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Reproductive pattern of the aggressive invader *Limnoperna fortunei* (Bivalvia, Mytilidae) in South America

Matías L. Giglio[a], María C. Dreher Mansur[b], Cristina Damborenea[c], Pablo E. Penchaszadeh[d] and Gustavo Darrigran[c]

[a]Instituto de Investigaciones Bioquímicas de La Plata, INIBIOLP, CONICET-CCT La Plata, Universidad Nacional de La Plata (UNLP), La Plata, Argentina; [b]Universidade Federal do Rio Grande do Sul, Centro de Ecologia, Grupo de Pesquisa em Ecotoxicologia e Biondicação, Porto Alegre, Brazil; [c]CONICET; División Zoología Invertebrados, Museo de La Plata, Facultad de Ciencias Naturales y Museo (UNLP), La Plata, Argentina; [d]Laboratorio de Ecosistemas Costeros-Malacología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", CONICET, Ciudad Autónoma de Buenos Aires, Argentina

ABSTRACT

Limnoperna fortunei is an aggressive freshwater invader species recorded for the first time in 1991 at Balneario Bagliardi, Río de la Plata Estuary, Argentina. Since then it has spread rapidly within South America. We tested the hypothesis that invasive species need a time span for the development of a reproductive pattern once settled in a new environment. We analyzed the reproductive cycle of a population of *L. fortunei* from Guaiba Lake (29°55′S; Rio Grande do Sul, Brazil) and compared it with previous studies at Balneario Bagliardi (34°52′S). We studied 834 specimens from Guaiba, following routine histological techniques. At any sampled period, the highest proportion of specimens were mature and gonads showed no resting periods. Female gonad maturation periods and oocyte growth were recorded throughout the year. Five periods of spawning of different intensity were differentiated. The reproductive pattern of the Guaiba population showed more spawning events and a remarkable recovery capacity of the female gonad compared to the Balneario Bagliardi population. The reproductive patterns of *L. fortunei* from Guaiba two years after invasion shows similarities with the reproductive pattern described previously from Balneario Bagliardi after two and three years of invasion.

Introduction

During the invasion process, invasive species must overcome a series of successive stages such as introduction, establishment, and dispersion beyond their natural range (Lockwood et al. 2007). Species that overcome these stages may become an important threat to the environment and economy of a region. Introduced species are present in geographic regions in which they did not evolve and where they are challenged by a set of new selective pressures and environmental tensions for which they are not adapted. Therefore, introduced species should have certain important features that may aid them to adapt rapidly, become established and proliferate, thus becoming successful invaders in the new environment (Prentis et al. 2008). The features that enable an invasive species to succeed in a new environment and become an invasive species include short lifespan (two to three years), rapid growth, rapid sexual maturity, high fecundity, and great genetic variability (Morton 1997).

Among the freshwater mollusks introduced in South America, *Limnoperna fortunei* (Dunker, 1857) (Bivalvia Mytilidae) – or golden mussel – is the most aggressive (Darrigran & Damborenea 2011), either in natural environments or man-made structures (e.g. drinking water-plants, hydroelectric power plants). This species is native to Southeast China and Korea (Iwasaki & Uryu 1998). It also invaded Hong Kong in 1968 (Morton 1973), and Japan (Kimura 1994) and Taiwan during the 90s (Ricciardi 1998). *Limnoperna fortunei* invaded South America in 1991 through the Río de la Plata Estuary, first settling at Balneario Bagliardi, 34°52′25′′S 57°48′35′′W, where it was recorded for the first time in the American continent (Pastorino et al. 1993). Twenty years later, the golden mussel has spread along the main rivers of one of the most important basins of the Neotropical Region, the Plata Basin (including the Río de la Plata Estuary and the Uruguay, Paraná and Paraguay Rivers) and other minor basins. In 1998, this species invaded a new basin in South America,

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CONTACT Gustavo Darrigran ✉ invasion@fcnym.unlp.edu.ar

Lake Guaiba (South Brazil) that is independent of the Plata Basin. In the same year it reached Corumbá in the Paraguay River in Brazil. Both records were the first for Brazil in independent basins (Mansur et al. 1999; Santos et al. 2005; Darrigran & Dreher Mansur 2009).

Since 1991, *L. fortunei* has spread upstream in rivers of the Plata Basin at a rate of 240 km/year (Darrigran 2010). Initial densities were low, about four to five specimens/m^2, but reached maximum densities of 150,000 specimens/m^2 after four years of invasion (Darrigran 2002). However, stabilization values are in a range of 40,000 specimens/m^2, depending on the inhabited environment (Darrigran & Damborenea 2005). Equivalent densities were analyzed by Santos et al. (2008) in the Guaiba Basin. The populations of *L. fortunei* became adapted to the environmental differences shown by their new geographic range in South America. The reproductive capability of this species and certain features of its life cycle are directly related to its invasive potential, rapid dispersion, and high densities (Callil et al. 2012).

The golden mussel can adapt to a wide range of environmental conditions (e.g. Santos et al. 2005; Karatayev et al. 2007; Pestana et al. 2008; Boltovskoy et al. 2009; De Oliveira et al. 2010a, 2010b, 2011; Cataldo et al. 2012; Darrigran et al. 2011, 2012). The main factors limiting the distribution of this mussel are temperature (Darrigran et al. 1999, 2003; Cataldo & Boltovskoy 2000; Cataldo et al. 2005; Santos et al. 2005; De Oliveira et al. 2010a), dissolved oxygen, salinity, flow and turbulence, ph, Ca^{++} concentration (Barbosa & Melo 2009; De Oliveira et al. 2010b; Darrigran et al. 2011, 2012; Xu et al. 2013), composition of the plankton (Sylvester et al. 2005), and abundance of predators (sylvester et al. 2007; Lopes et al. 2009).

In species of Mytilidae, temperature is considered a determining factor in gonad maturation and spawning (Lubet 1983). Likewise, in temperate climate areas, mollusks show a typically seasonal reproductive cycle with a sexual resting period immediately after spawning. In tropical climate areas in general there are no well-defined seasonal periods of gonad development and resting. This results in the continuous production of gametes and numerous partial spawning events during the year (Sastry 1979; Olive 1995).

Knowledge of the life cycle of *L. fortunei* is a fundamental tool for planning and implementation of control strategies (Iwasaki & Uryu 1998) and – eventually – dispersion deceleration programs in natural environments. Morton (1982) first studied the gonadal cycle of *L. fortunei* in invasive Hong Kong populations 15 years after its introduction. Choi and Shin (1985) and Iwasaki and Uryu (1998) studied populations in Korea and Japan.

The first study of this kind in South America was undertaken in the founding population at Balneario Bagliardi, Río de la Plata Estuary, Argentina. This study began two years after the first record of this species in the area (Darrigran et al. 1999). A subsequent study (Darrigran et al. 2003) on the same population undertaken 10 years after allowed recognition of a reproductive pattern similar to that described by Morton (1982) for the Hong Kong population. Earliest data on the larval stages of the life cycle of *L. fortunei* were published by Choi and Shin (1985), on the basis of populations from Korea. Santos et al. (2005) studied the larval stages of golden mussels from Guaiba Basin, the locality where the species was first recorded in Brazil.

According to Prentis et al. (2008), many invasive species show a rapid adaptation to new environments, pointing out that it can occur in 20 generations or less. Characteristics of the reproductive cycle of an invasive population are directly related to the time since the start of invasion. Invasive species need a time span for the establishment of a reproductive pattern regarding the new environment, as reported by Darrigran et al. (2003) for a population of *L. fortunei* in a temperate climate. To test this hypothesis, we studied the gonad cycle of a population of the golden mussel two years after initial invasion at a south latitude of 29°55′ (Lake Guaiba), and compared these results with those obtained in a population during a similar invasion period at 34°52′ south latitude (Balneario Bagliardi, Darrigran et al. 1999).

Materials and methods

Specimens of *L. fortunei* were collected at Camping das Pombas, near the Itapuã State Park, on the shores of Lake Guaiba, Viamão District, Río Grande do Sul State, Brazil (between 29°55′ and 30°24′ S; and between 51°01′ and 51°20′ W), attached to rhizomes of *Schoenoplectus californicus*. Samples were taken monthly from December 2000 to December 2001, recording air and water temperature as well as depth (Table 1). The mean air temperature at the sample site is 28 °C in warm months and 17 °C in cold months, while the mean water temperature is 25 °C in warm months and 18 °C in cold months. Rains are evenly distributed during the year with an annual average of about 1300 mm.

Maximum shell length was measured in 836 specimens of *L. fortunei*. These were subsequently fixed in Bouin's solution for 24 h, washed three times in 96% ethanol for two hours, and dehydrated in butanol (three washings of 24 h each). Specimens were embeded in Histoplast®. Sections (7 μm thick) were made with a manual microtome. Sections were stained with Mayer's hematoxilyn and eosin. Diameters of oocytes showing conspicuous nucleoli were measured using an ocular micrometer of a light microscope. Those loose in the acinus lumen and those attached to the acinus wall were considered. Up to 30 oocytes per

Table 1. Summary of the sampling conditions and sample analysis.

Month	Water temperature (°C)	Air temperature (°C)	Depth (cm)	Total N	Size range (mm)	Undifferentiated n	Males n	Females n
Dec-00	26	27	70	30	11–28	2	13	15
Jan-01	28	30	70	29	5–25	1	15	13
Feb-01	26.5	24.75	96.5	64	8–30	5	37	22
Mar-01	26.75	25.5	74	74	9–31	8	33	33
Apr-01	18.5	19	100	50	10–30	0	27	23
May-01	18.75	19.25	131	90	11–30	10	31	49
Jun-01	18.5	17	104	69	7–30	7	27	35
Jul-01	14.5	12.5	150	66	8–25	7	23	36
Aug-01	18.5	19.5	81	88	7–30	7	45	36
Sep-01	18.5	17.25	88	71	7–31	6	29	36
Oct-01	22.25	24	94	63	8–29	5	31	27
Nov-01	25	23.5	88.5	73	10–35	4	33	36
Dec-01	25	23	92	69	9–31	1	32	36
TOTAL				836	5–35	63	376	397
%						7.54	44.98	47.49

female specimen were measured. Oocytes less than 30 μm in diameter and attached by a narrow stalk to the epithelial wall of the acini were considered inmature. Oocytes bigger than 60 μm were detached, rounded, and filled the acini lumen. These were considered mature and ready to be spawned (Boltovskoy et al. 2015). Spawning intensity was estimated as a proportion between the frequency of oocytes greater than 60 μm released during spawning and the time elapsed. Males with the central lumen of the tubules filled with spermatozoa that were arranged radially with their heads projecting toward the periphery were considered males with mature sperm.

The percentage of pallial follicle occupation was estimated for each specimen (200x magnification) in three different sectors of the mantle (upper, middle, and lower): this was done by visual estimation of the fields of view. Following the criteria of Darrigran et al. (2003), specimens were considered as immature, mature, and spawned, and lysis periods were determined.

The characterization of the classical gonadal cycle during the year for females and males of *L. fortunei* (e.g. developing, maturing, mature, spawning) was obtained from the studies of Darrigran et al. (1999-Fig. 2, 2003-Fig. 4), Callil et al. (2012-Fig. 3) and Boltovskoy et al. (2015-Fig. 1). The results of this work were compared to those published by Darrigran et al. (1999, 2003) based on the founding population in South America. The methods used to evaluate the reproductive pattern of the Lake Guaiba population are the same as those used in both mentioned previous studies. The information available on gonadal cycle of this species performed in South America was also used for comparative purposes (Table 2).

Results

During the period of study, water temperature varied between 14.5 °C (July 2001) and 28 °C (January 2001) (Table 1).

Limnoperna fortunei is a dioecious species, although some hermaphrodite specimens were recorded (less than 0.5%). Forty-five percent of specimens studied were males and 47.50% females. Sex could not be determined in the remaining 7.54% (Table 1). Minimum size for sexual differentiation and maturation recorded for female specimens were 7 and 8 mm, respectively, whereas in males it was 5 mm for both parameters. Among the non-differentiated specimens, 80% were ≤12 mm length, reaching 5 to 10% in most months of the year. The exceptions were recorded in April 2001 (no undifferentiated specimens recorded), and in January and December 2001 (the proportion was smaller).

No rest periods were recorded during the study period, and acini maturation and oocyte growth were evident throughout the year. Sixty percent of oocytes was larger than 60 μm most of the year, except in June and August when oocytes less than 60 μm were more abundant (Figure 1).

Five periods of spawning – showing different intensities – were recorded during this study (Figures 2 and 5(b)). The longest spawning event was observed between January and March 2001. Two more intense spawning events occurred in May–June and July–August. In April–May and October–November, two events of less intensity were recorded. Likewise, oocyte growth was observed for most of the year (Figure 2, black bars).

Most males were mature throughout the year (Figure 3), with a percentage larger or equal to 73% of specimens with differentiated sperm cells. Mantle coverage by male follicles was always greater than 48% (Figure 4). The lowest percentage of mantle occupation was observed in May–June.

Variations observed in mantle occupied by female follicles coincided with spawning periods determined from variation in oocyte sizes. The minimum gonad coverage was 27%, but it was ≥50% during follicle growth periods (Figure 4).

Table 2. Summary of surveys on gonadal cycle *Limnoperna fortunei* in South America.

Locality	First record of the species in the area	Water body	Latitude Longitude	Period covered	Minimum-maximum temperature (°C)	Spawning periods during the sample period	Resting period	Source
Balneario Bagliardi, Río de la Plata (Argentina)	Sept. 1991 (Darrigran & Pastorino 1995)	Estuary (freshwater area)	34°52'25''S 57°48'35''W	Jul 1992–Oct 1993	8–24 (air mean temperature)	Sep–Oct 1992 Jan–Feb 1993 May–August 1993	Absent	Darrigran et al. (1999)
Balneario Bagliardi, Río de la Plata (Argentina)	Sept. 1991 (Darrigran & Pastorino 1995)	Estuary (freshwater area)	34°52'25''S 57°48'35''W	Dec 1993–Nov 1994	8–22 (air mean temperature)	Feb–March April–June Oct–Nov	Absent	Darrigran et al. (1999)
Balneario Bagliardi, Río de la Plata (Argentina)	Sept. 1991 (Darrigran & Pastorino 1995)	Estuary (freshwater area)	34°52'25''S 57°48'35''W	Aug 1998–Mar 2000	11–31 (water temperature)	Sep–Oct 1998 Feb–June 1999 Jul–Aug 1999 Oct–Dec1999	Absent	Darrigran et al. (2003)
Punta Piedras, Río de la Plata (Argentina)	End of 1993 (Darrigran & Ezcurra de Drago 2000)	Estuary (brackish area)	35°26'59''S 57°07'33''W	Oct 1998–Apr 2000	5–32	Oct–Nov April–June	Absent	Taraborelli et al. (2002a), after Damborenea & Penchaszadeh (2006)
Yacyretá reservoir, Paraná River (Argentina)	1998 (Darrigran & Ezcurra de Drago 2000)	Reservoir	27°28'57''S 56°42'42''W	Nov 1998–Oct 1999	18–30	Dec–Jan March–July	Absent	Taraborelli et al. (2002b), after Damborenea & Penchaszadeh (2006)
Jacuí River, Rio Grande do Sul (Brazil)	Nov 1998 (Darrigran & Dreher Mansur 2009)	River	30°00'S–51°15'W	Jan 2002–Dec 2002	No data	Continuous	Absent	Marcelo & Callil (2006), after Callil et al. (2012)
Paraguai River, Corumbá (Brazil)	1998 (Darrigran & Mansur 2006)	River	18°59'S–57°39'W	Aug 2004–March 2006	No data	Oct–Feb	June–August	Uliana & Callil (2006), after Callil et al. (2012)
Itaipu reservoir, Foz do Iguaçu, PR, (Brazil)	2001 (Zanella & Marenda 2002)	Reservoir	25°26'10''S 54°33'00''W	Jan-Dec 2005	21–30	Feb–June Aug–Oct Other minor registered between the two periods	No data	Silva (2006)
Paraná River, Parana (Brazil)	April 2001 (Darrigran & Mansur 2006)	River	22°43'S–53°10'W	July 2007–Apr 2008	No data	Nov Jan–May	Present	Gomes & Callil (2010)
Baía River, Paraná (Brazil)	No data	River	22°43'21''S 53°18'42''W	Oct 2006–Apr 2008	No data	Feb–May 2007	Absent	Pinillos & Callil (2010)
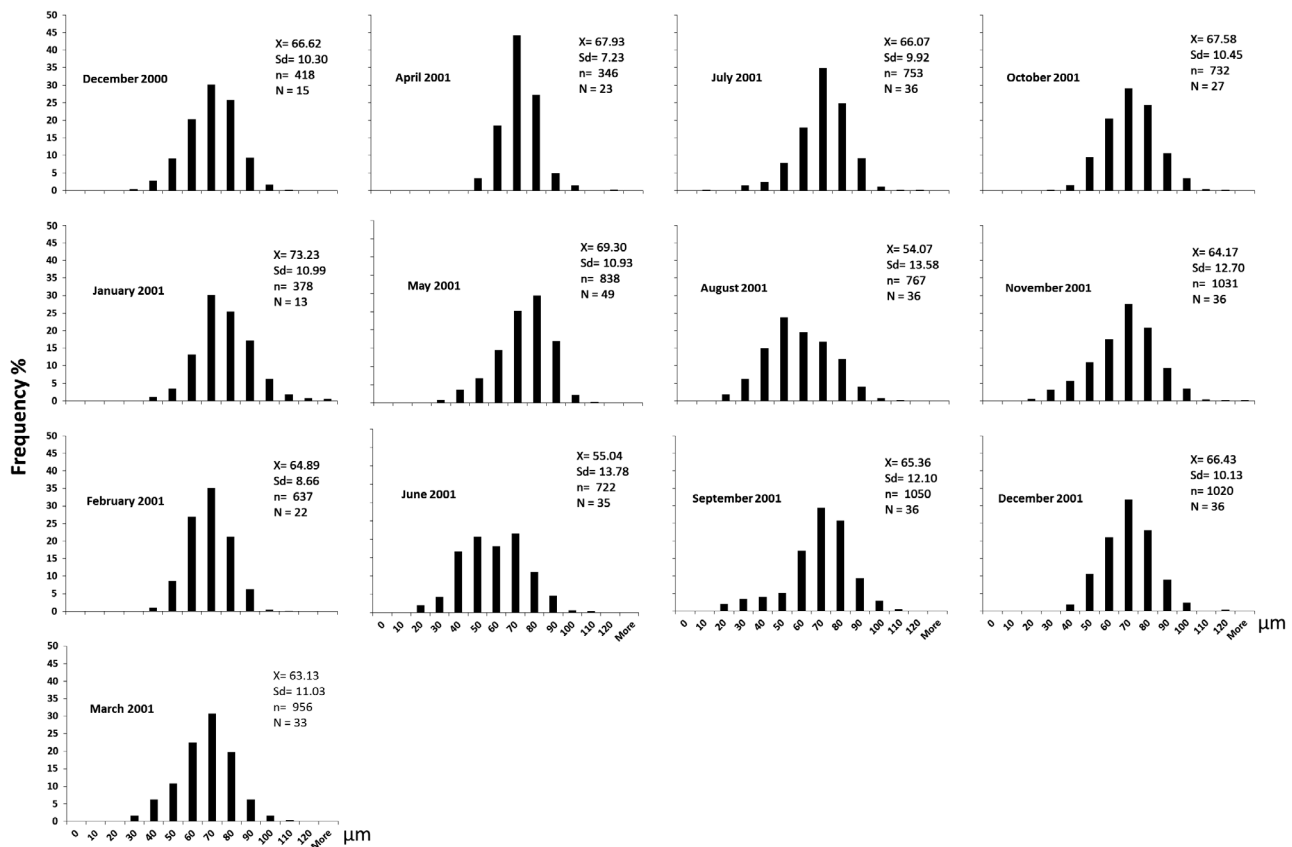
Figure 1. Frequency (percentage) of oocyte sizes (µm) in each sampling event. X, mean oocyte size; Sd, standard deviation; n, number of oocytes; N, number of females.

Lysis and oocyte resorption periods were observed between the months of June and September (Figure 2).

Discussion

During the invasion by a species, adaptation to the new environment is evident at all stages of the process (Lockwood et al. 2007). Nevertheless, some evolutionary processes are more important at some of these stages. For instance, the bottleneck effect, the effect of a significant reduction of a population, and genetic variation that could rapidly change its characteristics, is particularly frequent during the stages of introduction and establishment, where reproduction is one of the main barriers (Prentis et al. 2008). This applies to the group of first individuals that colonize a new region (founding population) and also to isolated populations derived from 'jump' dispersion. This dispersion mechanism is observed in South American populations of *L. fortunei* (Boltovskoy et al. 2006). The reproductive capacity and the tolerance of larvae and adults to extreme environmental conditions of this invasive species is one of the key factors that allow it trespassing barriers within the invaded habitats (Morton 1997).

The sexual processes in bivalves are related to several environmental factors, temperature being one of the most considered in reproductive studies (Lubet 1983; Olive 1995). Seasonality of reproduction varies according to climate. In temperate regions, bivalves usually have two spawning periods in the year, following the maximum production of phytoplankton in spring and autumn. Tropical species show less well-defined spawning periods, and they spawn for most of the year (Sastry 1979). Histological studies of the reproductive biology of *L. fortunei* indicate that spawning is related to temperature (Morton 1982; Choi & Shin 1985; Iwasaki & Uryu 1998) and Darrigran et al. (1999, 2003) observed that spawning is triggered by the rise and fall of temperature.

The first studies of the reproductive biology of *L. fortunei* in the Americas were undertaken by Darrigran et al. (1998, 1999) in the founding population at Balneario Bagliardi, on the Río de la Plata estuary two and three years after invasion (Figure 5(a) and (c)). Two years after invasion, the population showed continuous gonad activity, with maturation periods related to temperature changes (Darrigran et al. 1999); one major spawning event occurred from May to August 1993 and other partial events were observed from September to October 1992, and December 1992 to
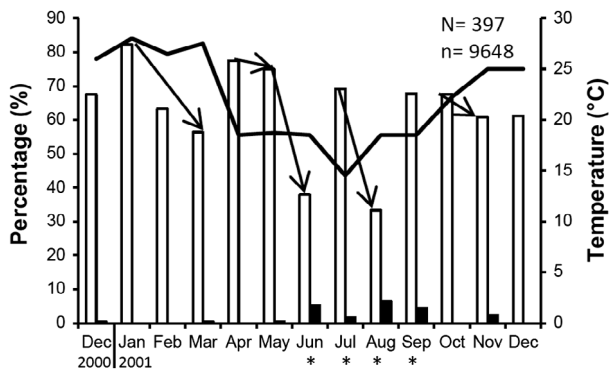
Figure 2. Relationship between water temperature (dark line) and monthly variation of the percentage of oocytes larger than or equal to 60 μm (white bars), and less than or equal to 30 μm (black bars). The arrows indicate moments of spawning and asterisks (*) show months with lysis. N, total number of females considered; n, total number of oocytes considered.
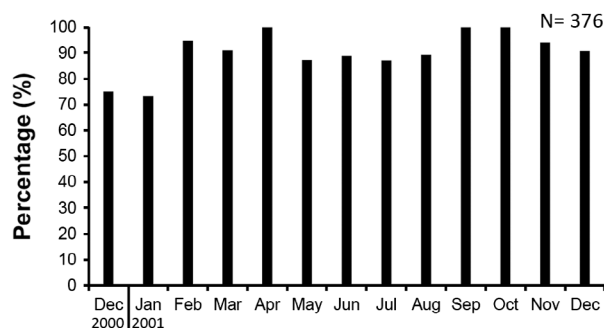
Figure 3. Monthly percentage of males with mature sperm. N, total number of males considered.
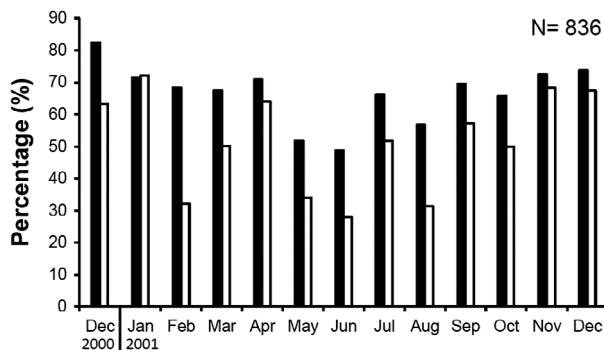
Figure 4. Temporal variation of mantle area occupied by gonad acini. Males: black bars; females: white bars; N, total number of specimens.

January 1993 (Figure 5(a)). Three years after invasion, the population showed three spawning events, February to March 1994, April to June 1994, and October to November 1994 (Figure 5(c)).

Ten years after the first record of the species in the area, Darrigran et al. (2003) undertook a study of the same

population (Figure 5(d)). By that time, one long spawning in summer and fall (February–June), and two minor spawnings in winter (July–August) and spring (October–December) were observed. In the present study, rest periods were observed; the specimens undergo a period of recuperation in which the mantle occupation by the gonads decreases, there is a higher predominance of oocytes smaller than 20 μm and the percentage of males with sperm cells is reduced.

In the Bagliardi population, after two years of invasion (Figure 5(a)) and after three years of invasion (Figure 5(c)) (Darrigran et al. 1999), oocytes larger than 60 μm and smaller than 20 μm appear during the entire sample period. Two years after the invasion, the proportion of both types of oocytes is similar when compared with the proportion observed after three and ten years of invasion (Figure 5(a),(c), and (d)). Ten years after the invasion (Darrigran et al. 2003), the proportion of oocytes larger than 60 μm varied between 75 and 10%. The proportion of oocytes smaller than 20 μm is always less than 40% (Figure 5(d)).

The present study was based, firstly, on the study of the reproductive pattern of the founding population of *L. fortunei* in the Guaiba Basin two years after the introduction into Brazil (Figure 5(b)), and on the reproductive pattern of this species two, three, and ten years after the introduction in the Río de la Plata estuary. The Guaiba population is the result of a jump dispersion from Buenos Aires Harbor, in the Río de la Plata estuary, by commercial trade (Darrigran & Dreher Mansur 2009). Therefore, the propagules that reached the Guaiba Basin in 1998, were from a population with at least 7 years of settlement in the South Hemisphere.

In the Gauiba population, two facts deserve attention: there are more spawning events than in the population at Balneario Bagliardi in all studies, and the proportion of oocytes larger than 60 μm is much higher than that of oocytes smaller than 30 μm. The Guaiba population shows similarities with the reproductive pattern observed two and three years after invasion in Balneario Bagliardi (Figure 5(a) and (c)) in the similar number of spawning events observed and the absence of gonadal resting periods (Darrigran et al. 1999). This could be owing to the initial stage of the invasion process in the new environment.

The observed density after two years of settlement in Guaiba Basin was approximately, 140,000 specimens/m² (Santos et al. 2005). At Balneario Bagliardi – during the same post-invasion period – it was of approximately 80,000 specimens/m², just reaching 150,000 specimens/m² after the fourth year of invasion (Darrigran 2002). The Guaiba population reached high densities sooner than in Bagliardi, probably due to the species had a previous period of adaptation in the Plata basin.
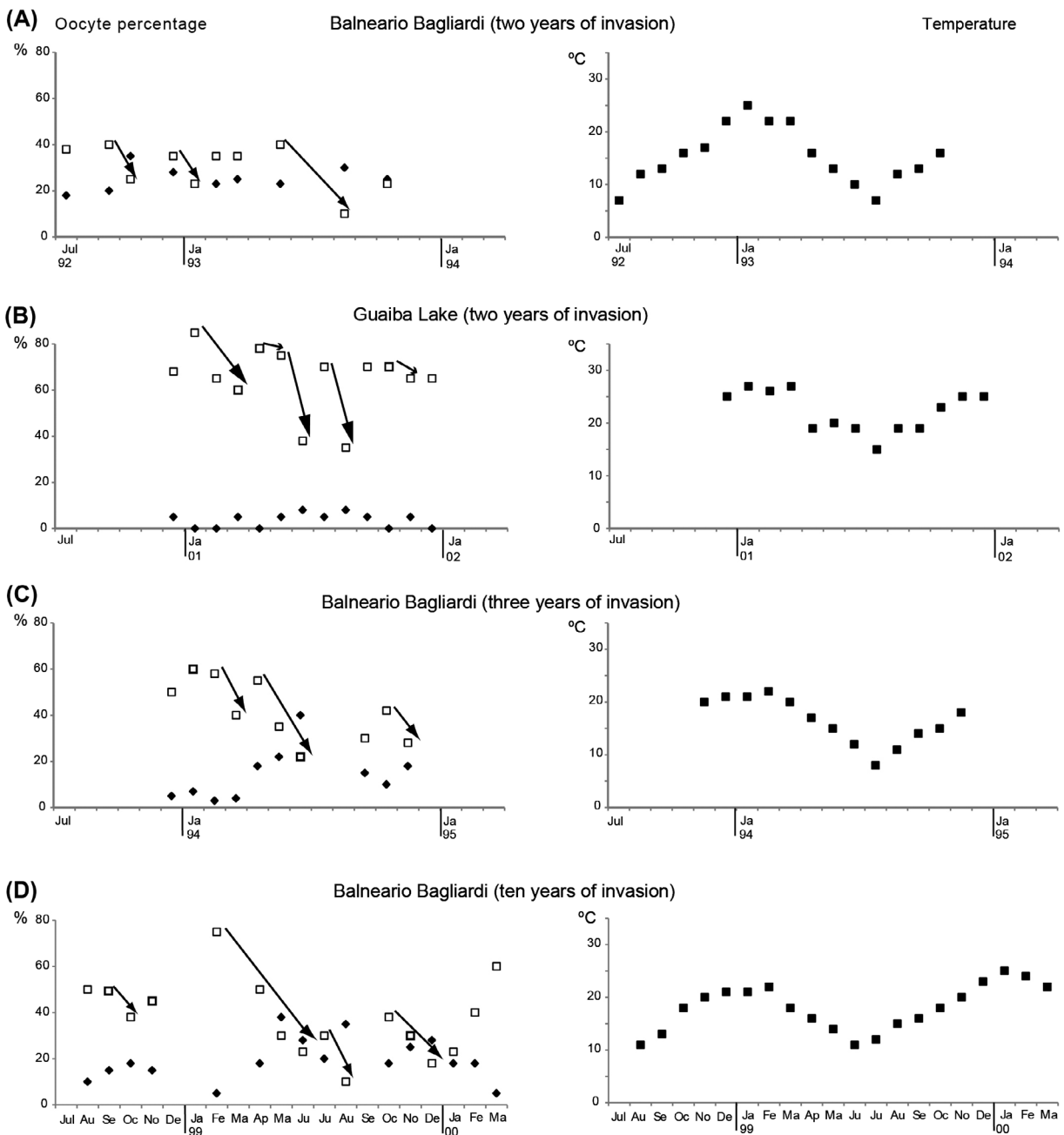
Figure 5. Temporal variation of oocyte size and temperature in different populations of *L. fortunei*. White squares, oocytes >60 μm; black diamonds, oocytes <20 μm (in a, c and d) and <30 μm in b. Arrows indicate moments of spawning. (a) Population in Balneario Bagliardi after 2 years of invasion (samples between July 1992 and October 1993) (Darrigran et al. 1999); (b) population of Lake Guaiba two years of invasion (present study, samples between December 2000 and December 2001); (c) population in Balneario Bagliardi after 3 years of invasion (samples between December 1993 and November 1994) (Darrigran et al. 1999); (d) population Balneario Bagliardi after 10 years of the invasion (samples between August 1998 and March 2000) (Darrigran et al. 2003).

In addition to evidence coming from gonad histology, the reproductive periods can be inferred based on seasonal changes in larval density (Boltovskoy et al. 2009). However, the larval information is not exactly comparable to that derived from gonad histology. This yields information on the reproductive situation of the population in a determined space and time. At the same time, the capability of larvae to swim or drift and live for several weeks until attaining metamorphosis renders evidence based on the presence of larvae in the water column only as a 'photograph' of an instant in a particular place. Thus, this piece of evidence is less precise and can respond to events occurred in far geographic areas. This kind of study was undertaken at several localities in which *L. fortunei* thrives (Boltovskoy et al. 2015). Santos et al. (2005) studied the larval density in the Guaiba basin and after four years

of the first settlement of the species there. These authors observed a relationship between temperature and the presence of larvae. These were registered in all months sampled, with a peak of larval density during October–December. However, Pestana et al. (2008) observed two peaks of seasonal larval density at two localities (Paraná and Iguaçu rivers, Brazil), with widely varying values.

Studies on the gonadal cycle of *L. fortunei* in South America are scarce (Table 2), and do not show temporal continuity. As the bibliographic information shows (Table 2), a definite pattern in the reproductive cycle cannot be established owing to multiple factors interacting, e.g. time since the settlement in the area, origin of the populations, temperature, and other environmental factors.

The observations on the gonadal cycle of *L. fortunei* in South America agree with Iwasaki and Uryu (1998) who supported the existence of geographic variations in the reproductive cycle, growth and longevity of the golden mussel. They pointed out the need of further studies across different areas invaded by this bivalve. These geographic variations would be influenced by the capability of the golden mussel of adapting to a wide range of environmental conditions (e.g. Santos et al. 2005; Karatayev et al. 2007; Pestana et al. 2008; Boltovskoy et al. 2009; De Oliveira et al. 2010a, 2010b, 2011; Cataldo et al. 2012; Darrigran et al. 2011, 2012).

Finally, Morton (1982) studied a population of Hong Kong six years after its introduction in the area and found two evacuation events in the year, one in spring–summer and the other in autumn–winter. Studies by Liu et al. (*fide* Morton 1982) on a population of *L. fortunei* from China, by Choi and Shin (1985) for a population from Korea, and by Iwasaki and Uryu (1998) for one from Japan, recorded only one spawning event coincident with decreasing temperature in the first case and with summer maximum temperature in all other cases.

In synthesis, the reproductive patterns of *L. fortunei* from Lake Guaiba (29° south latitude) two years after invasion and from the Balneario Bagliardi (34° south latitude) after three years of invasion are similar. Both patterns share a high proportion of oocytes bigger than 60 μm, particularly evident during the sample period of Gauiba population (Figure 5(b)) and in the first period in Balneario Bagliardi after three years of invasion (Figure 5(c)); and no resting periods are observed. These similarities are probably because the Balneario Bagliardi population was introduced to South America from the Northern Hemisphere, whereas the Gauiba population was introduced from Río de la Plata Estuary, so the Guaiba population probably needed a short time to adapt its reproductive pattern to the new environmental conditions. Furthermore, the golden mussel population at 34° south latitude, where mean winter water temperature is about 15 °C shows

seasonal variations in the proportion of oocytes bigger than 60 μm and smaller than 20 μm, and the proportions of both types of oocytes are reversed after the spawning periods. By contrast the population at 29° south latitude where the mean winter water temperatures is about 18 °C, the proportion of oocytes bigger than 60 μm is almost constant.

The golden mussel, as other invasive species, is opportunistic and therefore establishing a general reproductive pattern is difficult (McMahon 2002). However, reproductive studies such as this are a basis for predictive trends of the reproductive behavior of populations of *L. fortunei* in the different regions occupied by this species. This is crucial to establish protocols for controlling its range of invasion.

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