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Synchrony between Reproductive Phase and Flood Period: A Dispersion Mechanism for the Freshwater Clam *Corbicula fluminea* (Bivalvia: Corbiculidae) in a Brazilian Neotropical Floodplain

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This study evaluates the gonadal histology of *Corbicula fluminea* present in the upper Paraná River floodplain and samples of limnological variables to understand its reproductive cycle. *Corbicula fluminea* was monitored monthly from December 2013 to February 2015. Spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa were identified in the male follicles of the hermaphrodites. Oogonia, oogonial nests, previtellogenic oocytes, early vitellogenic oocytes, middle vitellogenic oocytes and full-grown vitellogenic oocytes were identified in the female follicles of the hermaphrodites and females. The reproductive phases were described as developing, active spawning/sperm releasing, regression and regeneration. Higher values of temperature, dissolved oxygen, total nitrogen and total phosphorous were identified during flood periods, while higher values of pH and conductivity were obtained during dry periods. The species either does not reproduce or reduces the intensity of reproduction in cold months, with the sex ratio not differing significantly between hermaphrodites and females with regard to month and reproductive phase. Thus, reproduction is synchronized with the flood period and its limnological characteristics and when the increase in connectivity between floodplain environments facilitates the larval dispersion of this non-native species into other environments.

Key words: Propagule pressure, Invasion, Bivalve reproduction, Spermatogenesis, Oogenesis.

BACKGROUND

The bivalve *Corbicula fluminea* (Müller 1774) is native to Asia (Santos et al. 2012). It invaded South America in the 1970s and dispersed through the Río de la Plata river basin (Ituarte 1981). It currently occupies several hydrographic regions of South America, and mapping reveals that it occurs from Venezuela to Argentina (Patagonia) (Darrigran 2002; Lasso et al. 2009; Santos et al. 2012; Crespo et al. 2015; Darrigran et al. 2020). Its densities in South American ecosystems vary widely. A total of 192 ind./m^2 occur in the Paraguay

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ecoregion (Callil and Mansur 2002), while more elevated densities of 10,000 ind./m^2 have been identified from the Lower Uruguay ecoregion (Castillo et al. 2007).

Brazilian aquatic ecosystems also support a great variety of *C. fluminea* densities (*e.g.*, 0.35–217.13 ind./m^2 from the Tapajos-Juruena River (Poleze and Callil 2015), 6.66–7.3 ind./m^2 from the Amazonas Estuary & Coastal Drainages (Beasley et al. 2003), 39.6–265.6 ind./m^2 from the Iguassu River (Meyer et al. 2017), and 6154 ind./m^2 from the Upper Paraná River ecoregions (França et al. 2007)). Moreover, a larger variety of *C. fluminea* densities have been recorded from within the latter ecoregion: 0–300 ind./m^2 (Rodrigues et al. 2007), 3223 ind./m^2 (Suriani et al. 2007), 0–1282 ind./m^2 (Vianna and Avelar 2010), 4–519 ind./m^2 (Luca et al. 2012), 12–235 ind./m^2 (Beghelli et al. 2014), 105.5–274.1 ind./m^2 (Oliveira et al. 2014), and 144.9 ind./m^2 (Ragonha et al. 2014).

High densities of invasive species are of great concern. Despite the potential positive impacts of *C. fluminea*, such as furnishing shelter and substrate for other species and being a source of food for pelagic and benthonic species, it has many negative impacts (Sousa et al. 2008 for a revision). According to these authors, this invader, for example, causes the dislocation of or reduction in the habitats available to other species, competes for benthonic food—thus possibly limiting planktonic food for other species—due to its elevated filtration rates, and can ingest a large number of spermatozoa of other species. A laboratory experiment with *C. fluminea* and *Unio delphinus* showed a decline in carbohydrate concentration in the latter taxon as a response to the higher density of *C. fluminea*. The ecological requirements of both species can sometimes overlap and may negatively interfere with native unionids and explain, in part, the loss of up to 30% of *U. delphinus* populations in native areas (Ferreira-Rodríguez et al. 2018). Moreover, economic impacts such as the shutdown of hydroelectric plants and urban water supply systems due to the obstruction of this species have also been recorded in Brazil (Santos et al. 2012).

Successful invasions depend on fast colonization and dispersion, and could be related to many individual characteristics. For example, invasive animal species guarantee dispersion and colonization success by associating with human activities (Sousa et al. 2008). These authors also comment that invasive species can possess a large geographic distribution with the capacity to colonize new habitats due to genetic variability and phenotypical plasticity, and physiological tolerance to abiotic changes. Furthermore, invasive species can have particular reproductive aspects that facilitate their dispersion, such as a short reproduction period, precocious sexual maturity, high fecundity rates and fertilized females capable of colonizing new environments alone (Sousa et al. 2008). Although what was mentioned by these authors occurred with *C. fluminea* on the coast of the Río de la Plata, where this species was first identified in South America, these environmental conditions are not necessarily constant over time and this variation can adversely affect invasive species. For example, the populations of *Corbicula* inhabiting the Río de la Plata are currently contracting (Reshaid et al. 2017).

Considering the reproductive aspects of invasive species, it is worth noting that gonadal alterations reflect the development of germ cells in different phases, which, in turn, allows for the characterization of different development stages. Thus, understanding the gametogenesis, reproductive phases and timing, and other aspects of the reproduction of invasive species could increase the evaluation precision of these population parameters. Moreover, invasion success can be understood using gametogenesis data, especially if this is related to environmental parameters. Knowledge of the reproductive cycle and the factors that influence reproduction could be an important tool to determine future control or management of this invasive species (*e.g.*, Giglio et al. 2016).

Other studies have described the gonadal cycle of *C. fluminea* in southern neotropical streams and potential environmental variables related to it (Cao et al. 2017). However, a proper evaluation of the relationship between the reproductive activities of *C. fluminea* and environmental variables is lacking, particularly in floodplain areas, which vary seasonally. In addition, evaluating the reproductive activities of *C. fluminea* in floodplain areas could increase our knowledge concerning the dispersion of this invasive species, since another invasive bivalve, *Limnoperna fortunei*, seems to synchronize its reproductive cycle with the flood to reach longer distances and colonize new habitats (*e.g.*, Ernandes-Silva et al. 2016). Investigations of *C. fluminea* and other bivalves in different environments in Ilha Grande National Park, Paraná State, Brazil, through the evaluation of factors such as physical composition of granulometric texture, organic matter and macrophyte banks that encourage the establishment of bivalves, showed that *C. fluminea* was found only in Jatobá Lake, with the high density of this invasive species associated with the low percentages of finer sediments that allowed the establishment of a connection to the Paraná River (Ragonha et al. 2014).

Thus, in order to better understand the reproductive characteristics of *C. fluminea* (by means of light microscopy methods and protocols) and verify the relationship between the abiotic parameters and

the reproductive phases of the invader, the present study aimed to: i) histologically identify the types of germ cells and reproductive phases of *C. fluminea*, ii) determine the species' reproduction timing through an examination of its reproductive phases, iii) verify the monthly sex ratio of females and hermaphrodites, iv) identify the sex ratio (females: hermaphrodites) in different reproductive phases and v) identify the main abiotic factors that may be related to the reproductive activities of *C. fluminea*.

MATERIALS AND METHODS

Sampling area

Corbicula fluminea was sampled in December 2013 and monthly from February 2014 to February 2015 at the mouth of "Garças Lake Channel," which connects this shallow floodplain lake to the Paraná River, Brazil (22°44'58"S; 53°15'36"W) (Fig. 1).

Sampling was carried out manually when the water level of the channel was low or with a Petersen grab during the flood season. A monthly average of 24 (minimum 17 and maximum 34) *C. fluminea* individuals were collected and taken to the laboratory of the Research Group in Limnology, Ichthyology and Aquaculture - NUPÉLIA (Porto Rico, Paraná State, Brazil). The length (mm), width (mm) and weight (g) of the shells were measured using a pachymeter and a precision scale (0.01 g). The valves were separated by incision of the anterior and posterior adductor muscles and the viscera with gonad removed. The viscera were weighed (g) and fixed in Bouin solution for 48 hours. The samples were preserved in 70% ethanol.

Histology and Light microscopy

The preserved gonad was prepared for routine light microscopy for morphological study in a series

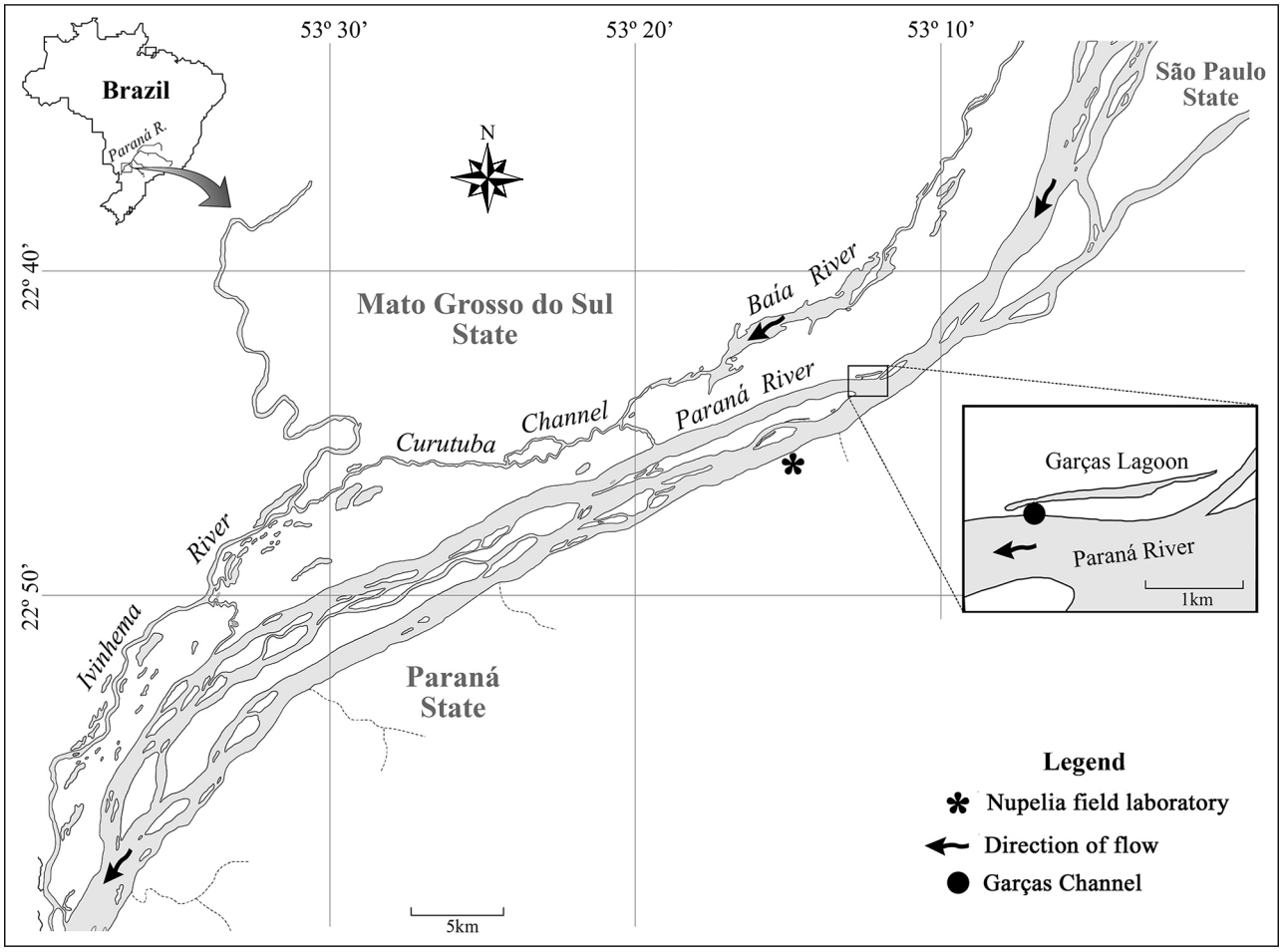

Fig. 1. Map of the upper Paraná River showing the sampling area of the bivalve *C. fluminea* originating at the mouth of "Garças Lagoon Channel," Paraná River (Porto Rico). (Courtesy of Jaime Luiz Lopes, 2019).

of ascending ethanol concentrations (70%, 80%, 90% and 95%) and embedded in historesin (Leica). The samples were sectioned at 5-µm thickness and the slides were stained using Periodic Acid-Schiff (PAS)/Metanil Yellow/Ferric Hematoxylin (Quintero-Hunter et al. 1991). The male and female germ cells were identified in most cases according to Park and Chung (2004). The reproductive phases were diagnosed according to the most advanced germ cell types, and abundance and distribution in the periphery of the germinal epithelium and follicular lumen.

The reproductive phases were categorized as developing, sperm releasing capable/active spawning, regression and regeneration. This scale was adopted because of its simplicity to diagnose the reproductive phases of another invasive bivalve: *Limnoperna fortunei* (Dei Tos et al. 2016).

Limnological variables

We measured the limnological variables monthly from February 2014 to February 2015. Hydrometric level data from the Paraná River were taken daily by means of a limnetic ruler installed at the field station on the banks of the Paraná River (22°45'53.91"S; 53°15'27.92"W). The hydrometric level measurements occurred in the morning and afternoon (daily average used in the present study). In addition, water temperature (°C), dissolved oxygen (mg l^{-1}), electrical conductivity (µS cm^{-1}) and pH were also measured in the field. Moreover, water samples were taken to later evaluate the concentration of total nitrogen (µg l^{-1}) (Bergamin et al. 1978) and total phosphorus (µg l^{-1}) (Mackereth et al. 1978).

Statistical analyses

Reproduction time was found through the number of hermaphrodites, females and grouped sexes per reproductive phases and month. The sex ratio for hermaphrodites and females with different sizes per month and in the different reproductive phases was estimated through the chi-square test using Statistica 7.1 (StatSoft Inc. 2005). In order to summarize the main abiotic factors that could affect the reproductive activities of the species, a principal component analysis (PCA) was also carried out using Statistica 7.1 (StatSoft Inc. 2005). The scores of the axes retained according to the Broken-Stick criterion were then grouped into flood period (December, January, February, March and April) and dry period (May, June, July, August, September, October and November). Lastly, analysis of variance (ANOVA) was used to verify significant differences between the periods as regards the PCA scores, adult abundance and the active spawning phase.

RESULTS

During the study period, we sampled 341 individuals (106 hermaphrodites and 235 females) of the bivalve *Corbicula fluminea*, whose shell length ranged from 0.98 to 3.11 cm among the hermaphrodites and 0.92 to 3.54 cm among the females. Total weight varied from 0.49 to 13.0 g in the hermaphrodites and 0.43 to 14.7 g in the females.

The spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa of hermaphroditic individuals were identified during the histological examination of the follicles and their germ cells (Table 1; Fig. 2). Moreover, it was possible to recognize four reproductive phases: developing, sperm releasing capable, regression and regeneration (Table 2; Fig. 3).

Regarding the females, the ovarian follicles were composed of germ cells such as oogonia, oogonial nests, previtellogenic oocytes, and early, middle and full-grown vitellogenic oocytes (Table 3; Fig. 4). Depending on the dominance of the germ cell types, four reproductive phases for females were also defined: developing, active spawning, regression and regeneration (Table 4; Fig. 5).

The reproductive period for females and hermaphrodites was long (almost one year) (Fig. 6A, B, C). There were no reproductive activities (or very few) in May, June and July. The number of individuals with gonads in the developing phase was higher in these months. Moreover, females occurred slightly more than hermaphrodites during the study period; however, according to the chi-square test, there was no significant predominance of one sex (*d.f.* = 1 and $\chi^2 > 3.84$ for $p < 0.05$; Table 5). The ratio between females and hermaphrodites, considering each reproductive phase during the study period, showed that neither sex predominated (*d.f.* = 1 and $\chi^2 > 3.84$ for $p < 0.05$; Table 6).

Principal component analysis (PCA) indicated that pH, electrical conductivity and total nitrogen were the main abiotic variables that may have affected the reproductive pattern of *C. fluminea*, considering the distribution of the samples on axis 1 of the analysis, with pH and electrical conductivity having positioned the collections negatively on the axis and total nitrogen having positioned them positively (Fig. 7). On the other hand, dissolved oxygen and temperature positioned the collections positively on axis 2, while total phosphorus positioned them negatively. This pattern explained 58.81% of the distribution of the *C. fluminea*

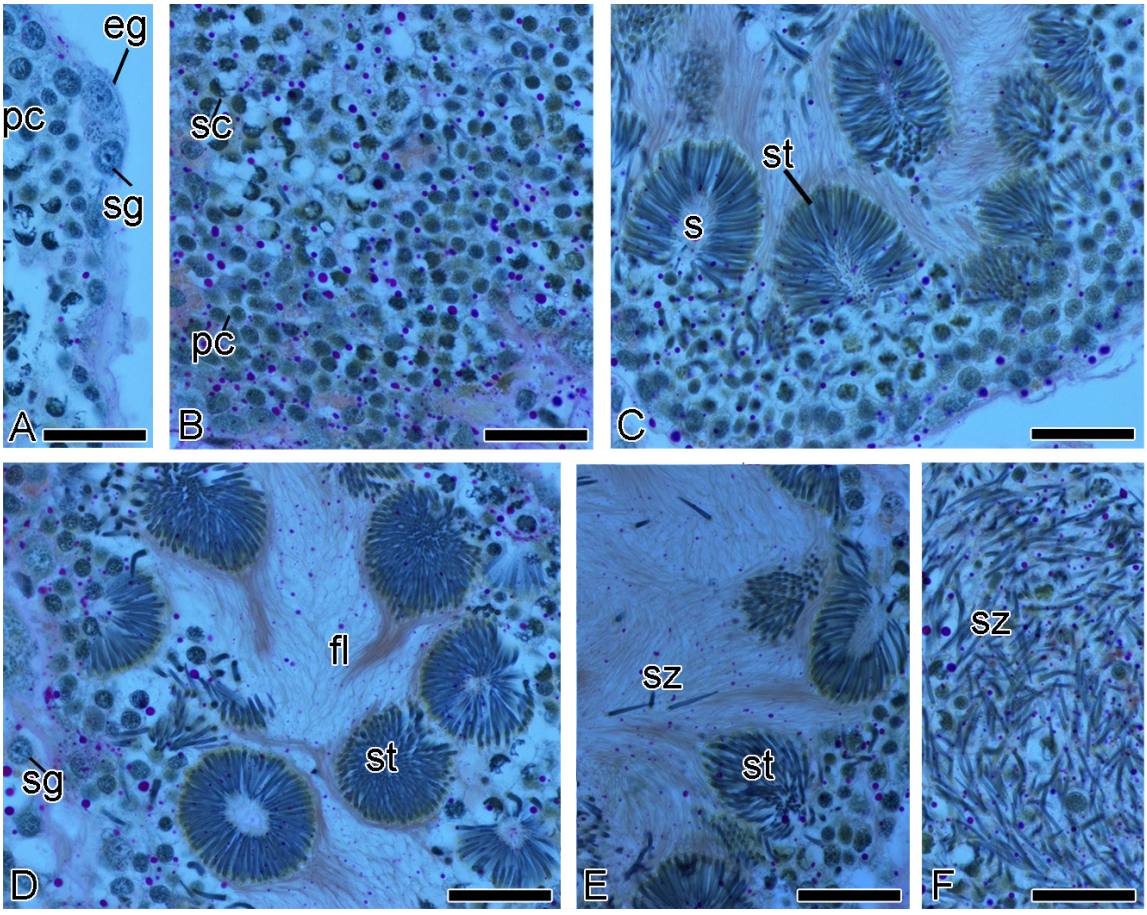

Fig. 2. Partial photomicrograph recording the germ cells of the germinal compartment of the follicles of the bivalve *C. fluminea*. A. Spermatogonia (sg), primary spermatocytes (pc), secondary spermatocytes (sc) and spermatids (st); B. Secondary spermatocytes and spermatids; C, D. Spermatozoa (sz) and their organization around Sertoli cells (S); Sertoli cell (S) and flagella (fl); E, F. Spermatozoa (sz). Germinal epithelium (ge). Light microscopy/Periodic Acid-Schiff /Hematoxylin /Metanil Yellow. Scale bar: A, B, C, D, E, F = 24 µm.

Table 1. Diagnosis of the germ cells present in the follicles of the testicles of the bivalve *C. fluminea* originating at the mouth of the Garças Lagoon Channel, Paraná River (Porto Rico)

Description of the germ cells	
Spermatogonia (sg)	the largest cells of the germinal lineage, located in the germinal epithelium of the testicles. They present slightly granular cytoplasm and a spherical or elliptical, central, basophilic nucleus and delicate nucleolar chromatin, with 1, 2 or 3 basophilic nucleoli (Fig. 2A). Spermatogonia enter into meiosis and originate primary spermatocytes.
Primary spermatocytes (pc)	spherical cells that have less cytoplasm than spermatogonia; therefore, they are smaller, with a condensed and basophilic nucleus (Fig. 2A). Primary spermatocytes have completed the first division of meiosis and originate secondary spermatocytes.
Secondary spermatocytes (sc)	frequent, spherical and similar in size to primary spermatocytes, with their nucleus possessing chromatin in the form of an umbrella (Fig. 2A, B, E) and after the second consecutive meiotic division originate spermatids.
Early spermatids (st)	spherical and smaller than primary and secondary spermatocytes. They possess scarce cytoplasm and a spherical nucleus (Fig. 2A, B, C, E). They do not divide any more, but transform morphologically through the process of spermiogenesis (become more elongated) into spermatozoa.
Spermatozoa (sz)	possess a conical head, with a long, condensed nucleus. Two flagella project from the basal region of the head. Spermatozoa form dense groupings that are spherical or semispherical or in the form of a bunch of bananas connected by slightly lilac Sertoli cells, with their flagella facing the lumen of the testicle. They detach from the Sertoli cells in the spawning period and are liberated into the lumen of the testicle tubules during spermiation (Fig. 2C, D, E).

Table 2. Diagnosis of the reproductive phases of the hermaphrodites of the bivalve *C. fluminea* originating at the mouth of the Garças Lagoon Channel, Paraná River (Porto Rico)

Diagnosis of the reproductive phases	
development	the male follicles are small and carry proliferating spermatogonia, primary and secondary spermatocytes and spermatids (Fig. 3A, B, C).
sperm releasing capable	the male follicles are expanded and branched and present spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa (Fig. 3D, E, F). The spermatozoa are abundant and organized in the form of a circle, semicircle or bunch of bananas stuck to Sertoli cells and/or dispersed in the lumen of the follicle (Fig. 3F).
regression	(equivalent to spawned), the branched follicles are smaller compared to the previous phase and possess spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa (Fig. 3G, H). The spermatozoa are scarce in this phase and dispersed in the lumen of the follicles (Fig. 3I).
regeneration	the follicles are smaller compared to the previous phase, with no evidence of lumen. Spermatogonia, primary and secondary spermatocytes and vestiges of spermatozoa occur (Fig. 3J, K, L).

Table 3. Diagnosis of the germ cells present in the ovarian follicles of the bivalve *C. fluminea* originating at the mouth of the Garças Lagoon Channel, Paraná River (Porto Rico)

Description of the germ cells		
Stages	Phases	Diagnoses
Oogonial proliferation stage	Oogonial nests	Oogonia (og) are the smallest germline cells, associated with the periphery of the germinal epithelium (Fig. 4A). They possess scarce cytoplasm, a basophilic nucleus and an evident nucleolus. Oogonial proliferation produces oogonial nests (Fig. 4B).
Early prophase stage	Leptotene, zygotene, pachytene and diplotene phases	Nest with germline cysts (Fig. 4B). Oocytes possess a voluminous, spherical nucleus with a regular scarce ooplasm. The distinct pattern of the chromatin indicates that the oocyte nest is in the pachytene phase of meiosis prophase I (Fig. 4C).
	Previtellogenic stage	Previtellogenic oocytes (pov) are larger than those from the previous phase and connected to the epithelium of the ovarian follicle. They possess basophilic ooplasm and a slightly basophilic nucleus with one, two or multiple nucleoli (Fig. 4D).
Vitellogenic stage	Early vitellogenic oocytes	Early vitellogenic oocytes (ev) are larger than those from the previous phase and connected by a peduncle to the epithelium of the ovarian follicle. They possess acidophilic ooplasm, indicating the start of yolk deposition. The nucleus is voluminous and contains 1, 2, 3 or 4 nucleoli (Fig. 4E).
	Middle vitellogenic oocytes	Middle vitellogenic oocytes (mv) are very similar to early vitellogenic oocytes; however, they are slightly larger. Their ooplasm is acidophilic and the nucleus voluminous and spherical or elliptical with 1 to 4 nucleoli that may be connected to or disconnected from the ovarian follicle (Fig. 4F, G, H).
	Full-grown vitellogenic oocytes	Full-grown vitellogenic oocytes (fg) are the largest germ cells. They possess acidophilic ooplasm replete with yolk granules. The nucleus is located in the center and is elliptical, semielliptical or almost spherical with one or 2 nucleoli (Fig. 4H, I). Full-grown oocytes may be linked to or unlinked from the epithelium of the ovarian follicle. The disconnected full-grown oocytes move toward the gonadal duct.

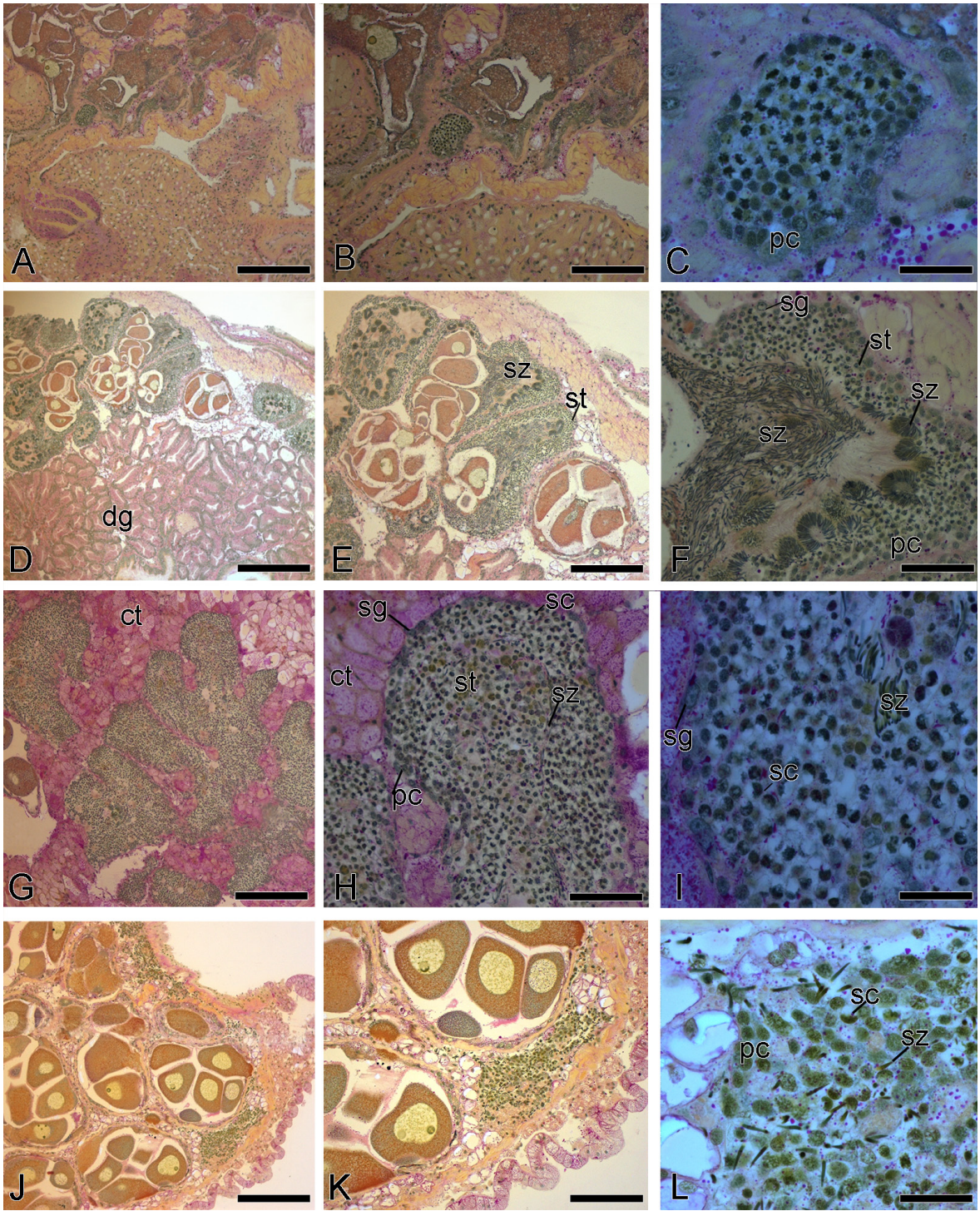

Fig. 3. Photomicrography illustrating spermatogenic follicles in different reproductive phases of the bivalve *C. fluminea* (hermaphrodite). A and B, General view of male follicles in developing phase. C, Detail of primary spermatocytes. Scale bar: A = 238 µm, B = 116 µm and C = 24 µm. D and E, General view of male follicles in sperm releasing capable phase. F, Detail illustrating spermatogonia, primary spermatocytes, spermatids and spermatozoa. Scale bar: D = 460 µm, E = 238 µm and F = 60 µm. G, Panoramic view of branched male follicles in regression. H and I, Follicles exhibiting spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa. Scale bar: G = 238 µm, H = 60 µm and I = 24 µm. J and K, General structure of male follicles in recuperation. L, Detail of a follicle in recuperation showing primary and secondary spermatocytes and vestiges of spermatozoa. Scale bar: J = 238 µm, K = 115 µm and L = 24 µm. Light microscopy/Periodic Acid-Schiff/Hematoxylin/Metanil Yellow. Spermatogonia (sg), primary spermatocytes (pc), secondary spermatocytes (sc), spermatids (st), spermatozoa (sz), interconnective tissue (ct) and digestive gland (dg).

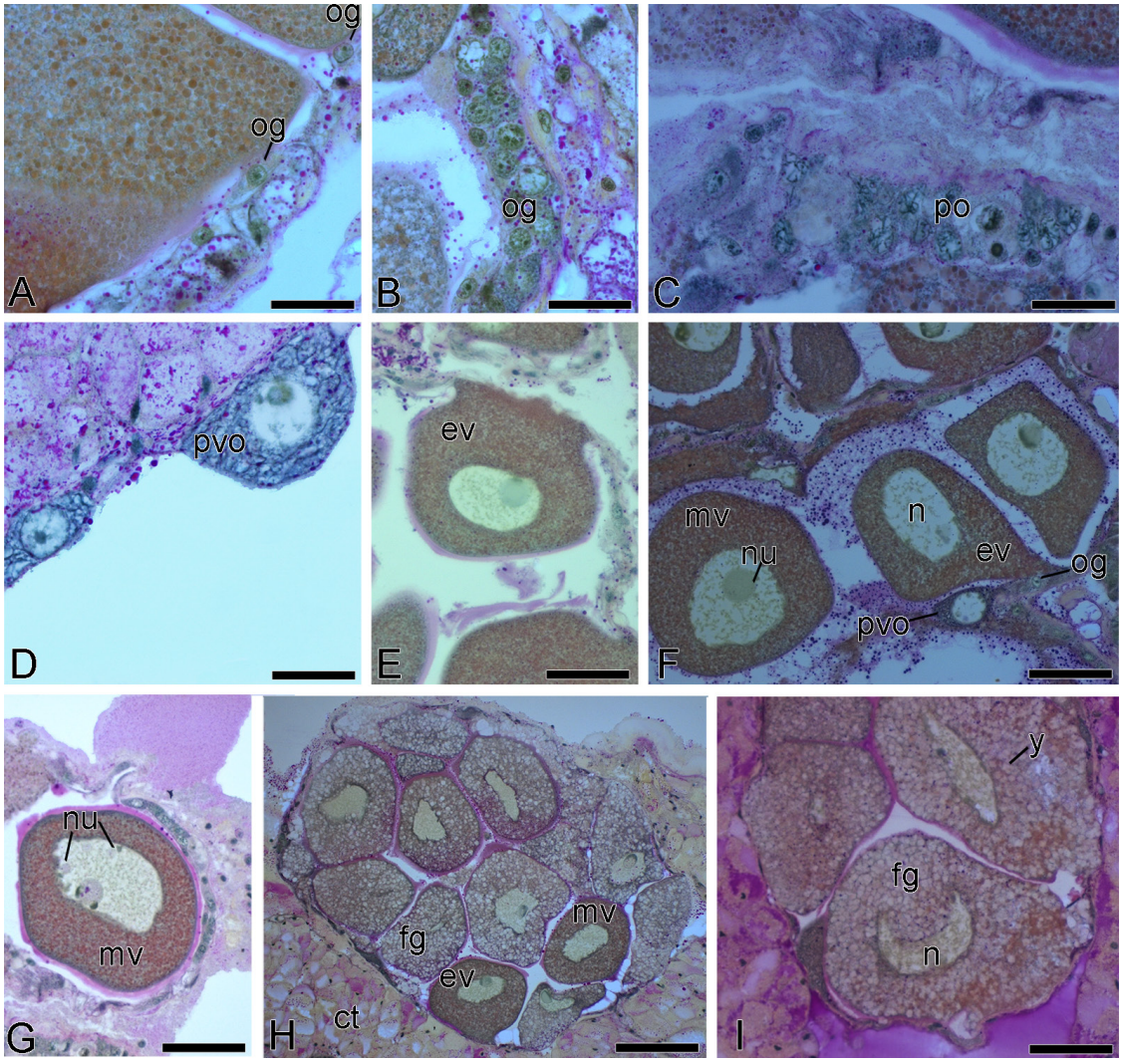

Fig. 4. Photomicrography illustrating germ cells of ovarian follicles of the bivalve *C. fluminea*. A, Germinal epithelium with oogonia. B, Oogonial cyst. C, Nest with pachytene oocytes. D, General view of previtellogenic oocytes. E, General view of early vitellogenic oocytes. F, Follicle showing oogonia, previtellogenic oocytes, early vitellogenic oocytes and middle vitellogenic oocytes. G, Follicle with middle vitellogenic oocytes. H, Follicle with early vitellogenic oocytes, middle vitellogenic oocytes and full-grown oocytes. I, General view of full-grown oocytes showing yolk granules and nucleus. Scale bar: A, B, C, D = 24 µm, E, F, G and I = 60 µm and H = 115 µm. Light microscopy/Periodic Acid-Schiff/Hematoxylin/Metanil Yellow. Oogonia (og), pachytene (pe), previtellogenic oocytes (pvo), early vitellogenic oocytes (ev), middle vitellogenic oocytes (mv), full-grown vitellogenic oocytes (fg) and interconnective tissue (ct).

Table 4. Diagnosis of the reproductive phases of females of the bivalve *C. fluminea* originating at the Garças Lagoon Channel, Paraná River (Porto Rico)

Diagnosis of the reproductive phases	
Developing	the ovarian follicles are expanding and previtellogenic oocytes and early and middle vitellogenic oocytes are found in them (Fig. 5A, B, C). Full-grown vitellogenic oocytes are absent.
Active spawning	the follicles are replete with full-grown oocytes that move toward the gonadal duct (Fig. 5D, E, F), but abundant early and middle vitellogenic oocytes can be seen in nearby tubules.
Regression	(equivalent to spawned), the ovarian follicles remain disorganized (Fig. 5G) and early and middle vitellogenic oocytes are found (Fig. 5H, I). There are also follicles with only oogonia and oogonial nests.
Regeneration	the follicles contain oogonia, oogonial nests, previtellogenic oocytes and early and middle vitellogenic oocytes (Fig. 5J, K, L).

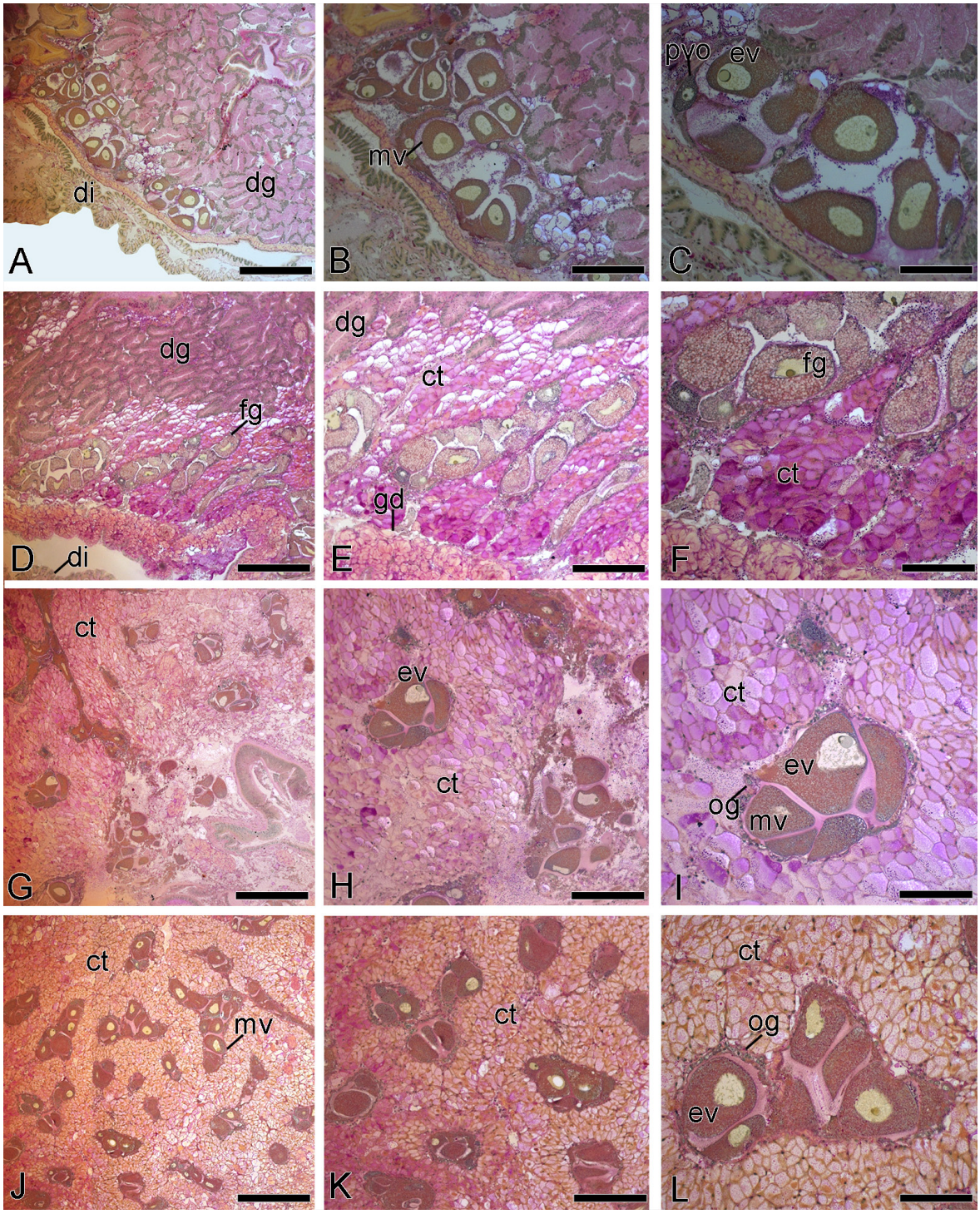

Fig. 5. Photomicrography showing the different reproductive phases of the ovarian follicles of the bivalve *C. fluminea*. A, General view of a female follicle in the developing phase. B and C, Details of previtellogenic oocytes and early vitellogenic oocytes. Scale bar: A = 460 µm, B = 238 µm and C = 115 µm. D and E, General view of female follicles in active spawning. F, Detail illustrating full-grown vitellogenic oocytes passing through the gonadal duct. Scale bar: D = 460 µm, E = 230 µm and F = 115 µm. G and H, Panoramic view of female follicles in regression. I, Follicle exhibiting oogonia, early and middle vitellogenic oocytes and empty follicles except for a germinal epithelium with oogonia. Scale bar: G = 460 µm, H = 238 µm and I = 115 µm. J and K, General structure of female follicles in recuperation. L, Detail of a follicle in recuperation showing oogonial cysts and early vitellogenic oocytes. Scale bar: J = 460 µm, K = 238 µm and L = 115 µm. Light microscopy/Periodic Acid-Schiff/Hematoxylin/Metanil Yellow. Oogonia (og), previtellogenic oocyte (pvo), early vitellogenic oocyte (ev), middle vitellogenic oocyte (mv), full-grown vitellogenic oocyte (fg), interconnective tissue (ct), gonadal duct (gd), digestive gland (dg), inner demibranch (id).

collections. The limnological variables monitored and used in the PCA also can be found in figure 8. In addition, evaluation of the scores of the PCA axes shows that the collections are grouped significantly (F = 10.62; p = 0.007), according to the flood and dry periods (Fig. 9), mainly in relation to the axis 2 scores.

The differences between the flood period and dry period in *C. fluminea* sampling showed that the mean abundance of the adults was higher in the flood period (F = 3.626; p = 0.05; Fig. 10). The active spawning phase follows the same pattern, with higher mean values found during the flood phase. However, despite having a clear tendency toward an increase in spawning during the flood period, the differences between the periods were not significant (F = 1.03; p = 0.33; Fig. 10).

DISCUSSION

Findings showed that the bivalve *C. fluminea* has a very long spawning period in females and hermaphrodites that is synchronized with the flood period of the upper Paraná River floodplain. The histological analysis of the gonads of *C. fluminea* showed hermaphrodites occurring with the simultaneous production of male and female gametes in separate follicles or in the same gonadal follicle, as well as the occurrence of females. However, functional males, *i.e.*, follicles having only male germ cells, were not recorded. In addition, the serial longitudinal sections of the gonads of the hermaphrodites and adult females showed that in reproductively active individuals the gonads are formed by a system of branched follicles whose gametes are liberated into the gonoducts or gonadal ducts.

Reproductive studies on *C. fluminea* carried out in Africa and Asia did not detect functional males either (Korniushin 2004). The histological studies that characterized the changes in the structure of the gonads of *Corbicula japonica* described the gonads as being

Table 5. Monthly absolute number, frequency and sex ratio of females and hermaphrodites of the bivalve *C. fluminea* sampled in the Garças Lagoon Channel, Paraná River (Porto Rico)

	Number		Frequency			
	Female	Hermaphrodite	Female	Hermaphrodite	Total	χ^2
Dec/2013	15	11	57.69	42.31	26	0.61
Feb/2014	20	9	66.67	30.00	29	0.51
Mar	17	12	58.62	41.38	29	0.58
Apr	17	6	73.91	26.09	23	0.54
May	23	6	79.31	20.69	29	0.61
Jun	17	7	70.83	29.17	24	0.52
Jul	14	8	63.64	36.36	22	0.51
Aug	21	3	87.50	12.50	24	0.76
Sep	13	4	76.47	23.53	17	0.57
Oct	12	8	60.00	40.00	20	0.56
Nov	13	6	68.42	31.58	19	0.50
Dec	17	5	77.27	22.73	22	0.58
Jan/2015	17	6	73.91	26.09	23	0.54
Feb	19	15	55.88	44.12	34	0.67
Total	235	106	68.71	30.99	341	0.50

Table 6. Total length and sex ratio of females and hermaphrodites in different reproductive phases of the bivalve *C. fluminea* sampled in the Garças Lagoon Channel, Paraná River (Porto Rico)

Reproductive Phases	Total length (cm)	Female (n)	Hermaphrodite (n)	Sex ratio	Total	χ^2
Developing	0.92–3.54	111	8	1.0–0.07	119	0.87
Spawning/ Sperm releasing adults	1.02–3.07	78	41	1.0–0.55	119	0.50
Regression	0.98–2.97	16	26	1.0–1.63	42	3.03
Regeneration	1.50–3.11	30	31	1.0–1.03	61	1.07
Total	0.92–3.54	235	107	1.0–0.46	342	0.50

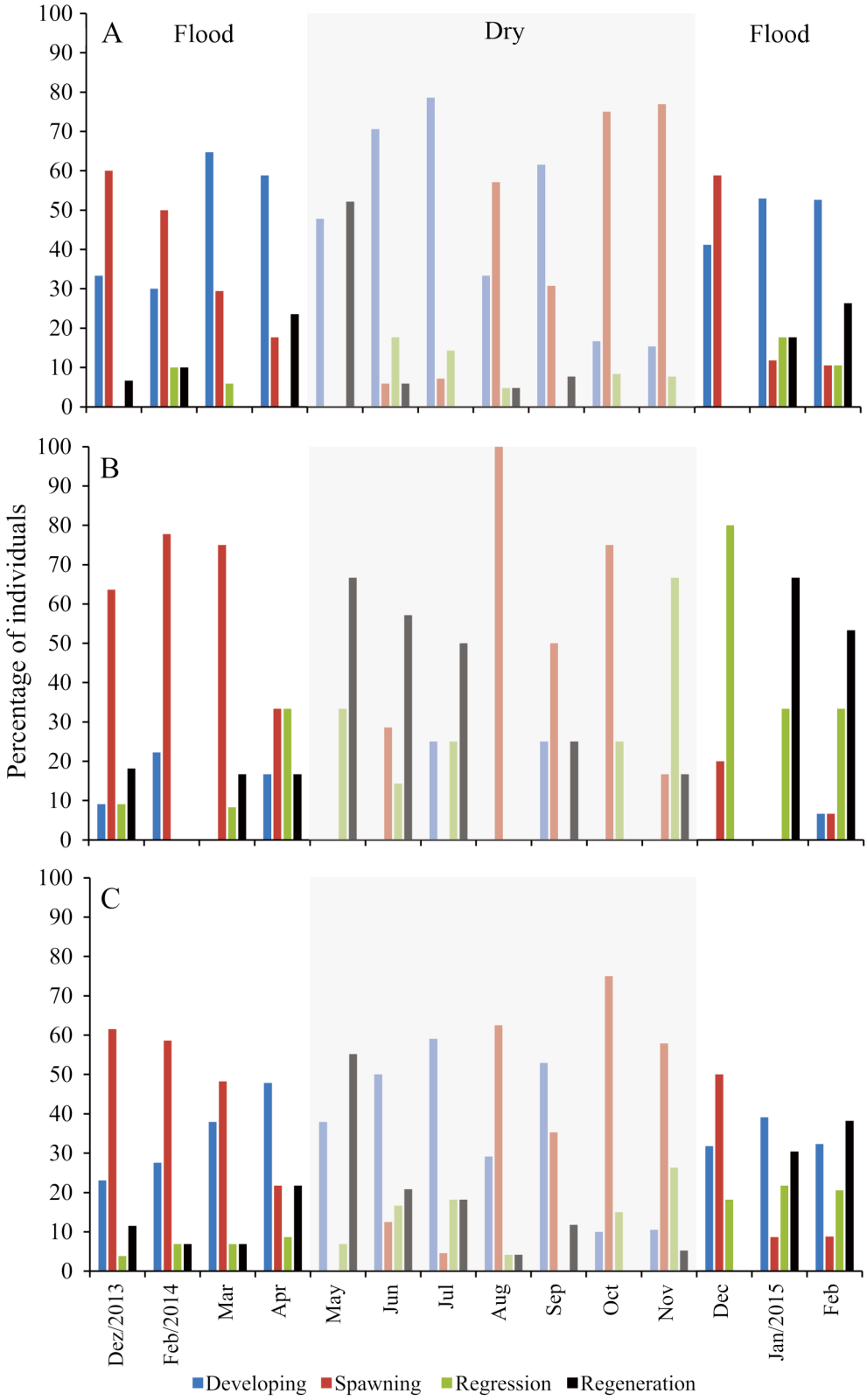

Fig. 6. Number of individuals: (A) females, (B) hermaphrodites and (C) grouped sexes of the bivalve *C. fluminea* sampled in December 2013 and from February 2014 to February 2015 in different reproductive phases in the Garças Lagoon Channel, Paraná River (Porto Rico). Flood = December, January, February, March and April; Dry = May, June, July, August, September, October, November. The gray area represents the dry period.

formed by ovarian or testicular tubules (Baba et al. 1999). In *C. japonica*, the gonads were also described as being composed of branched tubules whose terminal and lateral areas were called acini (Rybalkina et al. 2013). In regards to *Corbicula australis*, the terminology used to characterize the gonadal structure was also acini (Byrne et al. 2000). With respect to *Corbicula* sp. (Kennedy and Huekelem 1985), *C. fluminea* (Araujo et al. 1993; Park and Chung 2004; Levandowski et al. 2016) and *Corbicula leana* (Park and Chung 2004), the term used was follicles. Thus, comparisons with other studies should take into account potential differences in terminology.

The reproductive system of the bivalve *C. fluminea* consists of two gonads, one on each side of the animal. They occupy a large part of the surface of the visceral mass in the dorsal part of the mantle (Mansur 2012). Each gonad is formed by arborescent follicles that branch out through the stroma of the visceral mass and liberate their gametes via gonoducts that are externalized through a single pore on each side of the animal (Araujo et al. 1993; Mansur 2012). The ovaries are grayish green (Martins et al. 2006); however, in the hermaphrodites, part of the gonads is light gray, corresponding to the testicles (Mansur 2012).

The present study revealed that light microscopy can be used to recognize spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa. Similar findings for *C. fluminea* spermatogenesis were recorded by Park and Chung (2004), which described spermatogonia, spermatocytes, spermatids and spermatozoa using light microscopy. Male germ cells of the same type were described in Konishi et al. (1988) using a transmission electron microscope. With regards to the females, the follicles were found to possess oogonia, oogonial nests, previtellogenic oocytes, and early, middle and full-grown vitellogenic oocytes. Gametogenic studies of *C. fluminea* in North America characterized similar stages of oogonia, previtellogenic oocytes, early vitellogenic oocytes and mature oocytes (Park and Chung 2004).

The types of germ cells, their distribution in the follicles and abundance characterized the reproductive phases of developing, sperm releasing capable/active spawning, regression and regeneration of the *C. fluminea* hermaphrodites and females in the present study. Likewise, *C. fluminea*, occurring in Santa Catalina Stream (Argentina), underwent the gonadal developing phases immature, premature, mature, spawning and spawned in the male and female follicles (Cao et al. 2017).

The results show that hermaphrodites and females spawn during the same period, *i.e.*, reproducing synchronically. Although females occur in slightly higher proportions than hermaphrodites in the period, they do not differ from what is expected. The flood period (December, January, February, March and April) can be characterized as the reproductive period. However, studies carried out on *C. fluminea* juveniles (lower Paraná River, Argentina) revealed that the reproductive period could be in October and November (Cataldo and Boltovskoy 1999). In addition, there are records of spawning in May and September 2003, January–February and October 2004 and February–March 2005 (Cao et al. 2017).

Histologically, the hermaphrodites in active spawning showed asynchrony (n = 26) and synchrony (n = 15) in the development of the germ cells in the spermatogenic, oogenic or mixed follicles. The asynchronous hermaphrodites possessed spermatogenic

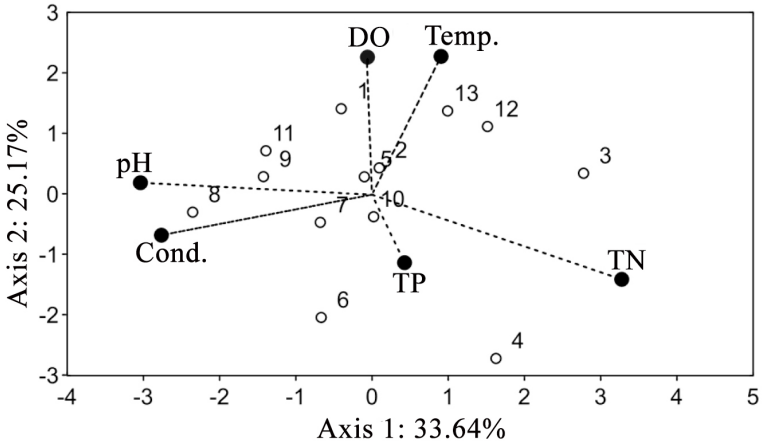

Fig. 7. Diagram of the principal component analysis, emphasizing the directions of the effects of the abiotic variables on the distribution of the collections of the bivalve *C. fluminea*. Cond. = Electrical conductivity; DO = Dissolved oxygen; Temp. = Temperature; TP = Total phosphorus; TN = Total nitrogen. The number at each point on the graph represents a series of collections, with number 1 being the month of the first collection (February 2014) and number 13 the month of the last collection (February 2015).

follicles in the sperm releasing capable phase with plenty of spermatozoa, in addition to spermatogonia, primary and secondary spermatocytes, and spermatids. The oogenic follicles contained oogonia, previtellogenic oocytes, and early and middle vitellogenic oocytes. On the other hand, the hermaphrodites possessing synchronous follicles during the active spawning phase and sperm releasing capable phase presented follicles abundant in spermatozoa, with spermatogonia, primary and secondary spermatocytes, spermatids and follicles abundant in full-grown vitellogenic oocytes, in addition to previtellogenic oocytes, early and middle vitellogenic oocytes, and oogonia.

Ecologically, the evaluation of the limnological variables showed that the two annual periods of flood and dry phases of the hydrological cycle of the upper

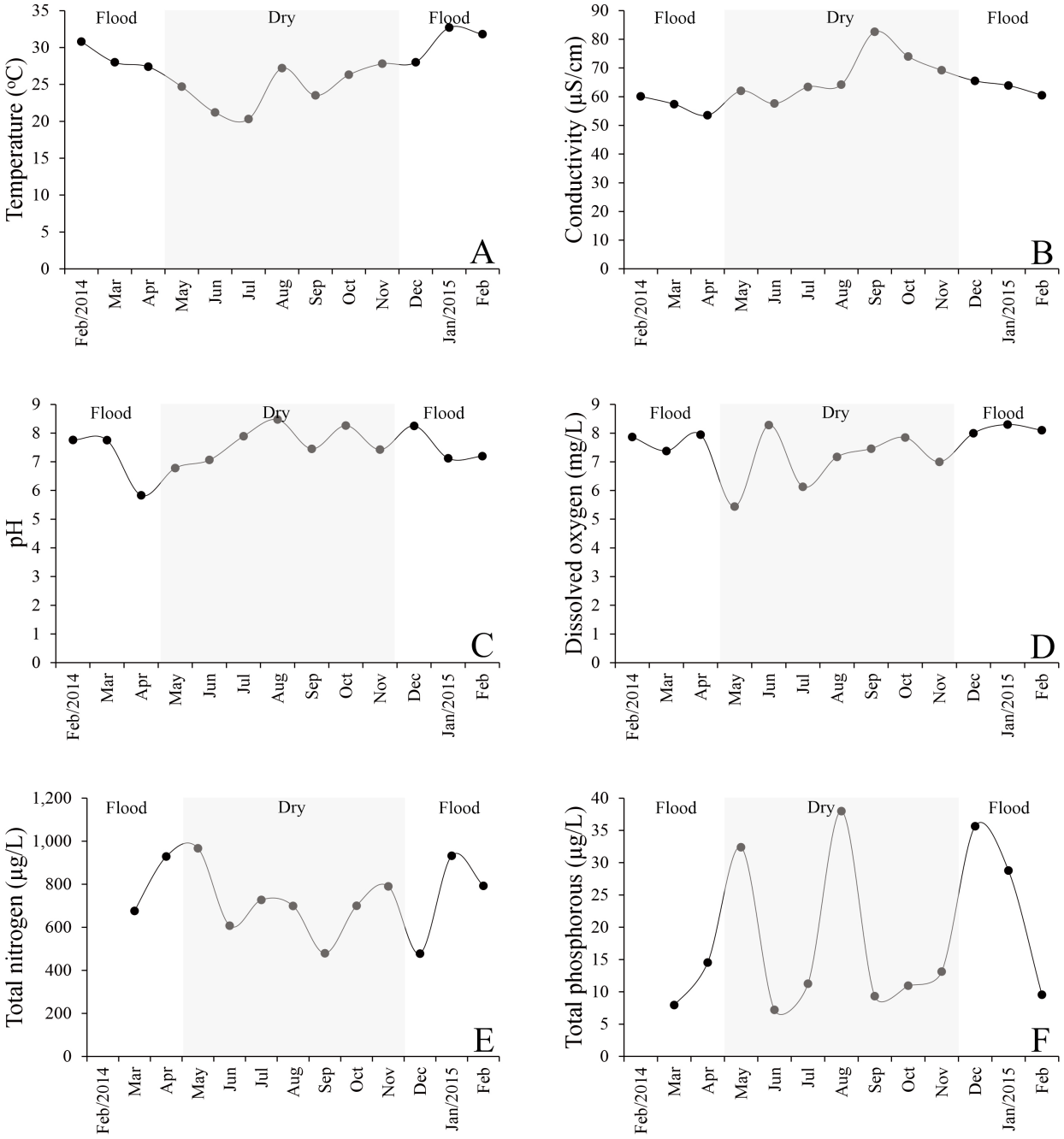

Fig. 8. Values of the monitored limnological variables (A) temperature, (B) conductivity, (C) pH, (D) dissolved oxygen, (E) total nitrogen and (F) total phosphorous.

Paraná River floodplain grouped the samples. During the dry period, relatively colder than the flood period, there is a fall in the number of adults, as well as a drop in their active spawning and sperm releasing capable phases. Most of the active spawning and sperm releasing capable phases thus seem to occur during the flood period. Temperature, total nitrogen and phosphorous were the most important variables during the flood period according to the PCA. These variables may increase food availability since nutrients are the main primary productivity resources. Increases in temperature and food availability can favor the increase of adults in active spawning and sperm releasing capable phases in the Rhine River (Germany), and the Waal and Lek Rivers (The Netherlands) (Rajagopal et al. 2000). The reproductive success of *C. fluminea*, associated with temperature elevation, also occurred in Lake Tahoe (California-Nevada border) (Denton et al. 2012). Here, there is also synchrony between the reproduction and flood periods, when there is an increase in connectivity between floodplain environments (Thomaz et al. 2007), which can facilitate the dispersion of the species between environments (Ernandes-Silva et al. 2016).

From an invasion point of view, the synchrony between the flood period and the active spawning and sperm releasing capable phases could cause an increase in pressure from invasive propagules of the species, thus elevating the probability of colonization success in new environments. In fact, other bivalves, such as the Asian species *L. fortunei*, possess similar reproductive dynamics, as most of the larvae in the early stage of development were recorded in the flood phase at the study site (Ernandes-Silva et al. 2016). Therefore, the results demonstrate that it is possible that the reproductive success of *C. fluminea* is related to food availability and the synchrony between the

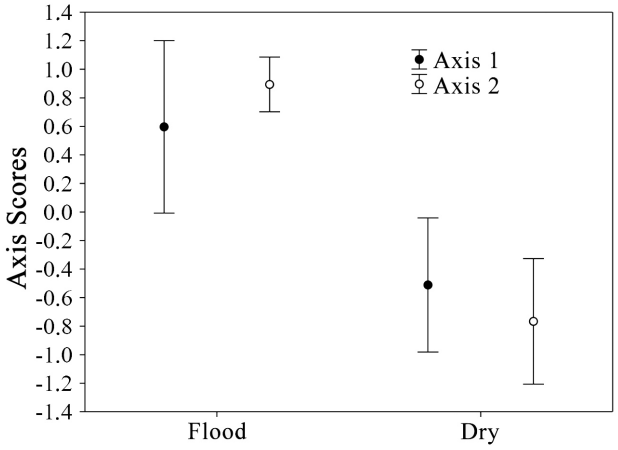

Fig. 9. Mean and standard error of the scores of the PCA axes, grouping the collections of the bivalve *C. fluminea* into flood period and dry period. Flood = December, January, February, March and April; Dry = May, June, July, August, September, October, November.

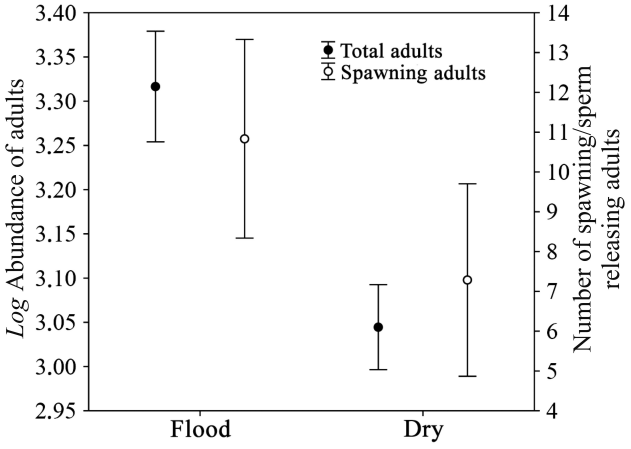

Fig. 10. Mean and standard error of the log of the abundance of the bivalve *C. fluminea* (adults), considering the flood and dry periods, as well as the mean and standard error of the number of adults in the active spawning/sperm releasing phase. Flood = December, January, February, March and April; Dry = May, June, July, August, September, October, November.

regional environmental factors and the reproductive characteristics of the species, in addition to the existence of synchronic hermaphrodites, which could facilitate and elevate the pressure from propagules in the study area even more.

CONCLUSIONS

Any control measure or management activity involving the species should be used during the dry period, when the number of reproducing adults is small. The change from the developing phase to the active spawning and sperm releasing capable phases can be avoided, thus reducing propagule pressure and the potential spread of *C. fluminea*.

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Ethics approval consent to participate: Not applicable.

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