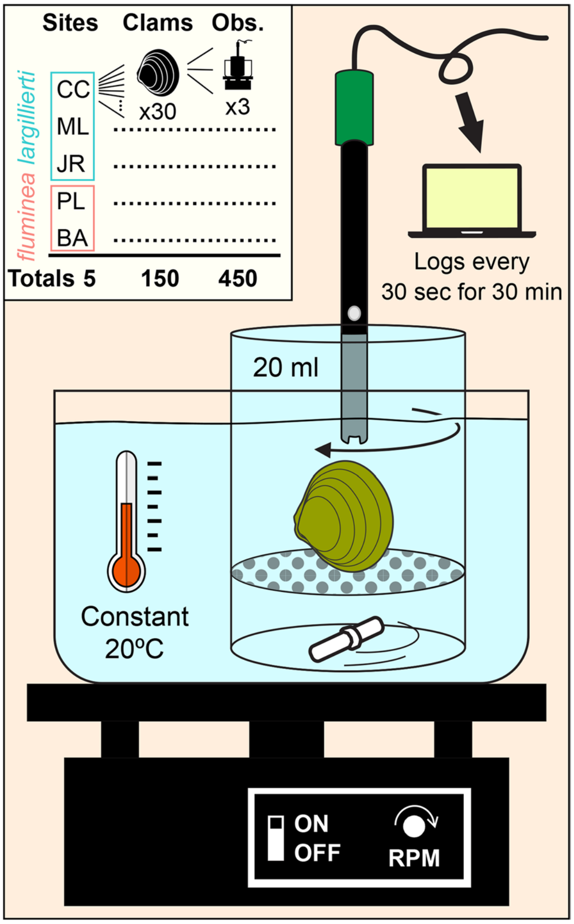

Fig. 2 Experimental setup of respirometric experiments conducted under controlled laboratory conditions on *Corbicula largillierti* (CC, ML, and JR) and *C. fluminea* (PL and BA) clams from five Argentine invasive populations in 2016–2017. The inset provides a schematic summary of the number of replicate individuals (Clams) and measurements (Obs.) conducted for each population (Site). Site codes as per Fig. 1

antibiotics to avoid bacterial oxygen consumption (100 mg l^{-1} each of streptomycin and ampicillin; Fig. 2; Paolucci et al. 2010). During the experiments, the water was gently mixed using a magnetic stirrer rotating beneath a perforated plate that supported the clams (Sobral & Widdows, 1997). During the measurements it was checked whether clams had open valves as an indicator for filtering activity. In this type of organisms this activity is considered standard metabolic rate (SMR), which is defined as the metabolic rate at rest, constant temperature, normoxia, and absence of food (Ortmann & Grieshaber, 2003). Between replicates, clams were placed in a different holding aquaria and fed again until 24 h before the next oxygen consumption measurement. The chamber temperature was kept constant at 20°C throughout the experiments using a water bath. The oxygen concentration in the chamber was measured using a calibrated oxygen electrode (Hanna® Instruments, EdgeHI764080) inserted through a sealed hole on the top of the chamber (Djangmah et al., 1980; Sobral & Widdows, 1997) at 30-s intervals for 30 min, or until oxygen saturation levels descending down to 40%, whichever comes first. It is important to clarify that it has been seen that *Corbicula* has mechanisms to keep the oxygen consumption rate constant at low concentration (~ 40%; Tran et al., 2000; Rodriguez et al., 2020), so the established limit should not generate errors in the measurement. Furthermore, only in 4 sub-replicas of 450 (i.e., ~ 1%) this value was reached. Sensors were calibrated at two points using a 5% sodium sulfite aqueous solution for oxygen-free water and gently stirred filtered water for oxygen-saturated water. Immediately after finishing the experiments, clams were fixed in 96% ethanol for subsequent morphological measurements. Three no-clam control experiments were conducted before measurements on each species in an identical fashion to the experimental runs but without clams to quantify eventual changes in the oxygen concentration due to bacterial or electrode activity. For comparative purposes with other studies OCR values were used to calculate SMR per clam (see Data analyses).

Morphometric measurements

Shell dimensions

Shell length (*L*), width (*W*), and height (*H*) were measured to the nearest 0.1 mm using a caliper (ESSEX, precision 0.01 mm) to estimate maximum anterior–posterior, lateral, and dorsal–ventral dimensions, respectively.

Weight measurements

Clam tissues were carefully dissected from the shells, dried individually in pre-cleaned porcelain crucibles for 48 h at 60°C, and weighed with a digital scale (precision 0.1 mg) to obtain the tissue dry weight (TDW). The shell weight (SW) was measured after drying the clam shell at room temperature.

We calculated a tissue: shell ratio (TSR) as the ratio between both mass measurements (TSR = TDW SW^{-1}) to have a measurement of relative structural allocation. Finally, we calculated a shell thickness estimator (ST) based on the works of Boulding (1984) and Johnson and Smee (2012) as the ratio between SW and general shell size (product of length of the three major body axes: $L \times H \times W$; cm^3; Novack-Gottshall, 2008).

Gill area

For each individual clam, the gills were dissected and placed in a Petri dish with ethanol 96%. Areas for the inner and outer demibranchs (gill area, GA) were delineated and calculated to the nearest 0.1 mm^2 using a dissecting microscope (Leica S8APO; 10X magnification) equipped with a digitizer and image processing software ''QCapture'' version 6.0 and AutoCAD 2000 (Paolucci et al., 2014). The relative gill area (RGA, mm^2 g^{-1}) was calculated as the ratio between the gill area and TDW to estimate the amount of gas-exchange surface per g of tissue mass.

Data analyses

Oxygen consumption and standard metabolic rates

We performed a linear regression of the slope of the oxygen concentration as a function of the time using Graphical Analysis software (Vernier Software, Sarasota, FL, USA), and converted the slope into a metabolic rate (Thuesen et al., 2005). Triplicate oxygen consumption measurements were used to calculate mean oxygen consumption rates (OCR, μmol O$_2$ h^{-1}) and standard metabolic rates (SMR, μmol O$_2$ $\mathrm{g_{TDW}}^{-1}$ h^{-1}) as the quotient between OCR and the TDW obtained per clam.

Allometry

We used the oxygen consumption rates and morphometric variables measured to study allometric relationships on both species. Allometric relationships were analyzed based on allometric equations expressed through the power function (1) (Huxley, 1932) obtained from the linear regression (2) based on the log-transformed values of our morphological and metabolic measurements:

$$Y_1 = aY_2^b \tag{1}$$

$$\log\left(Y_1\right) = \log(a) + b\log\left(Y_2\right) \tag{2}$$

where Y_1 and Y_2 are biological variables measured (e.g., body mass and physiological rates), and a and b are the allometric coefficient and allometric exponent, respectively (Huxley, 1932; Schmidt-Nielsen, 1975; Peters, 1983).

We fitted major axis (MA) or standardized major axis (SMA) regressions with selected pairs of biological variables (see below) for each species and compared their slopes (allometric exponent b) and intercepts (logarithmic of the allometric coefficient a, Warton et al. 2006) in R (R Core Team, 2020) using the "smart" package (Warton et al., 2012). We used reduced major axis regressions, MA and SMA, rather than alternative analyses based on ordinary least squares (OLS) since it is the recommended analysis in situations where the dependent and independent variables are arbitrarily assigned and the slope of the regression is applied to assess changes in shape across different sizes (RMA, following Warton et al., 2006; Smith, 2009; Legendre & Legendre, 2012). For comparison purposes only with data in the literature (often based on OLS) we calculate the OLS scaling exponent (b_{OLS}) using the "smart" package (Warton et al., 2012). Additionally, we tested the existence of isomorphy, i.e., preservation of the basic shape as organisms varies in size, between each pair of variables, comparing the scaling exponent b with theoretical values (see below). When both variables of the regressions were measured in the same units, we used MA; in any other case we used SMA.

The allometric exponent, b, which has deserved wide theoretical and empirical development (Niklas & Hammond, 2019), is interpreted as the ratio of the specific growth-rates of both variables in Eq. (1) (Huxley, 1932) hence it provides information about geometric growth. In organisms with isomorphic growth, a change in any one linear dimension is accompanied by a change in all other linear dimensions in exactly the same proportion ($b = 1$), while surfaces and volumes (or weights) scale at b values of 2 and 3 with respect to shell length, respectively (Schmidt-Nielsen, 1975; Okie, 2013). With respect to metabolic rates, surface area theory predicts that in an isomorphically growing organism surface area will decline relative to volume or mass at a rate $b = {}^2\!/_3$ and metabolism rates should decrease at the same rate because respiration is proportional to the surface area (Glazier, 2005; Okie, 2013; Hirst et al., 2014). Therefore, relative allometric growth patterns were classified as isomorphic when $b = 1$ for models relating shell linear measurements, $b = 3$ when evaluate relationship between length and mass (shell or tissue mass), and $b = {}^2\!/_3$ for relationships between gill area and tissue mass (TDW). With respect to metabolic scaling (relationship between oxygen consumption rate and TDW), we established a theoretical value of $b = {}^2\!/_3$ because we hypothesized that it should parallel gill area vs. tissue mass scaling.

The regressions performed, using $\log_{10}$-transformed data, were as follows: Inter-specific differences in metabolic rates were studied by comparing the relationship between oxygen consumption rate (OCR) and tissue dry weight (TDW); the relationship between gill area (GA) and TDW was performed to test differences in respiratory surface development as function of meaningful metabolic tissue; differences in relative growth between shell and tissue mass were studied by regressions of shell weight (SW) and TDW on shell length (*L*); and shell allometry were studied with regressions of width (*W*) and height (*H*) on length (*L*).

In the cases where significantly different slopes did not allow comparisons of intercepts between species, we applied the Johnson–Neyman (JN) technique to determine regions of non-significance between species along the range of the covariate (Johnson & Fay, 1950; White, 2003).

Shell thickness and tissue:shell ratio

To contrast structure allocation strategies between *Corbicula* species we analyzed their ST and TSR and their change across sizes, grouping the clams of each species into three size categories (small: 11.28–16.53 mm; medium: 16.61–20.45 mm; and large: 20.53–23.45 mm) based on their shell lengths. For it, we identified modal lengths components in clams sampled applying the Bhattacharya's method available in FISAT II software (Version 1.2.0, FAO-ICLARM Fish Assessment Tools; Gayanilo et al., 2002). To confirm each modal component, we used the NORMSEP method also available in the FISAT II software (Pauly & Caddy, 1985). We estimated two linear mixed-effects models (LMM), with package "lme4" (Bates et al., 2015) in R (R Core Team, 2020), in which the species (factor with two levels: *C. fluminea* and *C. largillierti*) and size (factor with three levels: small, medium, large) were incorporated as fixed effects and the ST or TSR as response variable. In both models we include population as a random effect. We assumed a Gaussian error structure of the response variables. The assumptions of the models were checked by means of graphics (QQ-plot and plot of predicts vs. residuals) and analytic inference (Levene test). When significant differences were found, we conducted Tukey HSD comparison tests between groups and corrected the *p*-values using the Bonferroni adjustment (Wright, 1992).

Results

Oxygen consumption and standard metabolic rates

Corbicula fluminea and *C. largillierti* showed on average a OCR of 4.3 ± 1.7 and 3.0 ± 0.8 µmol O_2 h^{-1}, respectively. Converted to SMR, *C. fluminea* presents average values of 134.0 ± 59.9 µmol O_2 $g_{TDW}{}^{-1}$ h^{-1} while *C. largillierti* averaged 115.8 ± 44.9 µmol O_2 $g_{TDW}{}^{-1}$ h^{-1} (Table 2).

Allometry

Corbicula fluminea presented a higher metabolic level (coefficient *a*) and a less steep slope (exponent *b*) than *C. largillierti* on the allometric scaling between OCR and TDW (Table 3). JN analyses showed that the non-significance zone was between 47.63 and 480.71 mg of TDW. For smaller clams, which accounted for all but six individuals in our samples, OCR inter-species differences were significant (Fig. 3A). For this relationship, the allometric exponent of *C. largillierti* clams (0.60) was not significantly different from the ${}^2\!/_3$ theoretical value (Table 3). In contrast, *C. fluminea* showed an allometric exponent of 0.47 significantly lower than ${}^2\!/_3$ (Table 3). The allometric relationship between gill area and TDW paralleled the metabolic scaling: *C. largillierti* had an allometric exponent (0.71) significantly higher than that of *C. fluminea* (0.57). For *C. largillierti*, this exponent was not significantly different from the ${}^2\!/_3$ value of isomorphy, while *C. fluminea* showed non-isomorphic growth

(Table 3; Fig. 3B). JN analyses showed a wide zone of non-significance between 0.38 and 492.96 g_{TDW} virtually ruling out GA differences between species.

The rate of increase in SW with respect to length was not significantly different between species (likelihood ratio test between allometric exponents; Table 3). Nevertheless, they showed marked differences on allometric coefficients (log (*a*), Table 3), having *C. fluminea* clams heavier shells than *C. largillierti* congeners of the same length (Tables 2, 3). SW showed isomorphic growth (*b* value not significant different for 3) in *C. fluminea* clams, while *C. largillierti* has a significant positive allometric growth (*b* value significantly higher than 3; Table 3; Fig. 4A). Allometric relationships between tissue mass (TDW) and length were also positive, but in this case *C. fluminea* presented positive allometry, while *C. largillierti* had an isomorphic relationship (Fig. 4B; Table 3). Thereby, allometric exponents were significantly different between species (Table 3). JN analyses identified a non-significant zone between 4.84 and 28.60 mm of shell length, which suggests there were no significant TDW differences between the two clam species.

With respect to width, both species showed isometric growth and no significant slope differences were detected. *Corbicula fluminea* presented inflated shells across all lengths (Fig. 4C), which was reflected in higher intercepts than *C. largillierti* (Table 3). The two species were almost similar in height except for the size distribution extremes (JN non-significance zone between 11.8 and 19.43 mm in length; Fig. 4D), although significant slope differences were found (Table 3). While *C. largillierti* showed isometric growth, *C. fluminea* showed a slightly positive allometry ($b = 1.12$; Table 3).

Shell thickness and tissue:shell ratio

Corbicula fluminea clams had thicker shells than *C. largillierti* congeners, and although these differences were maintained across all sizes, they were wider in the small clams, which is reflected in the significant interaction effect between species and size (Fig. 5A; Table 4). The opposite pattern was found for TSR, for which *C. largillierti* presented significant higher values in both small and medium clam size ranges, while for large clams no significant differences between species were found (Fig. 5B; Table 4).

Discussion

This is, to the best of our knowledge, the second time the metabolic rates of *C. largillierti* have been measured, and the first time direct metabolic comparisons between this species and its close congener *C. fluminea* have been attempted. Our most important finding is that, even though standard metabolic rates clearly correlate with clam size and gill area in both species, *C. fluminea* clams had consistently higher rates than *C. largillierti* in the species introduced ranges in Argentina, especially during early life. Physiological differences between the two species were reflected in morphometric differences with likely life-history consequences. *Corbicula fluminea* is the species that invests the most in shell development, especially during early development stages, which could be related to this species' slower growth but higher maximum size and longer life expectancy than *C. largillierti* (Darrigran & Maroñas, 1989). We propose that these differences can shape fitness,

Table 2 Descriptive statistics (mean ± standard deviation) (minimum; maximum values observed) of morphometric and metabolic variables measured on two *Corbicula* species collected from five populations in Argentina

Lineage	L (mm)	W (mm)	H (mm)	SW (g)	TDW (g)	GA (mm^2)	RGA (mm^2 mg^{-1})	OCR (µmol O$_2$ h^{-1})	SMR (µmol O$_2$ $g_{TDW}$$^{-1}$ h^{-1})
C. fluminea	17.0 ± 3.7	11.4 ± 2.4	15.6 ± 3.7	1.42 ± 0.89	0.043 ± 0.032	238 ± 102	7.03 ± 2.81	4.313 ± 1.662	134.0 ± 59.9
	(11.2; 23.4)	(7.9; 15.9)	(10.1; 22.7)	(0.36; 3.48)	(0.009; 0.134)	(101; 487)	(2.66; 13.3)	(2.238; 8.314)	(36.1; 263.2)
C. largillierti	17.0 ± 2.5	9.4 ± 1.4	15.4 ± 2.3	0.65 ± 0.29	0.029 ± 0.011	221 ± 63	7.99 ± 1.68	3.009 ± 0.810	115.8 ± 44.9
	(11.5; 21.8)	(5.8; 12.6)	(10.3; 19.9)	(0.13; 1.57)	(0.009; 0.058)	(111; 367)	(4.51; 12.5)	(1.398; 5.472)	(52.4; 310.5)

L length, *W* width, *H* height, *SW* shell weight, *TDW* tissue dry weight, *GA* gill area, *RGA* relative gill area, *OCR* oxygen consumption rate, *SMR* standard metabolic rate

Table 3 Allometric parameters (minimum; maximum 95% confidence interval) of the regressions fitted by major axis and standard major axis between log-transformed metabolic and morphometric variables measured in two *Corbicula* species collected from five populations in Argentina. *a*, allometric coefficient; *b*, allometric exponent; b_{OLS}, allometric exponent obtained from ordinary least squares regressions included for comparison purposes only with data in the literature; $r^2$and *p*, coefficient of determination and correlation test results of the global models, respectively

Regression	Lineage	Log (*a*)	WT (*p*)	*b*	b_{OLS}	Theoretical *b*	LR (*p*)	r^2	*p*
OCR vs TDW(mg)	*Fluminea*	− 0.11 (− 0.19; − 0.02)	45.63 (< 0.001)	0.47* (0.42; 0.52)	0.43	0.67	4.784 (0.029)	0.77	< 0.001
	Largillierti	− 0.39 (− 0.56; − 0.22)		0.60 (0.49; 0.73)	0.40			0.38	< 0.001
GA vs TDW(mg)	*Fluminea*	1.47 (1.37; 1.56)	8.493 (0.004)	0.57* (0.51; 0.63)	0.51	0.67	7.849 (0.005)	0.78	< 0.001
	Largillierti	1.31 (1.21; 1.42)		0.71 (0.63; 0.78)	0.64			0.82	< 0.001
SW vs *L*	*Fluminea*	− 3.60 (− 3.79; − 3.41)	1048 (< 0.001)	3.01 (2.86; 3.16)	2.96	3	2.854 (0.091)	0.96	< 0.001
	Largillierti	− 4.19 (− 4.44; − 3.94)		3.22* (3.02; 3.43)	3.10			0.93	< 0.001
TDW vs *L*	*Fluminea*	− 5.82 (− 6.20; − 5.45)	33.39 (< 0.001)	3.56* (3.26; 3.87)	3.37	3	12.94 (< 0.001)	0.87	< 0.001
	Largillierti	− 5.08 (− 5.36; − 4.80)		2.86 (2.64; 3.10)	2.67			0.87	< 0.001
W vs *L*	*Fluminea*	− 0.16 (− 0.21; − 0.11)	859.4 (< 0.001)	0.99 (0.95; 1.03)	0.98	1	1.437 (0.231)	0.97	< 0.001
	Largillierti	− 0.31 (− 0.39; − 0.22)		1.04 (0.97; 1.11)	1.00			0.91	< 0.001
H vs *L*	*Fluminea*	− 0.18 (− 0.23; − 0.13)	3.091 (0.079)	1.12* (1.08; 1.16)	1.11	1	15.33 (< 0.001)	0.98	< 0.001
	Largillierti	− 0.06 (− 0.10; − 0.02)		1.01 (0.98; 1.05)	1.00			0.98	< 0.001

Asterisks in the superscript of the *b* values indicate significant differences from the value obtained with the theoretical isometry (Theoretical *b*: 1, 0.67 or 3). Results of the Wald test (WT) and likelihood ratio tests (LR) and their significance (*p*) performed to compare intercepts (log(*a*)) and allometric exponents (*b*), respectively, between lineages are indicated in the columns next to the respective coefficients. Variable codes as for Table 2. Experimental temperature: 20°C

dominance, and ultimately, distribution patterns between the two species in their invasive ranges.

The respiration values measured for *C. largillierti* and *C. fluminea* (115.8 ± 44.9 and 134.0 ± 59.9 µmol O_2 g_{TDW}^{-1} h^{-1}, respectively) were widely variable and somewhat higher than those previously reported by other authors for the same species (9.4–87.5 µmol O_2 g_{TDW}^{-1} h^{-1}) (Table 1; Fig. 6). The high variability observed across studies is likely due to methodological differences, such as differences in the experimental temperature and size of the animals used (Table 1); still, most of the values reported in the literature fall within the limits of variation obtained in this study (Fig. 6). The values reported in the literature bearing the largest differences with the present results (Ortmann & Grieshaber, 2003; Xiao et al., 2014; Liquin et al., 2021) correspond to experiments conducted under sharply different conditions (e.g., lower temperatures), which likely at least partially account for some of the differences observed. Given the variability among reports, comparisons among species and populations must be performed on the same experimental and methodological grounds, as done in the present work, to be meaningful.

The studied populations of *C. largillierti* had metabolic rates consistently and significantly lower than those of their *C. fluminea* congeners, which could be associated with differences in other biological traits between the two species. Studies in South America have shown that *C. fluminea* has a lower growth constant *k* and a higher asymptotic length (0.377 and 51.99 mm, respectively) than *C. largillierti* (0.551

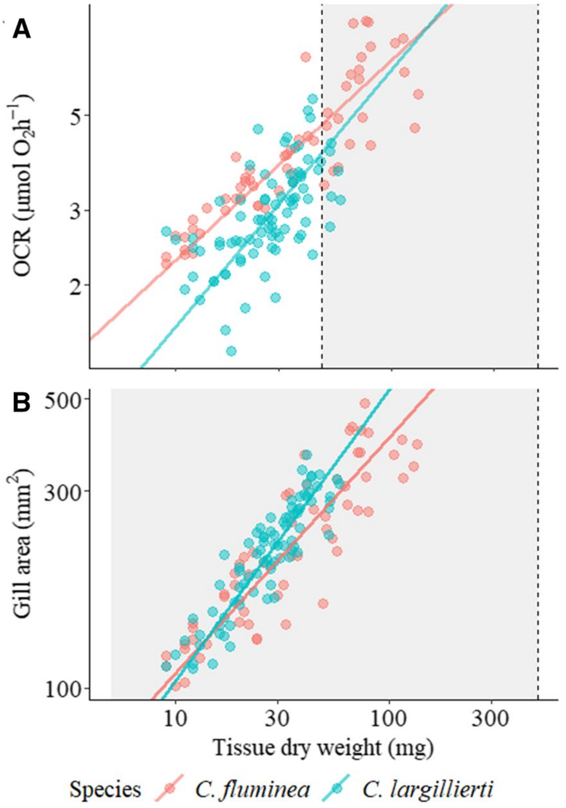

Fig. 3 Regression plots of clam oxygen consumption rates (OCR) (**A**) and gill area (**B**) as a function of shell-free dry weights for two *Corbicula* species collected from waterbodies in Argentina. Regression lines were drawn based on the results of model II regressions detailed in Table 3. Broken lines indicate the limits of the non-significance region determined by the Johnson–Neyman technique (shaded area). When missing, it is because they are outside the plotted range. Both axes are represented in a logarithmic scale

and 33.15 mm) (Darrigran and Maroñas 1989). For its part, *C. largillierti* presents a significantly higher average number of incubated larvae and a biannual reproductive cycle as opposed to the annual cycle of *C. fluminea* (Mansur et al., 2012).

Our results also indicate that *C. largillierti* clams have allometric relationships, of both gill area and oxygen consumption rates with respect to tissue dry weight, with a low intercept and a high slope compared to *C. fluminea*. In these organisms, the surface for gas exchange is chiefly determined by the gill area, which displayed allometric growth with the biomass ($b < 1$; West et al., 2001; for example, Hirst et al., 2014). In the case of *C. largillierti*, we found an isomorphic growth of the gill area with respect to clam biomass. The value of the allometric metabolic exponent found here ($b = 0.60$) was not different from the theoretical value established for the relationship between surfaces and mass ($b = {}^2/_3$; Okie, 2013). In contrast, *C. fluminea* showed non-isomorphic growth (i.e., $b < {}^2/_3$) both in the relationship between gill area and biomass ($b = 0.57$) and in the exponent of the allometric relation of metabolism ($b = 0.47$). Other studies have shown before this parallelism between metabolic and gill area allometries, although they have been interpreted as mediated by ecological factors (for example, selective predation by fishes over the amphipod *Gammarus minus* Say, 1818; Glazier & Paul, 2017; Glazier et al., 2020) and anatomical changes related with temperature (e.g., studies on the goldfish *Carassius auratus* (Linnaeus, 1758); Li et al., 2018).

The differences here found are large and fundamental, and perhaps also unexpected between organisms seemingly very similar. The metabolic rate is commonly considered the most fundamental biological rate of an organism, which can ultimately control and explain complex ecological processes related to species fitness (Fly & Hilbish, 2013; Schuster et al., 2021; although an alternative view may be found in Glazier, 2015). Yet, predicting the direction of the relationship between the standard metabolic rate and fitness is not straight forward (Burton et al., 2011). In the case of invasive species, high metabolic rates have been argued to explain invasive success (Lagos et al., 2017). This relationship, however, is not clearly established and the reverse pattern has also been reported (Baldwin et al., 2002; Steyermark, 2002), suggesting that the benefit of having high or low metabolic rates is context-dependent (Burton et al., 2011; Killen et al., 2016).

Differences in fitness may explain dominance and geographical and temporal patterns of replacement between species (Fly & Hilbish, 2013). For example, the replacement between freshwater invasive mussels *D. polymorpha* (Pallas, 1771) and *D. bugensis* Andrusov, 1897 in the Laurentian Great Lakes (Mills et al., 1999; Ricciardi & Whoriskey, 2004) has been attributed, among other factors, to differences in their metabolic capacities (Stoeckmann, 2003). *Dreissena bugensis*' lower respiration rates and greater shell growth and body mass likely confer this species an advantage over *D. polymorpha* in nutrient-poor environments (Stoeckmann, 2003). Similar differences

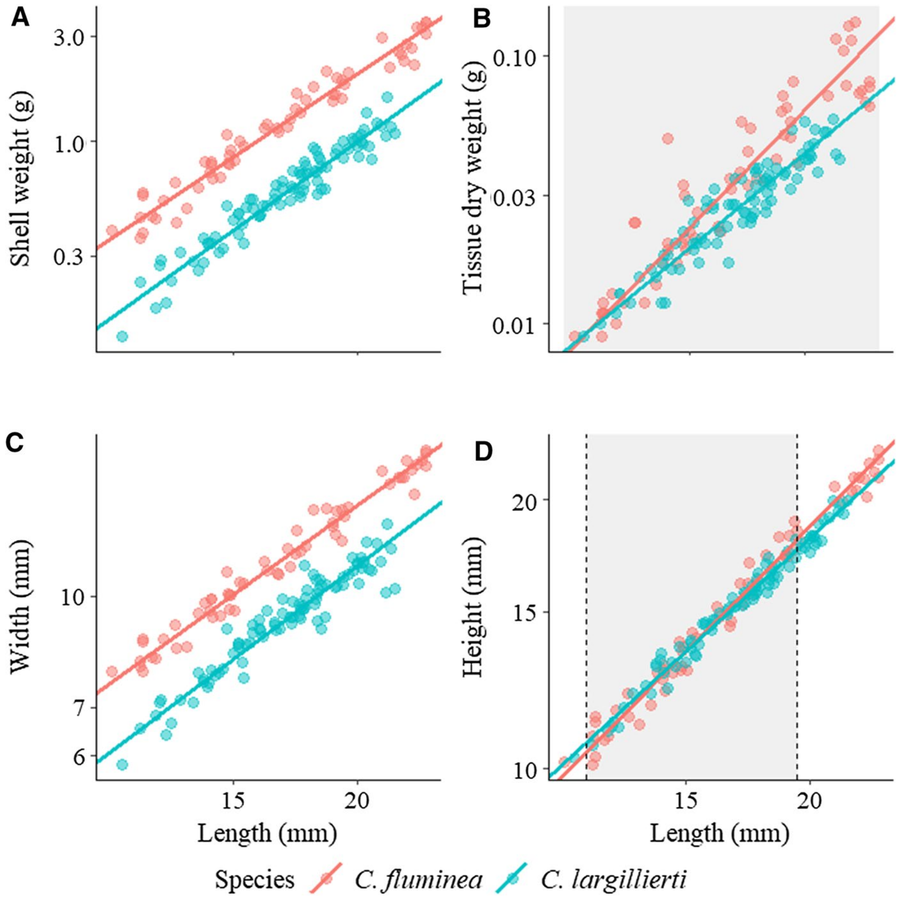

Fig. 4 Regression plots of shell weight (**A**), tissue dry weight (**B**), shell width (**C**) and shell height (**D**) as a function of shell length for two *Corbicula* species collected from five populations in Argentina. Regression lines were drawn based on the results of model II regressions detailed in Table 3. Broken lines indicate the limits of the of the non-significance region determined by the Johnson–Neyman technique (shaded area) (**B** and **D**). When missing, it is because they are outside the plotted range. Both axes are represented in a logarithmic scale

have been contended between shallow and profundal morphs of *D. bugensis*, but in this example the higher respiration rates, velocity of response, and tolerance to temperature changes of the shallow morph have been considered advantageous for life in the more variable epilimnion (Tyner et al., 2015).

Similarly, the displacement of *C. largillierti* clams by *C. fluminea* congeners in Río de la Plata estuary (Ituarte, 1985; Darrigran, 2002; Reshaid et al., 2017) could be at least partially related to the metabolic differences observed here. The lower sections of the Paraná-Río de la Plata watershed carry extremely large amounts of particulate organic carbon, which can match the metabolic demands of highly competitive filter-feeding invasive bivalves such as *C. fluminea* (Sylvester et al., 2005). Energetically costly traits such as the possession of thick shells can be afforded in such habitats (Chase & Bailey, 1999; McMahon & Bogan, 2001). We found that *C. fluminea* clams have heavier shells than *C. largillierti* congeners across all size classes, which likely confers the former species a more effective protection against the varied array of potential bivalve consumers inhabiting the Paraná-Río de la Plata watershed (Sylvester et al., 2007; Paolucci & Thuesen, 2015).

Limitations and caveats

A limitation of the study was the use of a single experimental temperature (20°C). While the physiological behavior can change at different temperatures, the use of a single experimental temperature was adequate for a first comparison, as in the present work, because it allowed a high number of replicates and measurements that increased accuracy and statistical power. This temperature was representative of temperature conditions during sampling, which roughly coincided with the annual averages in the collection sites (see Supplementary Information) and the

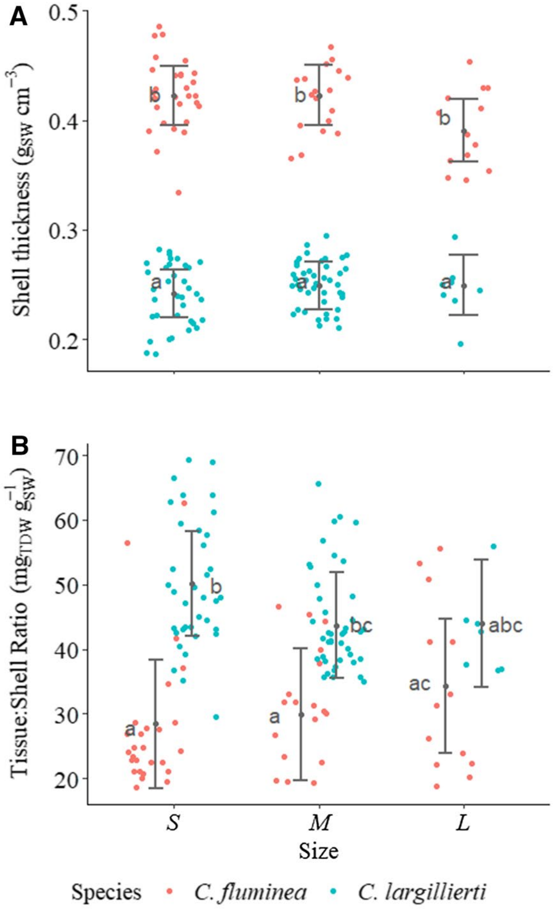

Fig. 5 Shell thickness (**A**) and tissue:shell mass ratio (**B**) of clams in three size groups belonging to two *Corbicula* species collected from waterbodies in Argentina in 2016–2017. *S* small, *M* medium, and *L* large shell length. Dots and error bars indicate mean and confidence interval values obtained from linear mixed models. Different letters indicate significant differences between groups obtained from Tukey HSD post-hoc comparisons. See Table 4 and the main text for model details

species metabolic optimum (Xiao et al., 2014; Rodriguez et al., 2020).

Another potential limitation was produced by the deliberate exclusion of individuals < 11 mm in shell length, as the relationship between OCR and size may differ across species (Glazier, 2005). This decision means that we can only confidently conclude for clams > 11 mm; but also presents the assets (superior in our view) of ensuring working with individuals in a similar state of sexual maturity (i.e., mature; Ituarte, 1984, 1985; McMahon, 2002) and keeping the oxygen consumption measurement error—disproportionately large in small sizes—reasonably low.

Lastly, given the segregated distribution of *Corbicula* species in Argentina, we cannot rule out sampling location confounding effects stemming from the influence of environmental variables or phenotypic differences or both on gill morphology. Environmental variables, relevant for the evaluation of relative physiological performances (Rodriguez et al., 2020), were not measured in the sites studied and thus their effects might wrongly be interpreted as interspecific differences. In an attempt to mitigate the likelihood of these effects, we have included more than one population from different types of habitats for each species. While we are confident that the differences here found between the two *Corbicula* species are meaningful, as they hold despite this diversity of populations, we acknowledge that some of the observed patterns might change if more populations are studied.

Conclusions

Our results show that *C. fluminea* has significantly higher metabolic rates than *C. largillierti* during a large part of the clams' lives. Our results seem to be consistent with previous data from other populations in Argentina, which collectively indicate that *C. fluminea* has a longer life expectancy and maximum size (= survival), while *C. largillierti* has a higher growth at initial stages, lower age at maturity, and greater brooding capacity (= reproduction). This evidence combined suggests contrasting ecological strategies between the two species with potential physiological and allometric bases and long-term distribution consequences. Important future research steps include conducting metabolism assessments at different temperatures, as well as studying the growth and reproduction of the same populations here surveyed to make direct comparisons with the morphologic and physiological patterns observed here.

Table 4 Estimated parameters and 95% confidence intervals (CI 95%) of the models made to compare differences in the shell thickness and tissue:shell mass ratios between two *Corbicula* clams species and three size categories [large, medium (M) and small (S)] collected from waterbodies in northern Argentina and Río de la Plata

Predictors	Shell thickness			Tissue:Shell mass ratio		
	Estimates	CI 95%	*P*	Estimate*s*	CI 95%	*P*
(Intercept)	0.39	0.36; 0.42	**< 0.001**	34.32	24.00; 44.65	**< 0.001**
Lineage [*largillierti*]	− 0.14	− 0.18; − 0.10	**< 0.001**	9.71	− 4.51; 23.93	0.181
Size [M]	0.03	0.01; 0.05	**< 0.001**	− 4.42	− 10.14; 1.30	0.130
Size [S]	0.03	0.02; 0.05	**< 0.001**	− 5.89	− 11.14; − 0.65	**0.028**
Size (M) * Lineage [*largillierti*]	− 0.03	− 0.06; − 0.01	**0.015**	4.07	− 4.78; 12.92	0.367
Size (S) * Lineage [*largillierti*]	− 0.04	− 0.06; − 0.01	**0.002**	11.99	3.58; 20.40	**0.005**
Random (Population, $N = 5$) effects						
σ^2	0.00			63.55		
τ_{00}	0.00			45.68		
VPC	0.35			0.42		
Observations	149			144		
Marginal R^2/conditional R^2	0.885/0.926			0.399/0.650		

The response variables of each model are indicated in bold in the first row. In all cases the factor "Population" was included as a random effect. For random effects, the value of the residual variance not explained by the model (σ^2) is indicated; the variance explained by the random effect (τ_{00}); and the variance partition coefficient (VPC). The number of observations (clams) used in each model and the values of the coefficients of determination (R^2) marginal and conditional are indicated

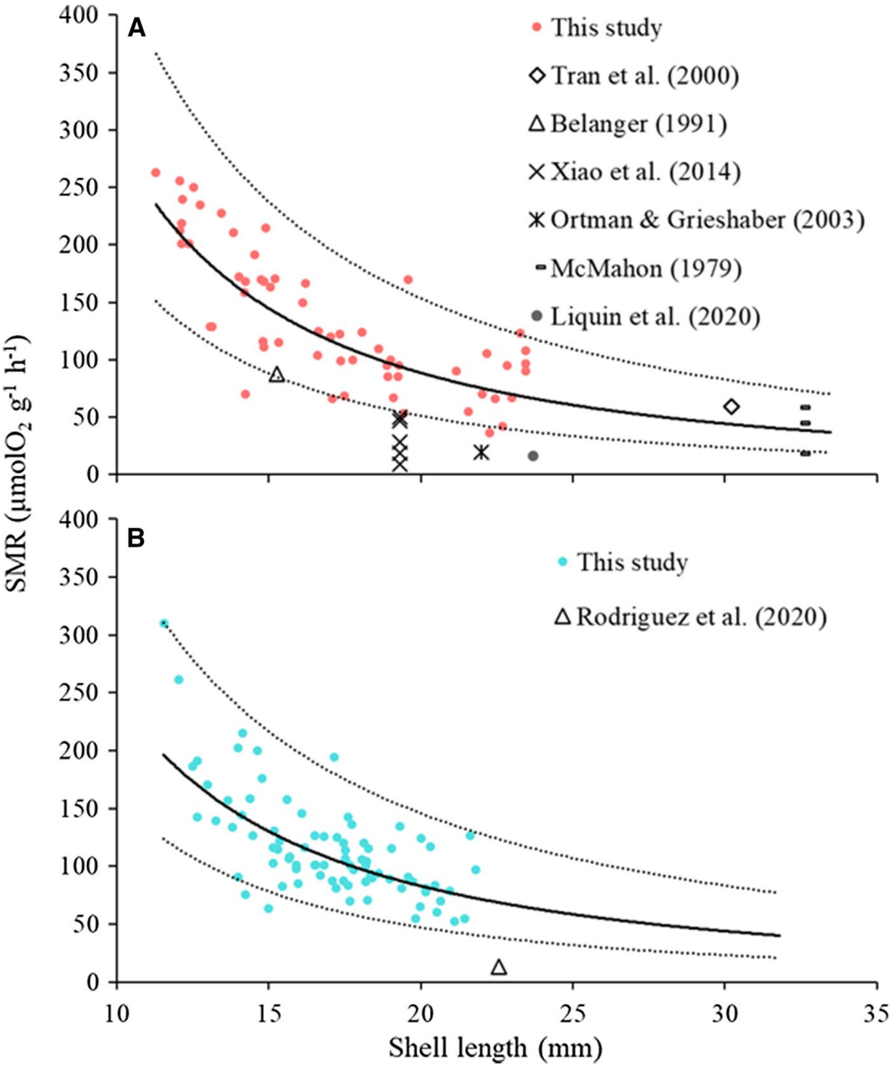

Fig. 6 Comparison between standard metabolic rates (SMR) as function of shell length for *Corbicula fluminea* (**A**) and *C. largillierti* clams (**B**) measured in this study and those reported in the literature. Black solid lines are fitted power regression lines for data obtained in the present study and dotted lines are upper and lower standard deviation limits. For details on the experimental design and mean SMR values obtained from the literature for each species see Table 1

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Author contributions All authors conceived the idea and designed the methodology used. LAH and FS collected the experimental organisms. LAH conducted laboratory procedures, led data analysis, and writing with supervision by FS and EMP. All authors contributed to the final version of the manuscript. FS and EMP provided funding acquisition, project administration, and resources.

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Data availability Row data generated in this survey are available from the Zenodo Digital Repository. https://doi.org/10.5281/zenodo.3542117 (Hünicken et al., 2019).

Code availability Not applicable.

Declarations

Conflict of interest The authors have no conflicts of interest to declare.

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