Gonadal Cycle of *Corbicula largillierti* (Bivalvia: Cyrenidae) in a Pampean Streams, Argentina

Cristina Damborenea\(^1,*,†\), Yeny Labaut\(^2\), Pablo Penchaszadeh\(^3,†\), Gonzalo A. Collado\(^4,†\) and Gustavo Darrigran\(^1,*,†\)

\(^1\) División Zoología Invertebrados, Museo de La Plata, FCNyM—CONICET, Paseo del Bosque s/n, La Plata 1900, Argentina

\(^2\) Instituto de Investigación en Paleobiología y Geología, CONICET-Universidad Nacional de Río Negro (Sede General Roca), Av. Roca 1242, General Roca R8332FDZ, Argentina; ylabautbetancourt@unrn.edu.ar

\(^3\) Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”, Lab. 80, Av. Angel Gallardo 470, Ciudad Autónoma de Buenos Aires C1405DJR, Argentina; pablopench@gmail.com

\(^4\) Departamento de Ciencias Básicas, Facultad de Ciencias, Universidad del Río-Bío, Av. Andrés Bello 720, Chillán 3800708, Chile; gcollado@ubiobio.cl

* Correspondence: cdambor@fcnym.unlp.edu.ar (C.D.); invasion@fcnym.unlp.edu.ar (G.D.)

† eMIAS (Grupo de Especialistas en Moluscos Exóticos de América del Sur).

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Abstract: The reproductive cycle of non-native species is indicative of their capacity for dispersal, invasion, and competition, and the alteration in biodiversity. *Corbicula* spp. are successful invaders of aquatic ecosystems. We studied the reproductive cycle of *Corbicula largillierti* from a Pampean stream, Argentina, at its southern distribution in South America. Specimens were collected monthly from January 2003 to April 2005 and processed using traditional histological techniques. Three gonadal stages (active gametogenesis, mature, and spawned) were recognized. In the studied population, most individuals were hermaphroditic with a dominant female gonadal portion. The three gonadal stages were observed during the whole sampling period with rapid gonadal recovery; no resting period was observed. Five oocyte spawning events were recognized, occurring in autumn and spring. Expanding the knowledge of reproductive features of the *Corbicula largillierti* allows the understanding of current distributions. The results highlight the difficulty of identifying patterns of gamete release and spawning behavior in this invasive species, as it is recorded for other *Corbicula* spp.

Keywords: freshwater clam; non-native species; reproduction; histology; hermaphrodite

1. Introduction

The genus *Corbicula* (Bivalvia, Cyrenidae) is composed of estuarine and freshwater clams native to Asia, Africa, and Australia, which are capable of reproducing both sexually and asexually (androgenetic lineages). Lineages of *Corbicula* have been introduced in freshwater ecosystems all over the world for almost a century and are considered major aquatic invaders, mainly because of their negative environmental impact and economic effects [1]. The first report of *Corbicula* species, *C. largillierti* (R. A. Philippi, 1844) and *C. fluminea* (O. F. Müller, 1774), in South America indicates the arrival through the Río de la Plata River, Argentina, in the late 1960s or early 1970s [2–4]. *Corbicula largillierti* is native to Asia [5]. Two potential vectors are mentioned in its introduction into South America: the release of living specimens brought from the point of origin as food on board vessels and the ballast water of transoceanic ships [6]. The vector for its dispersal in South America could be the same as that for *C. fluminea*, that is, by live transport as sports fishing bait [7] or by sand transport for the construction of artificial beaches [8]. *C. largillierti* is currently present in several localities of South America [9].

In Argentina, since *Corbicula fluminea* and *C. largillierti* entered the Río de la Plata, they have reached a wide distribution but with different patterns. Currently, *C. fluminea*...
dominates in the Río de la Plata estuary and the main rivers in East and South Argentina, being competitively superior to *C. largillieri* in these environments [6]. *C. largillieri* prevails in lakes, rivers, and streams of lesser order in the central and western regions of Argentina [10], but also occurs in the Plata basin [11–13]. In Brazil, *C. largillieri* occurs in the East and Northeast, from Ceará to Rio de Janeiro, in the upper and middle Paraná River, Paraguay and Uruguay basins, Laguna dos Patos e Mirim systems, and Tocantins River [14]. The species is also present in Uruguay [15]. *C. largillieri* has been also reported in the United States, in the States of Arizona, Florida, Iowa, Illinois, Kentucky, and Missouri, especially in the Illinois and Mississippi Rivers, but the status of the introduction is not clear [16]. In November 2004, the species was found for the first time in Europe, in the Ebro River [17].

Some introduced species have certain important features that aid them to adapt rapidly, become established, and proliferate, thus becoming successful invaders in the new environment [18]. The features that enable a species to succeed in a new environment include a short lifespan (two to three years), rapid growth, rapid sexual maturity, and high fecundity [19]. Despite this, studies focused on the gonadal cycle of *Corbicula* spp. are rare in South America [20]. Ituarte [21] studied, for the first time, the reproductive biology focusing on a population of *C. largillieri* in La Guardia Stream (34°48′50″ S–57°28′27″ W), near its mouth in the Río de la Plata, ten years after its introduction in South America. More recently, Mansur et al. [22] summarized the information available on the reproduction of *C. fluminea*, while Cao et al. [23] researched the gonadal biology of *C. fluminea* in Santa Catalina Stream (36°53′04.5″ S–59°55′25.22″ W), Argentina.

The sex composition of *Corbicula* spp. populations differs among regions. Dioecy, simultaneous hermaphroditism, gynodioecy, and trioecy were reported in the native range, while hermaphrodites with occasional male and/or female specimens were reported in the invaded area [23–26]. Furthermore, androgenetic hermaphroditic asexual reproduction has been described for the invaded areas [27]. Additionally, a wide spectrum occurs in the seasonality of the gonadal cycle [25] and in the development through free-swimming veliger larvae or incubation of juveniles in the gills [20,28]. The adaptive capacity of *Corbicula largillieri*, which allows its dispersion in different invaded ecosystems, must be in accordance with its reproductive strategy, allowing its successful establishment in new habitats.

However, there remains an incomplete understanding of the reproduction features and gonadal cycle of *C. largillieri* [20]. The goals of this study were to describe the gonadal cycle and the reproductive patterns of a population of *C. largillieri* from a stream located in the Argentine pampas through gonadal histology. Two aspects stand out: the study was carried out approximately 35 years after the introduction of the species in South America, and the population under study inhabits a different environment from the one studied by Ituarte [21]. These data allow us to infer the ability of *C. largillieri* to colonize and settle in different environments, expanding the understanding of reproductive features of *Corbicula* spp. in an invaded area, and can be applied to the development of control, monitoring, and management measures.

2. Materials and Methods

2.1. Study Area

The study was carried out in Santa Catalina Stream, Argentina (36°53′04.5″ S–59°55′25.22″ W). The Santa Catalina Stream basin spans 138 km². The sampling site was located in the lower part of the stream basin, at 151 m above sea level, in the depressed Pampa, where the topography is mostly flat with smooth relief and the drainage system is not well developed [29]. The highest temperatures are recorded between December and March (monthly average of 20 °C), while the lowest are recorded between June and August (monthly average of 8 °C); the average rainfall in the sampling point is approximately 840 mm per year; the most abundant rainfall is in spring–summer (monthly average around 100 mm) and the lowest occurs in autumn–winter (monthly average of 50 mm); and the area is affected by droughts and floods [30].
2.2. Field Sampling

Monthly samples of Corbicula largillierti were collected from January 2003 to April 2005. Due to environmental conditions, only two samplings could be carried out between November 2004 and February 2005. The sampling area was delimited by a cylindrical sampler of 0.07 m², which was pushed by hand into the sediment up to a depth of 0.10 m. The sampling site is one of the most vulnerable areas to flooding from Santa Catalina Stream because it is the lower part of the basin, and also due to the presence of a route forming a barrier that accumulates water [30].

The samples were washed in situ using a sieve (mesh size of 1 mm) and the specimens were transported to the laboratory. The species was identified according to valve morphology [31] (Figure 1). The collecting permit for research was provided by the Dirección de Fauna y Flora, Buenos Aires Province, Argentina. Because Corbicula largillierti is an invasive species, there is no restriction on its capture.

![Figure 1. Comparison of valve morphology of two Corbicula spp. recorded in Argentina. Corbicula largillierti, external (a) and internal (b) view. Corbicula fluminea, external (c) and internal (d) view.](image)

Physical and chemical parameters (i.e., water temperature, dissolved oxygen, oxygen saturation, conductivity, and total dissolved solids) were measured in the field using a multi water quality meter (Lutron WA SD). Daily rainfall, stream flow, and water level data were provided by the Instituto de Hidrología de Llanuras de Azul, Argentina (IHLLA).

2.3. Histological Study and Data Analysis

Between 20 and 30 specimens of representative sizes from each month (excluding November 2004 and January 2005) were selected for histological analysis. Individuals having an anteroposterior length (APL) < 6 mm were excluded. The valves were opened by incision of the anterior and posterior adductor muscles and fixed in Zenker’s solution [32] for 12 h. After fixation, the specimens were washed, dehydrated in a series of ascending ethanol concentrations, and preserved in butyl alcohol until being embedded in Paraplast®. They were then sectioned at 8 μm, and the slides were stained using Mayer’s hematoxylin and eosin and observed under a microscope (Axio Lab, Carl Zeiss, Germany).

According to previous studies [23], three types of follicles were recognized: oogenic, spermatogenic, and mixed (both oogenic and spermatogenic). The qualitative stages of gonadal development were established following the description by Ituarte [21] for C. largillierti and by Cao et al. [23] for C. fluminea, and some modifications were adopted to simplify its diagnosis (Table 1). All the gonadal stages observed in each specimen were recorded. The maximum diameter of oocytes with conspicuous nucleoli was measured in 380 specimens with a microscope under 400× magnification. According to the oocyte diameter, three size classes were recognized: small (<49 μm), medium (between 50 and
99 µm), and big (≥100 µm). The latter was considered to be full growth following Cao et al. [23]. The gills were examined to determine the presence of growing larvae.

Table 1. Concordance between gonadal development stages described by Cao et al. [23] for Corbicula fluminea in Santa Catalina Stream (Argentina), Ituarte [21] for C. largillierti in Río de la Plata River (Argentina), and the modification used in this study.

<table>
<thead>
<tr>
<th>Cao et al. [24]</th>
<th>Ituarte [21]</th>
<th>Present Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oogenic</td>
<td>Spermatogenic</td>
<td>Oogenic and spermatogenic</td>
</tr>
<tr>
<td>Immature</td>
<td>Immature</td>
<td>Virginal immature</td>
</tr>
<tr>
<td>Premature</td>
<td>Premature</td>
<td>Incipient maturation</td>
</tr>
<tr>
<td>Mature</td>
<td>Mature</td>
<td>Advanced maturation</td>
</tr>
<tr>
<td>Spawning</td>
<td>Spawning</td>
<td>Partial and successive spawns</td>
</tr>
<tr>
<td>Spawned</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Correlation (Pearson’s r) tests were performed to determine the relationship between gonadal characteristics.

3. Results

3.1. Habitat Characteristic

The main limnological variables monitored during the study are represented in Figure 2. The lowest water temperature recorded during the study occurred in June 2003 (8.2 °C) and the highest in February 2003 (26.5 °C) (Figure 2a). Mean oxygen concentration was 8.28 mg/L (n = 23; DS = 2.54), varying between 3.4 mg/L (February 2004) and 13.39 (July 2003) (Figure 2b). pH ranged between 7.4 (February 2004) and 8.6 (June 2004) and the average rainfall of the 30 days before sampling ranged from 0 mm (June 2003) to 4.8 mm (November 2003) (Figure 2c). Mean conductivity was 558.76 µS/cm (n = 21, DS = 66.39), varying between 401 µS/cm (October 2003) and 721 µS/cm (August 2003) (Figure 2d). The lowest and highest conductivity values were recorded during the first year. Additionally, the water level fluctuated more during the first year (Figure 2e).

Figure 2. Cont.
Figure 2. Values of the main limnological variables monitored during the study: (a) water temperature (°C); (b) dissolved oxygen (mg/L) and oxygen saturation (%); (c) pH and mean rainfall 30 days before the sample; (d) conductivity (µS/cm) and TDS (mg/L); (e) H water level (m). Sample date indicated as dd/mm.

3.2. Gonad Histology and Reproductive Cycle

During the study, 587 specimens were sectioned for histological study, ranging from 6 to 18 mm APL (mean size = 11.66 mm; SD = 2.59). These specimens were either without reproductive follicles or showed different types (Table 2). The absence of reproductive follicles was recorded in a small proportion, in specimens of 6 to 13 mm APL (Figure 3). Specimens with mixed follicles were observed in all clam sizes, from 6 mm long, being the most predominant type in big individuals (Figure 3). The spermatogenic follicles were recorded in all the size classes analyzed (between 6 and 18 mm), and always found in a low proportion. Specimens with only spermatogenic follicles (males) were absent.

Table 2. Proportion of different types of follicle found in the specimens during the gonadal analysis (n = 587).

<table>
<thead>
<tr>
<th>Follicle Types</th>
<th>Percentage of Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oogenic, spermatogenic, mixed</td>
<td>61.77</td>
</tr>
<tr>
<td>Only oogenic</td>
<td>21.33</td>
</tr>
<tr>
<td>Oogenic, spermatogenic</td>
<td>7.17</td>
</tr>
<tr>
<td>Oogenic, mixed</td>
<td>6.55</td>
</tr>
<tr>
<td>Only spermatogenic</td>
<td>0</td>
</tr>
<tr>
<td>Without reproductive follicles</td>
<td>3.24</td>
</tr>
</tbody>
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</tr>
<tr>
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<td>6.55%</td>
</tr>
<tr>
<td>Only spermatogenic</td>
<td>0%</td>
</tr>
<tr>
<td>Without reproductive follicles</td>
<td>3.24%</td>
</tr>
</tbody>
</table>

Figure 3. Follicle types (in percentage) in specimens of *Corbicula largillerti* in Santa Catalina Stream from January 2003 to April 2005, according to the specimen size (APL, anteroposterior length in mm).

Additionally, the different types of follicle were observed during the whole sampling period (Figure 4). Oogenic, spermatogenic, and mixed follicles were observed in more than 90% of specimens in February, July, and August 2003 and February 2005, but in less than 30% in February, April, June, July, and August 2004, when specimens with only oogenic follicles predominated.

Three gonadal stages, i.e., active gametogenesis (immature and premature), mature (mature and spawning), and spawned were recognized (Table 3 and Figure 5). The minimum size found with mature male and female follicles was 6 mm APL. The three stages were observed during the whole sampling period and a resting period was not detected. Oogenic follicles with active gametogenesis were abundant (more than 75% of follicle types) in November 2003, March 2004, and from July to September 2004. In spermatogenic follicles, active gametogenesis was not completely coincident with the oogenic follicles, being abundant (more than 75%) in September and October 2003, and September 2004 (Figure 6a). Oogenic follicles with active gametogenesis were absent in April and September 2003, while spermatogenic follicles in that stage were absent in March 2003 and February 2005 (Figure 6a).

Mature follicles were recorded throughout the whole sampling period. Mature oogenic follicles were observed in all samples except for December 2004, being abundant during 2003, while during 2004 they were abundant in May, June, and October. In 2005, there were some periods where their proportion was less than 40%. Mature spermatogenic follicles were less abundant than oogenic ones and their proportion showed great variation throughout the sampling period (Figure 6b). They were absent in September 2003, and August and September 2004. In general, oogenic and spermatogenic follicles were simultaneously mature during most of the sampling period.
(Figure 6a). Oogenic follicles with active gametogenesis were absent in April and September 2003, while spermatogenic follicles in that stage were absent in March 2003 and February 2005.

Figure 4. Monthly variation of the follicle types of *Corbicula largillierti* between January 2003 and April 2005, in Santa Catalina Stream. Sample date indicated as dd/mm/yy.
The presence of spawned oogenic and spermatogenic follicles was not coincident during the study. Spawned oogenic follicles were absent only in April 2003. During 2003, spawned spermatogenic follicles were abundant, except in September, when they were absent. They were also absent in February, March, August, and September 2004 (Figure 6c). A clear gonad cycle could not be determined.

The proportion of the follicle stages for the different types shows different patterns when comparing the first and second sampling periods (Figure 6), with mature follicles being more abundant during the first period (Figure 6b).

Mature and spawned follicles were observed simultaneously in the same specimen. The presence of active gametogenic follicles throughout the year indicates a quick gonadal recovery. Spawned spermatogenic follicles were strongly correlated with mature oogenic ones (Pearson’s $r = 0.6690$, $p = 0.0001$).

Larvae in the gills were detected in January (3.7%), March (4.5%), and December (45.0%) of 2004 in specimens between 11 to 16 mm (APL), and in February (75.0%) and March (78.6%) of 2005 in specimens between 10 to 17 mm. In 2003, no individual had larvae in gills. The presence of these larvae had no relation with the gametogenic stage of these individuals (Table 4).

Table 3. Diagnosis of the reproductive stages of *Corbicula largillierti* from Santa Catalina Pampean Stream, Argentina.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active gametogenesis (AG)</td>
<td>Reproductive follicles are small but expanding. In oogenic follicles, small oocytes, previtellogenic oocytes and early and middle vitellogenic oocytes are found, but full-grown vitellogenic oocytes are absent. Spermatogenic follicles carry proliferating spermatogonia, primary and secondary spermatocytes and spermatids.</td>
</tr>
<tr>
<td>Mature (M)</td>
<td>Oogenic follicles are replete with free full-grown oocytes, with no free space in the acini, or if partial release occurred, they are more sparsely. Spermatogenic follicles are expanded and branched. Spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa are present. The spermatozoa are abundant, organized in bundles and occupy the most area of the follicle.</td>
</tr>
<tr>
<td>Spawned (S)</td>
<td>The oogenic follicles are smaller compared to the previous stage and are disorganized. Early and middle vitellogenic oocytes are found. Spermatogenetic follicles are smaller compared to the previous stage. Spermatozoa are scarce and dispersed in the follicle lumen. Spermatogonia, primary and secondary spermatocytes, and spermatids are also present.</td>
</tr>
</tbody>
</table>

Oocytes with a nucleolus ranged between 5.7 and 280.0 µm in diameter ($X = 97.43$ µm, $SD = 30.02$, $N = 8655$). Oocyte diameter and specimen length had a significant correlation (Spearman’s $r = 0.91$, $p < 0.0001$).

The analysis of the proportion of oocytes bigger than 100 µm evidenced five oocyte spawning events during the sampling period (Figure 7 and online resource 1). Three of them occurred in autumn (March–April in 2003, April–May in 2004, and March–April 2005) and two in spring (September–November in 2003, and November–December in 2004) (Figure 7). During the first sampling period (2003), oocyte spawning events were minor compared to those in the second period, and the proportion of big oocytes was like that of medium ones. During the second sampling period (2004), the proportion of oocytes $\geq 100$ µm was greater than that of the other two classes, and spawning events were of greater magnitude.
Figure 5. Reproductive stages of *Corbicula largillierti* recognized in Santa Catalina Pampean Stream, Argentina. Active gametogenesis in mixed (a) and spermatogenic (b) follicles, where the strong hematoxylin staining shows the typical bunches of spermatids; spermatogenic follicles with abundant spermatozoa occupying the follicle center (c), and mature oogenic follicles replete with free full-grown oocytes with no free space in the follicle (d); mature female follicles partially spawned (e); spawned oogenic follicles with vitellogenic oocytes (f); spawned spermatogenic follicles with scarce spermatozoa, spermatocytes, and spermatids (g). Scale bar 50 μm.
Figure 6. Proportion of oogenic and spermatogenic follicles of *Corbicula largillierti* in Arroyo Santa Catalina, throughout the sampling period (n = 573), and water temperature: (a) active gametogenesis; (b) mature; (c) spawned. Sample date indicated as dd/mm.
Table 4. Number of specimens with larvae in the gills and their reproductive stage for oogenic and spermatogenic follicles during the sample period in Santa Catalina stream. AG, active gametogenesis; M, mature and spawning; S, spawned; n, number of specimens analyzed.

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>Specimens with Larvae</th>
<th>Oogenic Follicles</th>
<th>Spermatogenic Follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 January 2004 (n: 27)</td>
<td>1</td>
<td>AG</td>
<td>S</td>
</tr>
<tr>
<td>20 March 2004 (n: 22)</td>
<td>1</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>8 December 2004 (n: 20)</td>
<td>9</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>15 February 2005 (n: 20)</td>
<td>15</td>
<td>M</td>
<td>S</td>
</tr>
<tr>
<td>20 March 2005 (n: 14)</td>
<td>11</td>
<td>M</td>
<td>S</td>
</tr>
</tbody>
</table>

Figure 7. Temporal variation of oocyte size of Corbicula largillerti. In grey, proportion of oocytes > 100 µm, and in black, proportion of oocytes ≤ 49 µm. Arrows indicate spawning events. Water temperature (dotted line) on the right secondary axis. Sample date indicated as dd/mm.

4. Discussion

The success of invasive species is directly linked to their reproductive strategy [33]. The gonadal histological analysis of Corbicula largillerti allows the determination of its ability to adapt to new environmental conditions, and therefore its invasive capacity. Being a hermaphroditic population showing early sexual maturity and having mature gametes throughout the year, partial spawning, rapid gonadal recovery, and larval incubation are characteristics found in the studied population, which indicate this species’ ability to colonize different environments.

The populations of Corbicula species show a varied sex composition according to their distribution, from dioecious populations found in the native region, to hermaphrodites, with males or females in a low proportion, and which are characteristic of the invaded areas, although they are also found in native regions [24,26,34]. According to Pigneur et al. [27], free spawning for external fertilization in freshwater dioecious bivalves is considered a primitive condition (e.g., Corbicula sandai Reinhardt, 1878 and Corbicula japonica Prime, 1864). However, a low number of dioecious individuals of Corbicula fluminea was related to environmental factors in Asia [35].

Most hermaphrodite bivalve are protandrous [36], but Corbicula spp. are protogynous [27], while C. largillerti in South America was described as a simultaneous hermaphrodite where male and female tissues are not clearly segregated in the visceral mass [21]. Cross-fertilization between hermaphrodite individuals is suggested by the presence of sperm-containing mucous filaments connecting the siphons [36]. C. fluminea is capable of self-fertilization, which is also suggested for C. largillerti in South America [4,21].
The analysis of gonadal follicles indicated that the *Corbicula largillierti* population from Santa Catalina stream was mainly comprised of hermaphroditic individuals with a largely dominant female follicular portion, and a lower proportion of female specimens. The only previous study on the gonadal cycle of *C. largillierti* was conducted on a population from La Guardia Stream, at Río de la Plata River [21]. That was a hermaphroditic population where oogenic follicles predominated over spermatogenic ones, a characteristic also observed in other species of the genus [34,37], and which is coincident with our findings. Ituarte [21] mentioned that in some individuals bigger than 20 mm (APL), the spermatogenic portion of the gonad was bigger than the oogenic portion, while in this study the spermatogenic follicles were recorded in all the size classes analyzed but the oogenic portion always dominated.

In the present study, there were no *Corbicula largillierti* specimens with only spermatogenic follicles (functional males) recorded. This was also reported for *C. fluminea* in lotic environments, e.g., in Hong Kong [24], in Brazil [38], and in a study of five species of *Corbicula* (*C. fluminalis*, *C. purpurea* Prime, 1864, *C. consobrina* (Cailliaud, 1827), *C. africana* (Krauss, 1848), and *C. japonica* Prime, 1864) from malacological collections of museums from Africa, West and Central Asia, and China [39]. It has been stated that hermaphroditic *Corbicula* species are protogynous, with oocytes present during the whole year and spermatozoids produced in response to seasonal temperature changes [40]. The presence of female individuals in the Santa Catalina Stream population matches previous findings for other species of the genus. In South America, studies of a population of *C. fluminea* from the same locality and sampling events as the present study [23], and a population subjected to variations in flow rate on Upper Paraná River [41], recorded the presence of female specimens.

Different studies on the gonadal cycle of *Corbicula* spp. indicate that the number of reproductive events (spawning and fertilization) throughout the year is variable [14,23]. Several authors describe two annual reproductive events [21,26], while others report only one or three [25,42]. Two reproductive peaks were mentioned for *C. fluminea* in California and Arkansas, while in Kentucky, a region with a cooler climate, only one peak per year was noted [43]. These variations in the reproductive cycle could be due to environmental differences. In *C. largillierti*, the gonad was found to be active during the whole sampling period, with no seasonal resting pattern, and mature follicles in active gametogenesis throughout the year, partial spawning, and rapid gonadal recovery, similar to what was described previously [21]. This reproductive strategy would allow colonization and settling in unstable environments.

Free-living larvae are rare in freshwater environments, and occur in few bivalve species, such as the invasive *Dreissena polymorpha* (Pallas, 1771) and *Limnoperna fortunei* (Dunker, 1857). Some species of *Corbicula* use their inner demibranchs (on some occasions outer demibranchs) as a marsupium for the larval, such as *C. fluminea* [44] and *Corbicula possoensis* Sarasin & Sarasin, 1898 [20]. Ituarte [21] detected larvae in the inner demibranchs of *C. largillierti* and observed that, in the laboratory, pediveliger larvae in an advanced maturation stage leave the gills in two periods (spring and summer). In the Santa Catalina Stream population, two main oocyte spawning events (spring and autumn) were detected, besides other lower-magnitude events. However, the detection of larvae in the gills did not always match the major spawning events.

Rapid maturation and offspring incubation (e.g., gill marsupia) are general strategies that allow an increase in survival in the environment of the more vulnerable stages. Therefore, producing fewer offspring, but on these conditions, may be an effective role in the population survival [28]. This could be extrapolated to the protection of weak stages, such as larvae, as occurs in *C. fluminea* in South American temperate regions, where individuals are released from the branchial chamber almost as juveniles, whereas in subtropical regions the release occurs in the earliest larval stage [23]. Some authors mention that gametogenesis processes where gonads degenerate and gametes decrease may be inhibited during larval incubation and release [44]. However, no evidence of this was found in the *C. largillierti*
population studied, where the presence of larvae had no influence on the gametogenesis of these individuals.

Different environmental variables have been mentioned as determinants in the reproductive cycle of bivalves, including temperature [45]. Throughout the entire study period, mature oocytes ready for spawning were recorded (Figure 7 and Figure S1), similar to what was reported for *C. largillierti* [21] and for *C. fluminea* [23], while oocyte spawning occurred due to changes in water temperature (highest to lower and vice versa). On the other hand, Kraemer et al. [36] note that spermatozoa in *C. fluminea* seem to be released in response to seasonal temperature changes.

*Corbicula largillierti* presents a characteristic reproductive pattern of invasive species that would allow it to invade environments and prevail in them. However, there are still many questions regarding the reproductive pattern of this species and the variations observed in *Corbicula* spp.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d16060357/s1, Figure S1: Oocyte frequency during the sample period, Santa Catalina Stream, Buenos Aires Province, Argentina.

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