


# The role of temperature and oxygen availability on the distribution of *Corbicula largillierti*

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## Abstract

The invasiveness of *Corbicula largillierti* has been underestimated, but this bivalve is currently receiving more attention. The first study of the distribution of this species along one of the major basins of central Argentina showed that it was completely absent in the major central reservoir, but was present along its tributary and effluent rivers. Ten years later, this discontinuous distribution has not changed, despite the bivalve's high dispersion capacity. The main environmental differences between the reservoir and the rivers are that the former has finer sediment grain size, higher temperatures, and lower oxygen concentration during summer months. This study aimed to evaluate whether one of these factors could be limiting the distribution of *C. largillierti*. We assessed the metabolic response and burial behavior (under different grain sizes) of *C. largillierti* over 8 weeks at 30°C against a control condition at 20°C. We also analyzed the oxygen consumption ( $vO_2$ ) as a function of environmental oxygen concentration ( $pO_2$ ) at 25°C, estimating its metabolic regulation capacity ( $R$ ). This showed the existence of an environmental oxygen concentration threshold ( $P_t$ ) that could be an indicative of physiological limiting concentrations that compromise species survival and settlement. After 8 weeks at 30°C, the metabolic rate of individuals of *C. largillierti* increased and their burial behavior was slightly compromised but mostly for coarser sediments. Concerning environmental oxygen availability, *C. largillierti* is an oxyconformer with a low  $R$  (39.9%), a clear pattern of negative regulation, and a low  $P_t$  (48.4% air saturation). The lack of any regulation capacity of *C. largillierti* under environmental oxygen limitations and the extremely low  $pO_2$  at the bottom of the reservoir during summer months may explain its failure to colonize and survive there.

## KEYWORDS

burial behavior, freshwater bivalve, hypoxia, invasive species, oxyconformer

## 1 | INTRODUCTION

As a consequence of globalization, species movement has increased noticeably in the last century, spreading faster and farther from their native ranges (Ricciardi, 2015). Invasive species have a variable

number of characteristics that allow them to colonize and establish themselves in new environments (Richardson et al., 2011). Among these, “being able to function in a wide range of environmental conditions” (Ehrlich, 1986) might be one of the most controversial ones. On the one hand, it has been assumed that tolerance to a wide range

of physical conditions allows invasive species to inhabit different environments. On the other hand, authors such as McMahon (2002) argue that there is a lack of evidence to indicate that invasive species have such a physiological capacity. However, the main limitation to elucidating this controversy is that the experimental knowledge of the physiological limits of invasive species, particularly in freshwater bivalve species, is still limited (Feng & Papes, 2017).

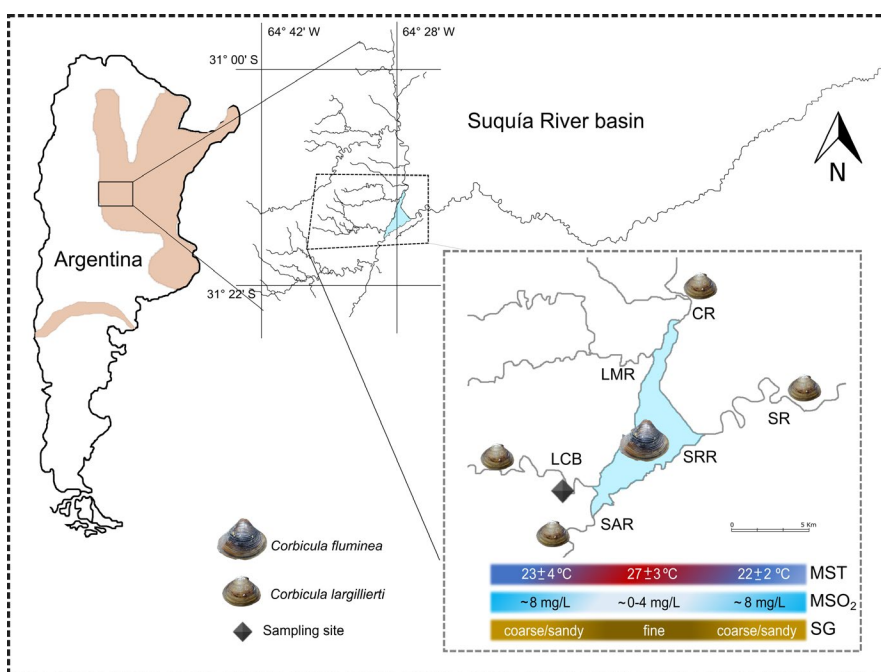
In recent decades, highly invasive bivalves, such as *Limnoperna fortunei* (DUNKER 1857), *Dreissena polymorpha* (PALLAS 1771), *Dreissena rostriformis bugensis* (ANDRUSOV 1897), and the Asian clams *Corbicula largillierti* (PHILIPPI, 1844), *Corbicula fluminalis* (MÜLLER, 1774), and *C. fluminea* (MÜLLER, 1774), have invaded wide areas of America and Europe (Boltovskoy, ; Gallardo et al., 2013; Korniusin, 2004; Reyna et al., 2018). These benthic freshwater species reach high densities in short periods of time, altering not only the substrate but also the carbon and energy flux among the components of the invaded system (Karatayev et al., 2007; McMahon, 2002; Sousa, Gutiérrez, & Aldridge, 2009). Because of their worldwide distribution and negative ecological and economic impact, extensive knowledge has been gathered about the distribution, morphology, growth, reproduction, and physiology of the most problematic of these (Boltovskoy, ; Karatayev et al., 2007; Orlova et al., 2005; Sousa et al., 2008). However, although *C. largillierti* has been shown to be a potential invader of a variety of ecosystems (Reyna et al., 2018), there are almost no studies about basic aspects of its biology, reproduction, or physiological traits (Azevêdo et al., 2016; Ituarte, 1984; Martins et al., 2006; Reyna et al., 2013). Filling the gaps of fundamental physiological information on this species is crucial to better understand its invasion capacity, predict the magnitude of its impact, and elaborate suitable management policies (Blackburn et al., 2014).

Temperature and environmental oxygen concentration ( $pO_2$ ) are among the most important factors that determine bivalve distribution

(Castañeda et al., 2018; Gama et al., 2017; Le Moullac et al., 2007; Pereira et al., 2017; Reyna et al., 2018; Schmidlin & Baur, 2007). Temperature determines, to a large degree, the metabolic rate of organisms. Such metabolic changes are likely to result in variations in the fitness of populations and, on a macro-ecological scale, determine species distribution (Crespo et al., 2015; McMahon, 1979; Rosa et al., 2012; Xiao et al., 2014). Concerning oxygen concentration, many aquatic sessile animals tolerate hypoxic conditions up to certain limits, and this has been related to their ability to tolerate and invade a particular system (Lagos et al., 2017). As has been summarized by Galic et al. (2019) even for tolerant species, hypoxia directly affects respiration, reproduction, growth, and feeding rates which, in the end, could result in altering population structure, biomass, and distribution. Determination of these specific environmental oxygen thresholds is fundamental to estimating the capacity of an organism to settle in a certain area (Suárez-Mozo et al., 2018; Zamorano et al., 2007).

*Corbicula largillierti* and *C. fluminea* are two widely spread species in Argentina which are found along the Suquia River basin (Córdoba, central Argentina). However, in this watercourse, these two species do not cohabit as they do in other areas (Reyna et al., 2013). While a small population of *C. fluminea* has been recorded only in the San Roque Reservoir (the main water body of the basin), *C. largillierti* has been found only in tributary and effluent rivers, but never in the reservoir itself (Figure 1). This particular distribution was observed for the first time by Reyna et al. (2013) in 2009 and remains the case to date (P.B. Reyna, unpubl. data).

Both species are usually spread accidentally by anthropogenic vectors, through aquaculture, in ballast waters, transported as bait for fishing activities or attached to recreational boats (Ilarri & Sousa, 2012). Natural spread of their veliger larvae and juveniles occurs by their floating downstream, or upstream by zoochory dispersal



**FIGURE 1** Map of the Suquia River basin location in central Argentina. Shadow areas in the Argentina map indicate recorded corbiculid distribution. The current distribution of *Corbicula largillierti* and *C. fluminea* in the basin is indicated with the corresponding valve shape. Main streams and water bodies of the basin are indicated as Cosquín River (CR), Las Mojarras River (LMR), Los Chorrillos brook (LCB), San Antonio River (SAR), San Roque reservoir (SRR), Suquia River (SR). Diamond indicates sampling site. Schematic representation of main hydrological differences between reservoir and surrounding river are represented under the map: mean summer temperature (MST), mean summer oxygen concentration (MSO<sub>2</sub>), and sediment granulometry (SG)

by water birds or fishes (Coughlan et al., 2017; Darrigran, 2002; Rosa et al., 2014). As there are no clear barriers to its dispersion, the lack of any record of *C. largillierti* in the reservoir suggests that environmental factors may be the main cause of this exclusion. There are clear hydrological differences between this reservoir and the surrounding rivers, especially during summer when the reservoir has mean temperatures of  $\sim 27 \pm 3^\circ\text{C}$  and the rivers remain  $\sim 22 \pm 4^\circ\text{C}$ . The high temperatures of the reservoir during summer months are within the natural maximum temperature range described for this species' distribution (Reyna et al., 2018). Nonetheless, the effect of long-term exposure to these temperatures may signify an increase in metabolic rate, with detrimental consequences on behavioral performance, growth, reproduction, and ultimately survival (Hochachka & Somero, 2002).

Additionally, this summer temperature enhancement in the reservoir, combined with anthropogenic nutrient inputs, generates a eutrophication process that leads to very low oxygen concentrations. Oxygen levels decrease to values ranging from 4 mg/L on the surface, to values lower than 1 mg/L on the bottom, whereas rivers maintain higher concentrations throughout the year ( $\sim 8$  mg/L; Alberto et al., 2001; Hued & Bistoni, 2005; Wunderlin, 2018; Wunderlin et al., 2001). In addition, water flow speed increases in the rivers during summer precipitations (up to  $15 \text{ m}^3/\text{s}$ ), but it is practically nil in the reservoir (Vázquez et al., 1979). As a consequence of water flow speeds, the rivers have a mix of rocky, gravel, and sandy patches, whereas mostly finer sediment particles settle in the reservoir (Hued & Bistoni, 2005; Wunderlin, 2018).

Considering the environmental differences mentioned above, our hypothesis is that the particular distribution of *C. largillierti* in the Suquía River basin is restricted by one or a combination of these environmental factors. The goal of the present study was therefore to assess how these factors affect metabolism and behavioral performance in *C. largillierti*, which would ultimately determine distribution of the species. To determine the effect of exposure to the reservoir summer temperature, we evaluated the metabolism and burial performance of individuals of *C. largillierti* exposed long term to high temperatures ( $30^\circ\text{C}$  for 8 weeks). We also evaluated burial behavior in sediments of different granulometries, which could determine burrowing capacity, to consider the effects of differences in sediment grain size between the reservoir and the rivers (Alexander et al., 1993; Downing et al., 2000). Additionally, we assessed the metabolic response of individuals of *C. largillierti* to different environmental oxygen concentrations, to determine whether low reservoir concentrations represent a possible threshold for the species' settlement and survival. The results are discussed in the framework of previous knowledge of species of *Corbicula* and their potential invasiveness.

## 2 | METHODS

### 2.1 | Clams and sediment sampling

Sediment and individuals of *C. largillierti* ( $n = 160$ ) were manually collected from Los Chorrillos brook ( $31^\circ 24' \text{S}$ ,  $64^\circ 30' \text{W}$ ; Figure 1)

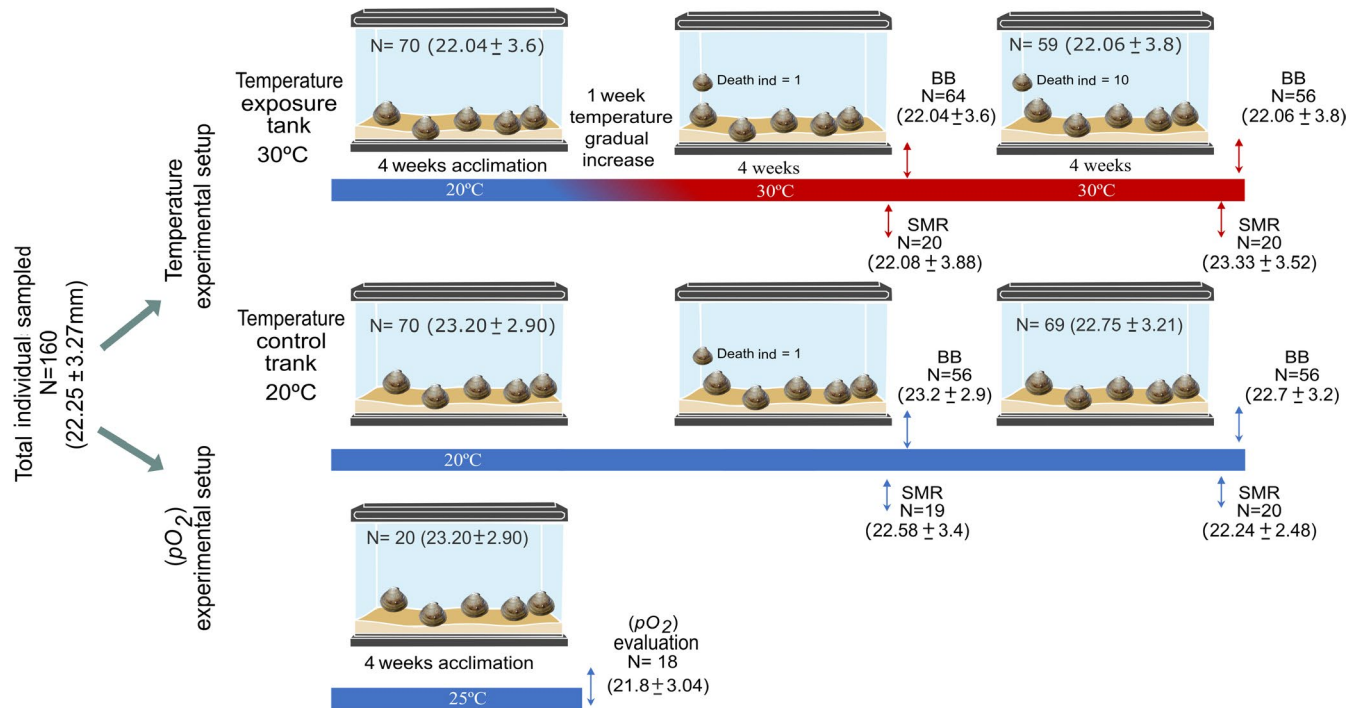
in the upper part of the Suquía River basin during December 2017. Individuals with a shell length (SL) of  $22.25 \pm 3.27$  mm (mean  $\pm$  SD) were selected. For this and the following individual measurements, a digital caliper accurate to 0.01 mm (Digimes Stainless Hardened DME 200) was used. Immediately after collection, all individuals and sediment were carefully transported to the aquarium. The experimental design was approved by the Institutional Committee for the Care and Use of Laboratory Animals of the Instituto de Diversidad y Ecología Animal (CONICET-Universidad Nacional de Córdoba, Argentina, number 11/2019).

### 2.2 | Temperature exposure

Groups of individuals were randomly chosen and were placed in two 50-L tanks ( $n = 70$  per tank, mean SL  $23.3 \pm 2.9$  mm and  $22.04 \pm 3.6$  mm, respectively) and acclimated for 4 weeks to aquarium conditions ( $20^\circ\text{C}$ ; 12:12 hr light: dark photoperiod; natural sediment and dechlorinated water [carbon-filtered tap water], continuously aerated). The control condition tank was kept at  $20^\circ\text{C}$ . After acclimation in the treatment tank, its temperature was gradually increased ( $1.5^\circ\text{C}$  per day), reaching  $30^\circ\text{C}$  by the end of the fifth week. In order to test long-term responses to the temperature increase, individuals were kept at each temperature for 8 weeks (Figure 2). The  $20$ – $30^\circ\text{C}$  interval fits the temperature sensitivity coefficient  $Q_{10}$  calculation and does not correspond to lethal temperatures for this species (Reyna et al., 2018).

During the acclimation and temperature exposure periods, water was renewed twice a week and animals were fed every other day. Food supply included a mix of ground TetraMin® Tropical Flakes (Tetra Holding GmbH) and commercial lyophilized *Chlorella vulgaris* (Organikal S HGL). At each change of water, ammonium and nitrate levels were checked with commercial tests (Tetra  $\text{NH}_3/\text{NH}_4^+$  and Tetra  $\text{NO}_3^-$ ; Tetra Holding GmbH).

After 4 weeks of temperature exposure, 20 individuals were randomly chosen from each temperature tank and relocated to a different tank at its corresponding temperature, without sediment and with filtered and dechlorinated water (carbon and  $0.1\text{-}\mu\text{m}$  mesh filtered tap water) for standard metabolic rate (SMR) determination. Immediately after individual SMR measurement, each individual was restored to its corresponding temperature tank. The following day, between 56 and 64 individuals were randomly chosen from each temperature tank and relocated to a different tank with dechlorinated water at the corresponding temperature to evaluate burial behavior. Four weeks later (treatment week 8), this procedure was repeated. Mortality was recorded daily (Figure 2). At the end of each measurement, each individual's fresh mass, SL, and volume were recorded (there were no significant differences between size ranges of the compared groups). At the end of the experiment, animals were sacrificed and dried at  $60^\circ\text{C}$  to obtain their constant dry mass. The dry mass for all previous measurements was estimated from biometric ratios calculated at the end of the experiment, resulting in the following equation:



**FIGURE 2** Schematic representation of experimental design to study response to different environmental conditions by individuals of *Corbicula largillierti*. The sequence of acclimation and treatment periods is shown, as well as number of individuals ( $n$ ) and corresponding shell length (mean  $\pm$  SD) in each temperature, and behavioral and physiological measurements, including standard metabolic rate (SMR), burial behavior (BB), and metabolic response to progressive hypoxia ( $pO_2$  evaluation). Two-way arrows indicate when individuals were returned to their corresponding temperature tanks once the measurement was finished. The number of deaths per individual per temperature per period is also included

$$\text{Estimated dry mass} = 0.0019e^{0.201 \times SL} \quad (1)$$

### 2.3 | SMR evaluation

The SMR is of functional and ecological importance, because it represents the basic cost of living at a certain temperature (Hochachka & Somero, 2002). SMR was measured based on the oxygen consumed per gram of estimated dry mass without shell per hour ( $\text{mg O}_2 \text{ g}^{-1} \text{ hr}^{-1}$ ). For SMR determination, 20 individuals were randomly chosen from each temperature treatment tank after 4 weeks of exposure and transferred to a 30-L tank at the corresponding treatment temperature, without sediment and with filtered (carbon and 0.1- $\mu\text{m}$  mesh), dechlorinated tap water. In each SMR tank, specimens were placed individually in 60-ml open chambers for 24 hr for chamber acclimation before the measurement. Each chamber and the three control chambers (without animals) were hermetically closed before starting the measurement procedure. No signs of manipulation stress (e.g., valves closed or syphons not observable) that could interfere with the respiration rate were observed during the measurements. After 1 hr, a 20-ml water sample was taken from each chamber and the oxygen concentration was determined by the Winkler spectrophotometric method, following Labasque et al. (2004), modified by Torre et al. (2012), with an LKB Biochrom 4060 spectrophotometer (Biochrom). Oxygen consumption was estimated as the difference in oxygen concentration between the experimental and the control

chambers. Once SMR measurement was finished, each individual was measured (SL, fresh mass) and restored to its treatment tank. Four weeks later (treatment week 8), this procedure was repeated with the same group of individuals (Figure 2).

### 2.4 | Burial behavior

Bivalve distribution is constrained by the grain size of the sediment in which they are able to burrow (Alexander et al., 1993; Downing et al., 2000). Burrowing is a mechanism that allows bivalves to continue feeding while avoiding harmful environmental factors or predators. Temperature influences bivalve burial behavior (Amyot & Downing, 1997), affecting their ability to evade predators, or to reestablish contact with water after burial by sediment deposition (Alexander et al., 1993; Fiori & Carcedo, 2015; McKeon & Barshis, 2015). Evaluating burial behavior in *C. largillierti* may thus represent a good proxy for performance at different granulometries and temperatures.

One day after SMR evaluation, burial performance in sediments of different grain sizes was evaluated following the methods of Fiori and Carcedo (2015) and Nel et al. (2001). Because the amount and type of organic matter in sediments may directly influence oxygenation and pH, and thus affect bivalve burial behavior (Cottrell et al., 2016; Cummings et al., 2009), the sediment used was first placed in a muffle furnace for 5 hr at 450°C to remove any remaining

organic matter. The sediment was then sieved to obtain the following granulometries: coarse sand (0.5–1 cm), thin sand (0.2–0.5 cm), fine sand (0.1–0.2 cm), and very fine sand (<0.1 cm). This laboratory-controlled approach is usually performed to evaluate the specific influence of granulometry and avoids any other unpredictable characteristic of natural sediments (organic matter fraction, the presence of bacteria or other small organisms, geochemical variability, etc; Alexander et al., 1993; De la Huz et al., 2002; Nel et al., 2001), which in general could be far from being homogeneously distributed (Béjar et al., 2018). Sediment containers of 250 ml, filled with one of the four sediment sizes, were placed into 50-L filtered water tanks at the corresponding temperature (20 or 30°C). Animals were taken from their temperature tank and immediately placed in the center of one of these sediment containers. Individual burial time behavior was evaluated for 30 min. Burial time is the period from when the bivalve introduces its foot in the sediment and the valve is positioned perpendicular to the sediment surface until the posterior end of the valve becomes level with the sediment surface. The measurement was repeated until a representative number of individuals ( $n = 14$ – $16$ ) per grain size and temperature was tested. Each animal was allowed to bury in only one size of sediment particles per test period (weeks 4 and 8). Because not all the specimens evaluated completed or even initiated burrowing, the degree to which individuals buried was also categorized as all buried, partially buried, or not buried. Bivalves were removed from the containers immediately after the burrowing test, the SL was measured, and the individuals were restored to their temperature treatment tank with natural sediment. Four weeks later (treatment week 8), this procedure was repeated (Figure 2). Finally, the burial rate index (BRI) was calculated from the burial time using the Stanley (1970) equation modified by Alexander et al. (1993):

$$\text{BRI} = \frac{\text{Individual mass}^{0.33}}{\text{Burial time}} \times 10^4 \quad (2)$$

The BRI normalizes the burial time according to the size of the individuals, since larger individuals take proportionally more time to bury completely than smaller ones.

## 2.5 | Metabolic response to progressive hypoxia

The amount of oxygen consumed per unit of time ( $v\text{O}_2$ ), plotted as a function of environmental oxygen levels ( $p\text{O}_2$ ), is one of the typical approaches to study the hypoxia tolerance of aquatic invertebrates to their environment. To evaluate the effect of environmental oxygen concentration ( $p\text{O}_2$ ) on performance in *C. largillierti* ( $n = 18$ ; mean SL,  $21.86 \pm 3.04$  mm), the oxygen consumption rate ( $v\text{O}_2$ ) of individual clams was measured at 25°C, using an OXY-4 channel PreSens Oxygen Ingress Measurement system (Regensburg), following Tremblay and Abele (2015). Since the number of devices connected (see below) increases water temperature, individuals used in this particular evaluation were acclimated from the beginning for

4 weeks to 25°C. All the measurements were performed at that temperature (Figure 2).

The system was equipped with four chambers for the simultaneous measurement of three animals and a control blank (to assess background oxygen demand from bacteria in the water). All chambers were placed in a 30-L tank with constantly aerated, filtered, and dechlorinated water (to keep 100% air saturation). Cylindrical chambers of 30 ml were used. Specimens were placed in the chambers 24 hr before measurement for acclimation. Water circulation was maintained with pumps to achieve homogeneous conditions among chambers. Following the oxygen sensor instruction manual (Oxygen Sensor Spots PSt3 Instruction Manual, www.presens.de), aeration was stopped between 10 and 20 min before starting measurement to avoid oversaturation. Water circulation was stopped by hermetically closing each chamber. Chambers were equipped with a magnetic stirrer in the bottom to achieve homogeneity of oxygen concentration and a 1-mm mesh gauze to separate the stirrer from the clams. Data recording was started simultaneously in all chambers while closed, at a  $p\text{O}_2$  of ~100% air saturation, and the oxygen concentration in each chamber was measured every minute. The duration of the measurements lasted 2.5–6.5 hr, and measurements were stopped individually when the  $p\text{O}_2$  reached 0%. No signs of manipulation stress (e.g., valves closed or syphons not observable) that could interfere with the  $v\text{O}_2$  were observed during the measurements. After measurements, the animals were sacrificed and weighed (with and without shell) to determine the fresh mass, and the SL was measured. Fresh body mass without shell was dried at 60°C to obtain the final dry mass. To analyze oxygen regulation capacity and hypoxia sensitivity,  $v\text{O}_2$  was estimated as the oxygen consumed per gram of estimated dry mass without shell per hour ( $\text{mg O}_2 \text{ g}^{-1} \text{ hr}^{-1}$ ) for each  $p\text{O}_2$  (100%–0%).

## 2.6 | Data analysis

Normality and homoscedasticity were evaluated by the Shapiro-Wilk and Levene's tests, respectively. For lack of normality, SMR and BRI were  $\log_{10}(x + 1)$  and  $\log_{10}$  transformed, respectively. To detect differences in the parameters evaluated, covariance analysis (ANCOVA) was performed with SMR as the dependent variable and temperature and time combination as independent variables. In the case of BRI, temperature, time, and granulometry were considered as independent variables. In all cases, individual size (SL) was considered as a covariable. Significant ANCOVA differences ( $p < .05$ ) were evaluated with a Bonferroni post hoc test. One of the individuals measured for the SMR control group in the fourth week was excluded from analysis because it was much larger than the rest of those compared. The degree of burial among temperatures, time of exposure, and granulometry was analyzed with a chi-squared test. The degree of granulometry selectivity was determined according to Alexander et al. (1993), by analyzing the kurtosis of the burial rate profiles. All statistical analyses were performed with Infostat 2016 (Di Rienzo et al., 2016).



The temperature coefficient ( $Q_{10}$ ) reflects the rate of variation of chemical or biological systems as a consequence of a 10°C increase in temperature. In biological systems, a  $Q_{10}$  value ~2 means that the system is being evaluated within the normal temperature range for that system. A  $Q_{10} = 1$  means that the process is not influenced by temperature, whereas  $Q_{10} < 1$  implies that the system is not within its normal temperature range. Smaller  $Q_{10}$  values indicate that the system is more strongly susceptible to the effects of higher temperature (Hochachka & Somero, 2002). To analyze temperature sensitivity and thermal compensation in SMR and BRI,  $Q_{10}$  was calculated as:

$$Q_{10} = \frac{r_{T_2}}{r_{T_1}} \quad (3)$$

where  $T_2$  and  $T_1$  stand for 30 and 20°C, respectively, and  $r$  is the measured rate SMR or BRI.

The traditional method of analysis of  $vO_2/pO_2$  behavior assumes that in an oxyconformer (a non-regulator)  $vO_2$  declines proportionally with  $pO_2$ , whereas in an oxyregulator  $vO_2$  remains constant, down to the critical  $pO_2$  ( $P_{crit}$ ). Because there are many cases in which data deviate from this traditional model of regulation, a different approach has been developed that evaluates a more general definition of this particular  $pO_2$ , defined as threshold  $pO_2$  ( $P_t$ ). Because  $P_t$  is the  $pO_2$  at which organisms shift their metabolic response, it is of importance for determining physiological distribution thresholds (Suárez-Mozo et al., 2018; Zamorano et al., 2007).

To analyze the effect of  $pO_2$  on the physiology and distribution of *C. largillierti*, we performed the following analysis. First, the regulation value ( $R$ ) was calculated as the integrated area under the curve described by  $vO_2$  as a function of  $pO_2$  (100%–0%), expressed as a percentage of the total possible area. Values of  $R$  between 100% and 50% are indicative of oxyregulation, with higher values indicating greater regulatory ability, whereas  $R$  values <50% suggest extreme hypoxia sensitivity (Alexander & McMahon, 2004; Lencioni et al., 2008). This is an integrative method for estimating and comparing overall oxygen regulatory capacity, but does not provide information about critical or threshold oxygen tensions ( $P_t$ ). To determine these thresholds, we calculated  $P_t$  as the  $pO_2$  at which there is a breaking point in the relationship between  $pO_2$  and the rate of  $vO_2$ , with  $V_t$  being the  $vO_2$  at  $P_t$ . We used the respR package (Hariato et al., 2019) in R (R Core Team, 2011) to calculate  $P_t$  by classical broken-stick regression and the segmented, or nonlinear, broken-line regression.  $P_t$  are values at which there is a greater change in trend but may not capture important aspects of the response pattern. Instantaneous regulation,  $\rho(x)$ , was plotted and analyzed in comparison with classical regulation models (ideal regulators and conformers) following Cobbs and Alexander (2018). Considering  $f(x)$  as the function that describes the relationship between standardized  $vO_2$  and  $pO_2$ ,  $\rho(x)$  is described by the following equation (Cobbs & Alexander, 2018):

$$\rho(x) = \frac{f(x)}{x} - f'(x) \quad (4)$$

In this relationship,  $\rho(x) < 0$  indicates negative regulation,  $\rho(x) = 0$  indicates no regulation, and  $0 < \rho(x) < (x-1)$  indicates partial positive regulation. The regulation profile (a plot of  $\rho(x)$  versus  $x$ ) describes the direction, intensity, and pattern of regulation over values of  $x$ . From this profile,  $P_t$  could be redesignated as  $P_{min}$ ,  $P_{zero}$ , and  $P_{max}$ , which are the values of  $x$  for which  $\rho(x)$  is at its minimum, zero, and its maximum, respectively. Total positive regulation ( $T_{pos}$ ) and total negative regulation ( $T_{neg}$ ) were also estimated by integrating the  $\rho(x)$  function (Cobbs & Alexander, 2018).

### 3 | RESULTS

There was a significant relationship between SMR and temperature, with a significant interaction with time (Table 1). At 30°C, SMR was always higher than at 20°C but only significantly greater at 30°C in the long term, together with greater percentage of accumulated mortality (~10 times greater; Figure 3, Table 1).  $SMR_{Q_{10}}$  values were 1.25 and 1.83 for four and eight weeks, respectively.

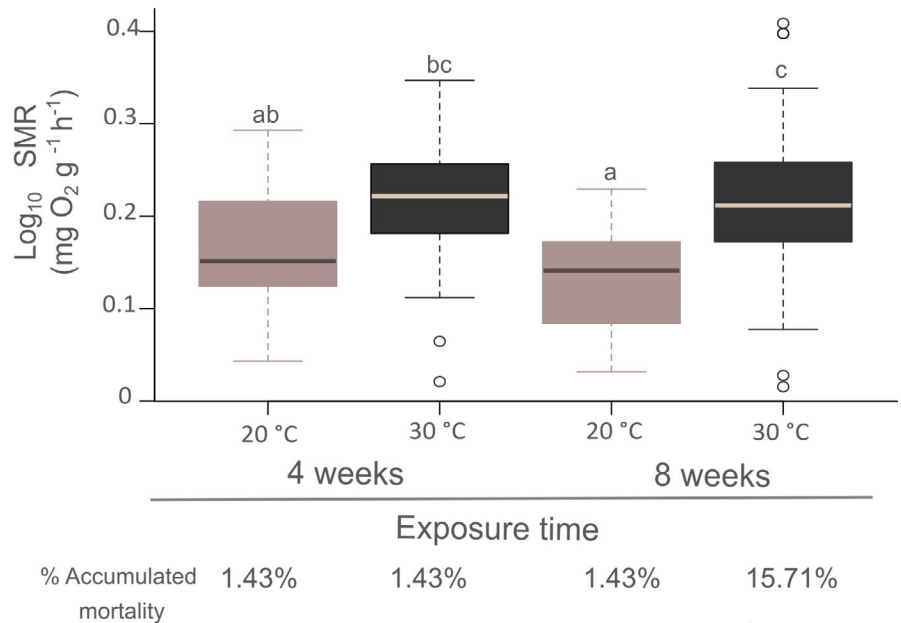
In the absence of any statistically significant effect of temperature or time on BRI, data from different temperatures and time were pooled together to analyze the effect of granulometry ( $BRI_{Q_{10}}$  values at 4 and 8 weeks were ~1). BRI strongly depended on granulometry, indicating that *C. largillierti* buried faster in finer sediments (Figure 4, Table 1). The burial rate profile kurtosis ( $K = -2.04$ ) indicated that clams were generalist in terms of granulometry selection, at least in the grain sizes chosen for this work (data not shown). At 30°C,

**TABLE 1** ANCOVA results for the effect of temperature, exposure time, and sediment grain size on standard metabolic rate (SMR) or burial rate index (BRI), with size (shell length) as a covariate

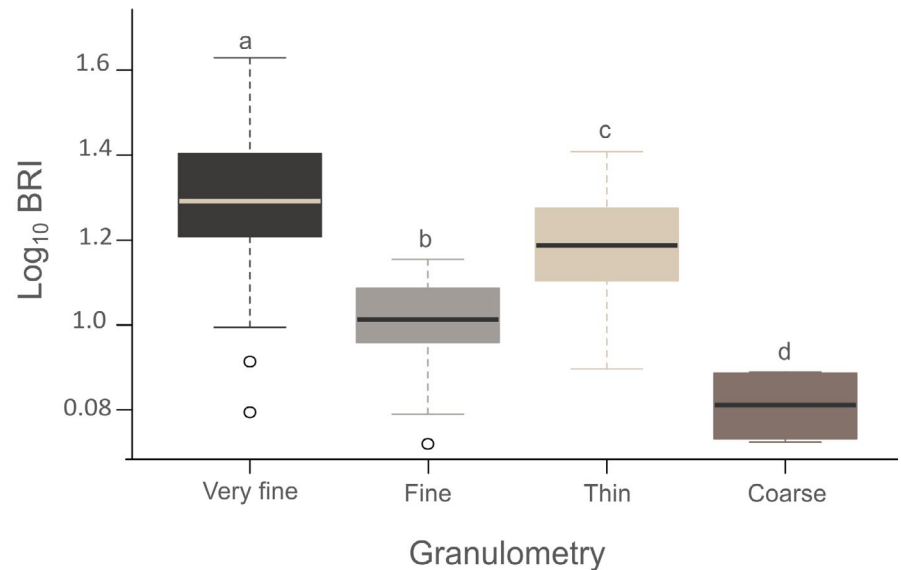
Comparison	F	p
<b>SMR</b>		
Temperature	23.36	<.0001
Time	0.2	.659
Temperature × Time	4.02	<.0001
Size (shell length)		<.0001
<b>BRI</b>		
Temperature	0.45	.504
Time	0.1	.751
Grain size	21.28	<.0001
Temperature × Grain size	1.83	.165
Temperature × Time	2.66	.106
Temperature × Time × Grain size	1.88	.158
Size (shell length)		<.0001
<b>BRI<sup>a</sup></b>		
Grain size	47.09	<.0001
Size (shell length)		<.0001

<sup>a</sup>In the absence of any statistically significant effect of temperature or time on BRI, data from different temperatures and time were pooled together to analyze only the effect of grain size.

**FIGURE 3** Relationship between standard metabolic rate (SMR) and temperature in *Corbicula largillierti*. Plot shows  $\log_{10}(\text{SMR} + 1)$ , corrected for shell length as milligrams of  $\text{O}_2$  per gram of dry mass without shell per hour ( $\text{mg O}_2 \text{ g}^{-1} \text{ hr}^{-1}$ ), for individuals at 20 and 30°C. Different letters indicate significant differences between measurements ( $p < .05$ ). Accumulated mortality (%) for individuals at 20 and 30°C is tabulated under the corresponding SMR box



**FIGURE 4** Burial rate at different sediment granulometries in *Corbicula largillierti*. The burial rate index (BRI) normalizes burial time to dry mass (calculated from shell length). Plot shows  $\log_{10}$  BRI at different granulometries (very fine, fine, thin, coarse). Different letters indicate significant differences ( $p < .05$ )

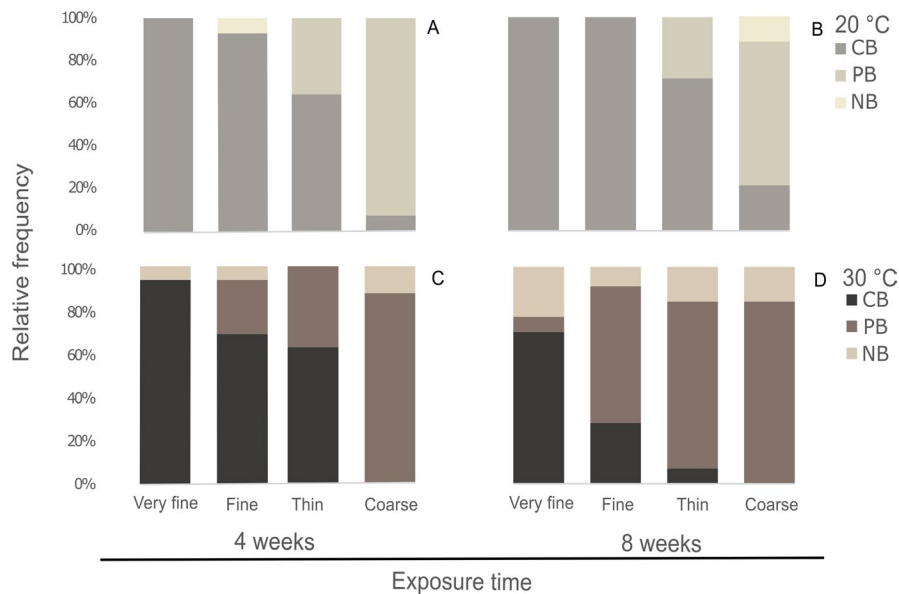


the proportion of clams that completed burial was significantly less than the proportion at 20°C ( $\chi^2 = 31.63$ ;  $p < .0001$ ) after 8 weeks (Figure 5).

Under progressive hypoxia, individuals of *C. largillierti* performed as oxyconformers, with an  $R$  value below 50% (39.9%), which also indicated negative regulation. This species did not appear to be a classical oxyconformer, because the respiration profile ( $v\text{O}_2$  versus  $p\text{O}_2$ ) showed two perfectly observed segments separated by an environmental  $p\text{O}_2$  threshold of  $P_t = 48.4\%$  air saturation (Figure 6a). The relative  $v\text{O}_2$  versus  $p\text{O}_2$  profile ( $f(x)$  plot) and its related instantaneous regulation profile ( $\rho(x)$  plot; Figure 6b,c) showed that only negative regulation occurs in the first segment (from maximum  $p\text{O}_2$  to  $P_t$ ) with a  $T_{\text{neg}} = 0.52$ . The rest of the profile presented no regulation, except when  $p\text{O}_2$  approaches 0, leading to a very small  $T_{\text{pos}} = 0.06$ . The  $\rho(x)$  plot (Figure 6c) showed that  $P_t$  is in fact a  $P_{\text{min}}$ .

## 4 | DISCUSSION

The present study is one of the first to assess the metabolic and behavioral responses in individuals of *C. largillierti* to temperature increase and to evaluate its metabolic response to progressive hypoxia. Temperature affects organism physiology at every level, from molecular structures to every metabolic and physiological rate (i.e., filtration, respiration, excretion, growth, and reproduction; Christophersen & Strand, 2003; Clarke, 1998; Hochachka & Somero, 2002; Pörtner, 2002; Saucedo et al., 2004). Many invasive species can survive acute thermal stress in freshwater environments, which is probably a key factor in their dispersion (Bates et al., 2013). For instance, *C. fluminea* can tolerate temperatures higher than 40°C for short periods and survive for a long time between 30 and 35°C (Cataldo & Boltovskoy, 1999; Lucy et al., 2012). Nonetheless,



**FIGURE 5** Burial behavior associated with sediment type and temperature in *Corbicula largillierti*. The degree to which individuals buried themselves in sediment was classified into three categories: not buried (NB), partially buried (PB), and completely buried (CB). Plots show the relative frequency of each of these categories when clams were placed in sediment of different grain sizes (very fine, fine, medium, or coarse) at two different temperatures. **A,B.** Exposure to 20°C after 4 (A) and 8 (B) weeks. **C,D.** Exposure to 30°C after 4 (C) and 8 (D) weeks

adaptation over the long term is one of the major challenges for these species to colonize and invade a wider environmental range (Frankham, 2005; Lee et al., 2005; Roman & Darling, 2007; Stapley et al., 2015). It is generally accepted that a  $Q_{10}$  value around 2 indicates no compensation at all and complete temperature dependence (Lurman et al., 2014). Our results show that *C. largillierti* is not a perfect compensator. The  $SMR_{Q_{10}}$  during the exposure indicates that *C. largillierti* is not able to compensate for temperature effects. Indeed, mortality in long-term exposure to 30°C was 10 times higher than at 20°C, indicating that, at least for a small fraction of the population, this temperature is unbearable.

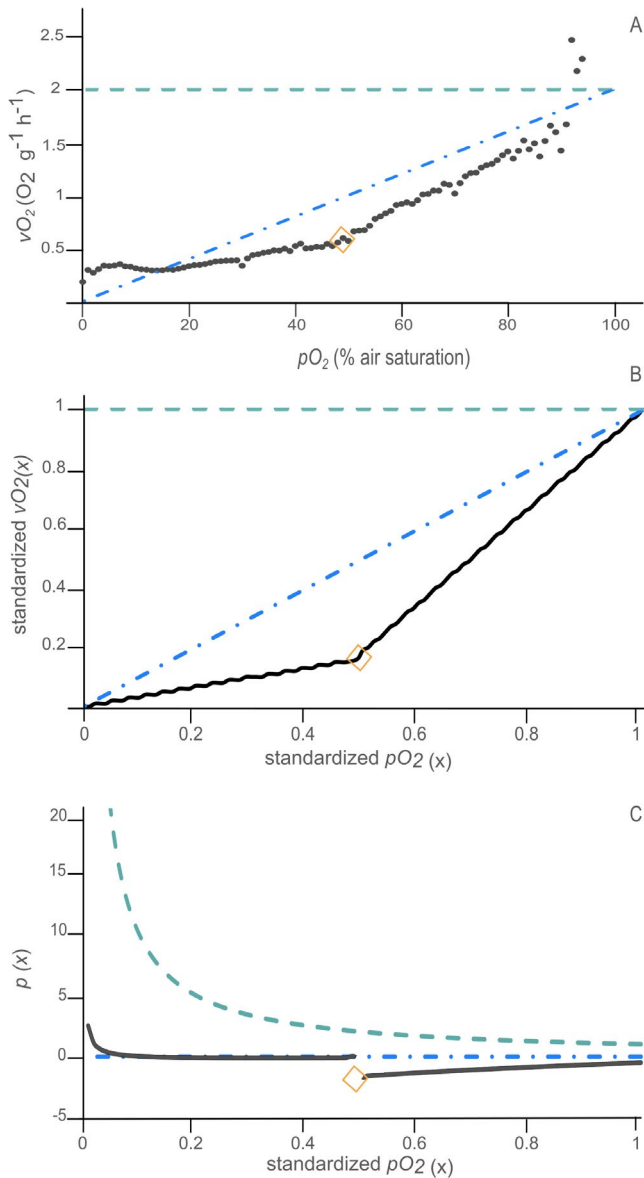
We also observed a temperature effect on burial behavior that is not evident by BRI analysis (which only takes into consideration individuals that completed burial). As has been observed in other bivalve species exposed to high temperatures (Savage, 1976), individuals of *C. largillierti* were progressively less able to complete burial at 30°C. The inability to bury negatively affects population establishment, because it increases exposure to predators or to other adverse environmental conditions (Bowers et al., 2005; Saloom & Duncan, 2005; Tallqvist, 2001). Thus, even when the temperatures tested are in the range of the maximum temperature for this species' distribution record, there seems to be an impact when the exposure to these higher temperatures is prolonged. The summer conditions described for the reservoir are more or less constant throughout the summer months (Wunderling, 2018). Thus, even when our results show that exposure to 30°C has no massive lethal effect, it has an energy cost that is reflected in a higher SMR. This physiological cost is then evidenced in mortality and burial failure.

Regarding granulometry differences between the reservoir and the rivers, our results indicate that the thinner sediments that characterize the reservoir bottom would not be a limiting factor for corbiculid settlement. Contrary to expectations, BRI differences between granulometries indicate some preference for the finer ones, but not strong enough for *C. largillierti* to be considered a specialist

(Alexander et al., 1993). The burial profile analysis classifies them as generalists, meaning they can bury at all granulometries tested. This matches the distribution of *C. largillierti* along the Suquia basin rivers, where it can be found inhabiting coarse and sandy sediments (Reyna et al., 2013). This may also be the case for *C. fluminea*, a species in which preference for fine sediments has been shown experimentally, but which is almost equally distributed along the fine and coarse sandbanks of the River Rhine (Schmidlin & Baur, 2007) and of the St. Lawrence River (Castañeda et al., 2018). In fact, it has been assumed that the presence of *C. fluminea* on higher grain size sediments is indeed related to the degree of oxygenation, which is always higher in coarse sediments (Castañeda et al., 2018; McMahon, 1999). Our results are consistent with the latest study of Pereira et al. (2017), which suggests that sediment characteristics are weaker predictors of corbiculid distribution per se.

Oxygen availability is another limiting factor of aquatic environments, particularly in the San Roque Reservoir, as explained above. Our results show that in *C. largillierti*, metabolic demands are not able to be sustained under progressive hypoxia, when individuals are completely dependent on  $pO_2$ . The  $R$  value and a  $vO_2/pO_2$  profile that passes below the perfect oxyconformer line, even at very high  $pO_2$ , is a pattern that has been previously described for hypoxia-sensitive species by Alexander and McMahon (2004). Significantly reduced respiration rates could also be an indication of metabolic suppression (Herreid, 1980; Tremblay et al., 2020). Low levels of negative regulation are quite common in aquatic invertebrates, as in this case (Cobbs & Alexander, 2018). Probably, the capacity to reduce energy expenditure during low oxygen events is a primary mechanism facilitating low oxygen tolerance (Seibel et al., 2016). This mechanism could be important for surviving emersion events, especially during the dry season. It has been described that other corbiculids survive emersion by alternating longer periods of valve closure with very reduced oxygen uptake rates and very short periods of higher aerial oxygen





**FIGURE 6** Metabolic response to progressive hypoxia in *Corbicula largillierti*. **A.** Respiration rate ( $vO_2$  mean  $\pm$  SE) in milligrams of O<sub>2</sub> per gram of dry mass without shell per hour (mg O<sub>2</sub> g<sup>-1</sup> hr<sup>-1</sup>) as a function of dissolved oxygen concentration ( $pO_2$ , % air saturation). **B.** Hypoxia plot  $f(x)$  as standardized  $vO_2$  as a function of standardized  $pO_2$ . **C.** Instantaneous regulation plot  $\rho(x)$ . The (---) and (-.-) lines in each graph indicate ideal regulator and conformer responses, respectively. Diamond in the plots indicates  $P_t = P_{min}$

exchange (McMahon & Williams, 1984). Because  $pO_2$  is the limiting factor under environmental hypoxic or anoxic conditions, adjusting  $vO_2$  through opening and closing of the valves makes no difference in these cases. In fact, *C. fluminea* is better at surviving short periods of anoxic than of hypoxic conditions (Johnson & McMahon, 1998; Mathews & McMahon, 1995). The positive regulation seen in *C. largillierti* at very low  $pO_2$  could be related to these minimum  $vO_2$  levels that corbiculids could sustain during valve closure (McMahon & Williams, 1984). As in the case of

most oxyconformers, the general performance of *C. largillierti* is heavily dependent on environmental oxygen tension (Crocker & Cech, 1997; Pörtner, 2010; Pörtner & Grieshaber, 1992). Therefore, inhabiting poorly oxygenated environments will compromise their biological functioning (i.e., growth and reproduction) and probably their survival (Galic et al., 2019). Additionally, if the limited hypoxic or anoxic tolerance in this species has evolved to survive during dry (cold) season emersion events, it is probable that individuals could not survive the San Roque Reservoir summer eutrophication events (27°C on average during summer months).

This may be one of the reasons for the absence of *C. largillierti* in the San Roque Reservoir. The presence of *C. fluminea* in the reservoir seems to indicate that, even though it is also a very hypoxia-sensitive species (McMahon, 1996, 1999), it is at least more tolerant than *C. largillierti*. *Corbicula fluminea* is also distributed at a higher range of temperatures than *C. largillierti* (Crespo et al., 2015; Gama et al., 2016; Lucy et al., 2012; Reyna et al., 2018), indicating better temperature tolerance. Given that the conditions of the reservoir are more extreme and that both species are able to coexist in other water bodies (Callil & Mansur, 2002; Martins et al., 2004; Reshaid et al., 2017), the absence of *C. fluminea* in the rest of the basin may be the result of a more recent invasion event. Nonetheless, the reservoir conditions are difficult enough to prevent colonization of *C. largillierti* and apparently dispersion of *C. fluminea*, presumably because these conditions affect growth and reproduction in these clams (Belanger, 1991). In conclusion, sediment granulometry seems not to be a determining factor in *C. largillierti* distribution in the Suquía River basin per se. Temperature may be a limiting factor but is not determinant, whereas oxygen availability seems to be the most important factor. Additionally, a combination of high temperatures and extremely low oxygen concentrations in the reservoir during summer eutrophication events may together act as a critical factor preventing the settlement of *C. largillierti*.

The results obtained in the present study contribute to filling the gaps of some biological aspects associated with the still poorly known and underestimated invasive species, *C. largillierti*. Knowledge of the physiological constraints that affect and restrict the establishment of species of *Corbicula* in freshwaters may help to better understand their invasiveness as well as to predict their distribution patterns. This may also aid the design of control strategies for mitigating species impact through efficient management policies.

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are openly available in [www.pangea.de](http://www.pangea.de) at <https://doi.org/10.1594/PANGAEA.921015> (Torre et al., 2020c), <https://doi.org/10.1594/PANGAEA.921017> (Torre et al., 2020a), and <https://doi.org/10.1594/PANGAEA.921013> (Torre et al., 2020b).

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