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REPRODUCTIVE STABILIZATION OF *LIMNOPERNA FORTUNEI* (BIVALVIA MYTILIDAE) AFTER TEN YEARS OF INVASION IN THE AMERICAS

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ABSTRACT *Limnoperna fortunei* (Dunker, 1857) or golden mussel invaded South America through the Río de la Plata estuary in 1991. Ten years later, the golden mussel lives in the main rivers of the Plata Basin. The gonadal cycle and the population settlement in a temperate climate are discussed in this article. This basic knowledge is needed to assist industries that may suffer the effects of macrofouling and also increment the ability to predict potential invasions of other countries. The study of population density and reproductive cycle was performed in Río de la Plata estuary, Argentina. The temporal variation of population density from data of settlement and age structure collected between 1991 and 2001 is presented. The reproductive cycle between August 1998 and March 2000 was analyzed. Through the analysis of oocyte percentages four gonad spawning events were observed. The spawning events appear regulated by temperature changes. After the initial increase in population density following the invasion, there was a decrease. The population appeared stabilized at one third of the initial peak.

KEY WORDS: invasion, *Limnoperna fortunei*, freshwater, bivalve, reproductive cycle, Neotropical Region

INTRODUCTION

Limnoperna fortunei (Dunker, 1857), or golden mussel, is a freshwater invasive bivalve, from the southeast of Asia. It invaded South America in 1991, through the Río de la Plata estuary. This represents the first record of *L. fortunei* for the American continent. Ten years later, the golden mussel lives in the main rivers of one of the most important Basins of the Neotropical Region (Bonetto 1994), the Plata Basin (the Rio de la Plata, and the Uruguay, Paraná, and Paraguay rivers). Since 1999, this species invaded the Guaiba Basin in the south of Brazil (Mansur et al. 1999). The golden mussel spreads 240 km/year, upstream along the Plata Basin, (Darrigran & Ezcurra de Drago, 2000). AU1

The golden mussel attaches to every available hard substrate. This lifestyle (epifaunal) is atypical in local freshwater bivalves. The attachment capability and the great adaptability and reproductive capacity of these mussels make this species very effective invaders (Darrigran 2000). The mussels impact on the natural environment (displacement of native species—Darrigran et al. 1998b, Darrigran et al. 2000—or change of native fish diet—Penchaszadeh et al. 2000) as well as on human activities (macro fouling in fresh water Darrigran 2000, Darrigran & Ezcurra de Drago 2000). AU2

Detailed information about the life cycle of this harmful invasive species provides a basis for the development and application of control strategies. The impact caused by this species in human activities (plugging of water intake for industrial cooling, power generation, and potable water) resembles what happened in the north hemisphere with the zebra mussel *Dreissena polymorpha* (Pallas 1771). The study of reproductive cycle, age structure and temporal density variation, is essential to generate sustainable techniques for golden mussel prevention and control.

Details of the reproductive cycle, and the population settlement in temperate climate are discussed in this article. This type of knowledge is not only essential to assist biologists and ecologists in the industries which may suffer from this new economic-environmental problem in the Neotropical Region, but it is also necessary for predicting potential invasions of other countries in the north hemisphere such as USA (Ricciardi 1998) and southern Europe.

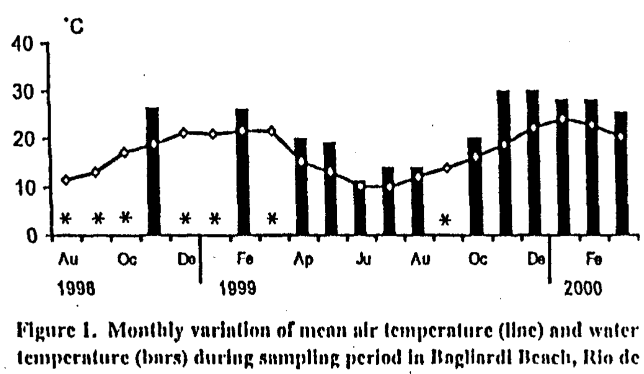

Figure 1. Monthly variation of mean air temperature (line) and water temperature (bars) during sampling period in Bagliardi Beach, Rio de la Plata. *Without data.

TABLE 1.

Date and number of specimens histologically processed per sample.

Date	N	Size range	Males	Females
23/08/98	27	0,6–2,5	17	10
25/09/98	30	0,6–2,6	23	17
30/10/98	29	0,4–2,5	18	11
27/11/98	17	0,5–2,6	14	13
23/02/99	14	0,5 2,9	13	11
19/04/99	20	0,8–2,2	7	13
15/05/99	24	0,7–2,2	14	10
30/06/99	29	0,7–1,9	13	16
26/07/99	25	0,7–2,1	10	15
27/08/99	28	0,5–1,8	19	9
21/10/99	32	0,6–2,1	22	10
27/11/99	34	0,5–1,7	23	11
16/12/99	31	0,5–1,7	14	17
26/01/00	27	0,6–2,1	16	11
22/02/00	35	0,7–2,1	25	10
12/03/00	29	0,6–2,2	18	11
Total	431		266	195

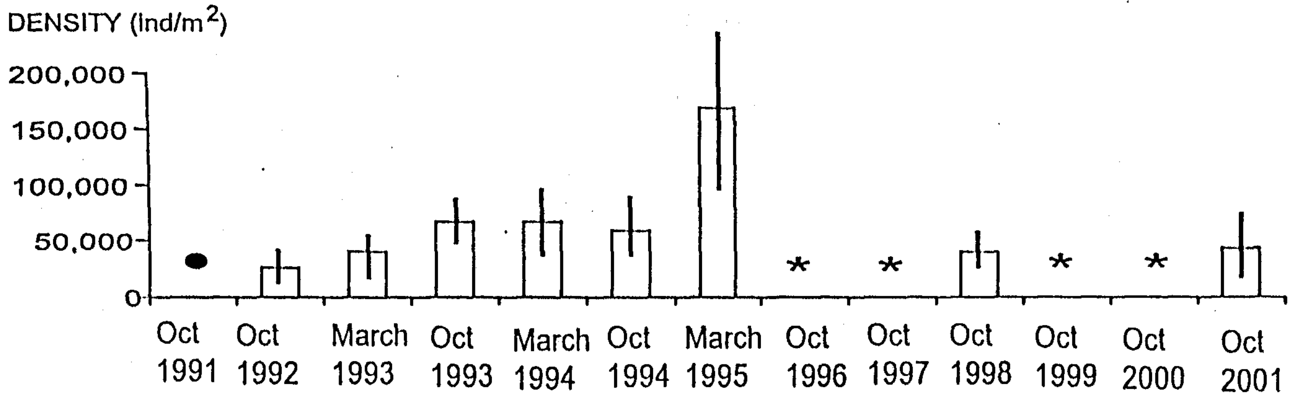

Figure 2. Temporal variation of mean density (bars) and standard deviation (lines) of *Limnoperna fortunei* in Bagliardi Beach, Río de la Plata. 4–5 ind/m². *Without data.

MATERIAL AND METHODS

To study the golden mussel population density and reproductive cycle, samples were collected along the rocky banks of Bagliardi Beach, (34°55'S; 57°49'W), Río de la Plata estuary, Argentina, South America, is where the mussel was found for the first time in 1991 (Pastorino et al. 1993). The water temperature in this locality has a temperate regimen ranging from approximately 11°C to 31°C (Fig. 1). The physicochemical features of the Río de la Plata may be found in Darrigran (1999). The density data were obtained partly from Darrigran, et al. (1998b) and through sampling carried out *ad-hoc* (October 1998 and October 2001) in Balgliardi Beach. Samples of mussels were collected for density analysis from the fringes with macrobenthos from a rectangular

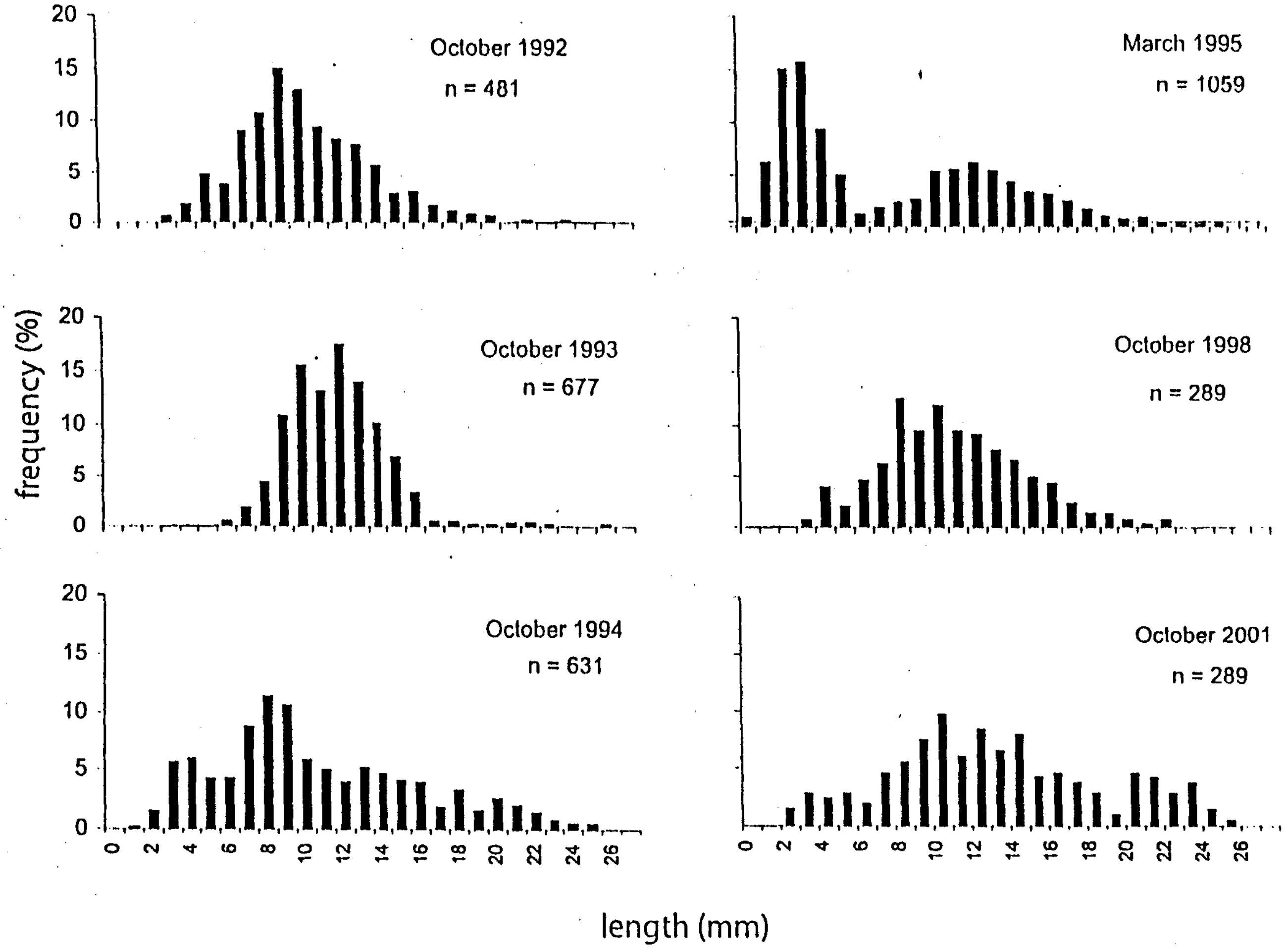

Figure 3. Size frequency (%) of *Limnoperna fortunei* in Bagliardi Beach, Río de la Plata.

area, variable in size, according to Darrigran et al. (1998b). For the age structure analysis, the maximum shell length was measured and the length frequency distribution was made at 1 mm class intervals (see Fig. 3 later).

The dates of sampling for reproductive cycle analysis, per-
[T1] formed at low tides, may be observed in Table 1. The maximum shell length of the 431 collected individuals was taken. The material was fixed in Bouin solution and the histologic processing was performed according to Darrigran et al. (1999).

Approximately 25 oocytes with conspicuous nucleolus, both free in the follicular lumen and attached to the follicle wall, for each gonad were measured. The percentage of males with spermatozoids and the percentage of follicular occupation on the mantle were calculated for each sample. The latter was calculated using magnification (×200) in three different sections of the mantle, (upper, middle, and lower) through the visual estimation of field. The lysis periods were determined by microscopical analysis.

RESULTS

The temporal variation of population density found on the rocky litoral zone of Bagliardi Beach between 1991 and 2001 is given in Figure 2. From 1991 to 1995, the density increase was [F2]
remarkable (from four to five individuals/m^2 to over 100,000 ind/m^2). The population density then decreases and stabilizes at approximately 40,000 ind/m^2. In Figure 3 it is shown that since [F3]
1994 the population has had an age structure where most size class intervals are represented.

The female and male follicles grow in the mantle and in the visceral mass. During this study 0.25% hermaphrodite specimen, with female, male, and mixed follicles were recorded.

The gonad growth is characterized by growing follicles. In this stage the follicles are small and there exists an abundant connective tissue between them. A more developed stage shows young oocytes on the wall, many stalked oocytes (Fig. 4A) and abundant [F4]
spermatogoniums in the males (Fig. 4D). In a later stage the follicles are bigger and the follicular lumen contains abundant oocytes half-grown and also almost fully grown oocytes (60–80 μ). When fully mature, the female and male follicles reach the maximum size. Male follicles are packed with spermatozoa (Fig. 4E) and females' follicles with fully-grown (80–100 μ) oocytes.

When the gonads are spent and partially spent, the follicles contain large spaces. Partially spent gonads retain genital products.

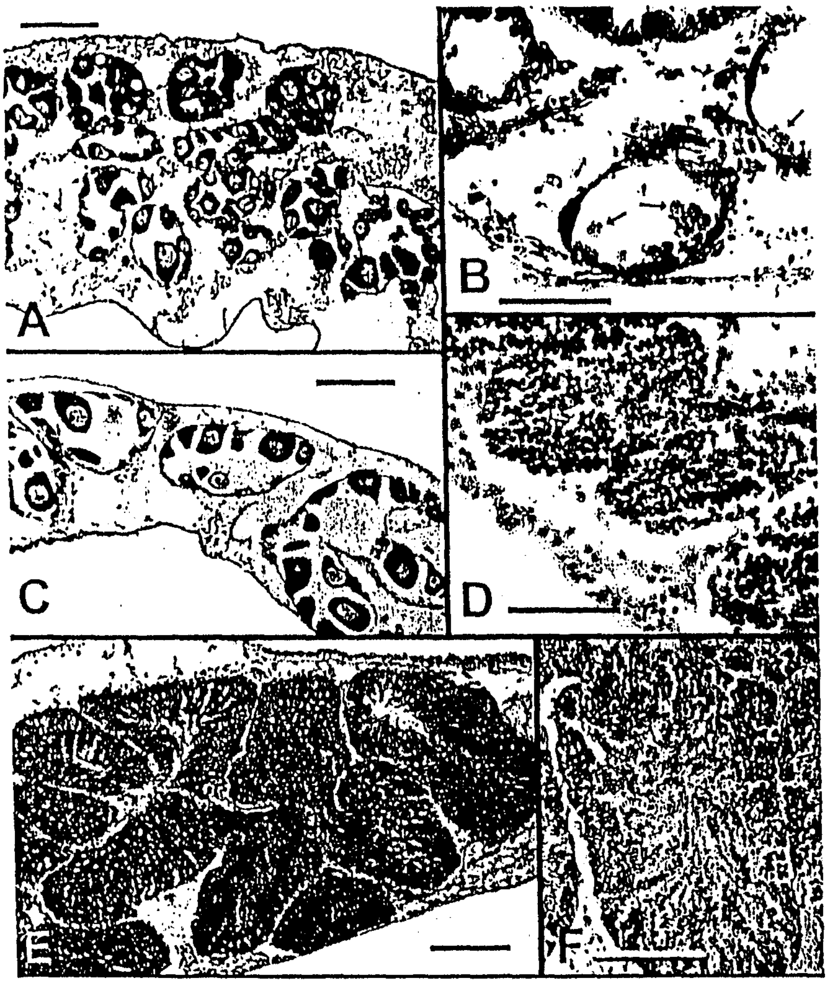

Figure 4. Female and male follicles in different development stages. (A) Female follicle partially grown with young oocytes on the wall and many stalked oocytes, scale bar = 100 μ. (B) Spawned female follicles with abundant yellow bodies (arrows), scale bar = 50 μ. (C) Female follicles partly spawned, scale bar = 100 μ. (D) Developing male follicles, scale bar = 50 μ. E. Fully developed male follicles, scale bar = 100 μ. F. Male follicles partly spawned, scale bar = 50 μ.

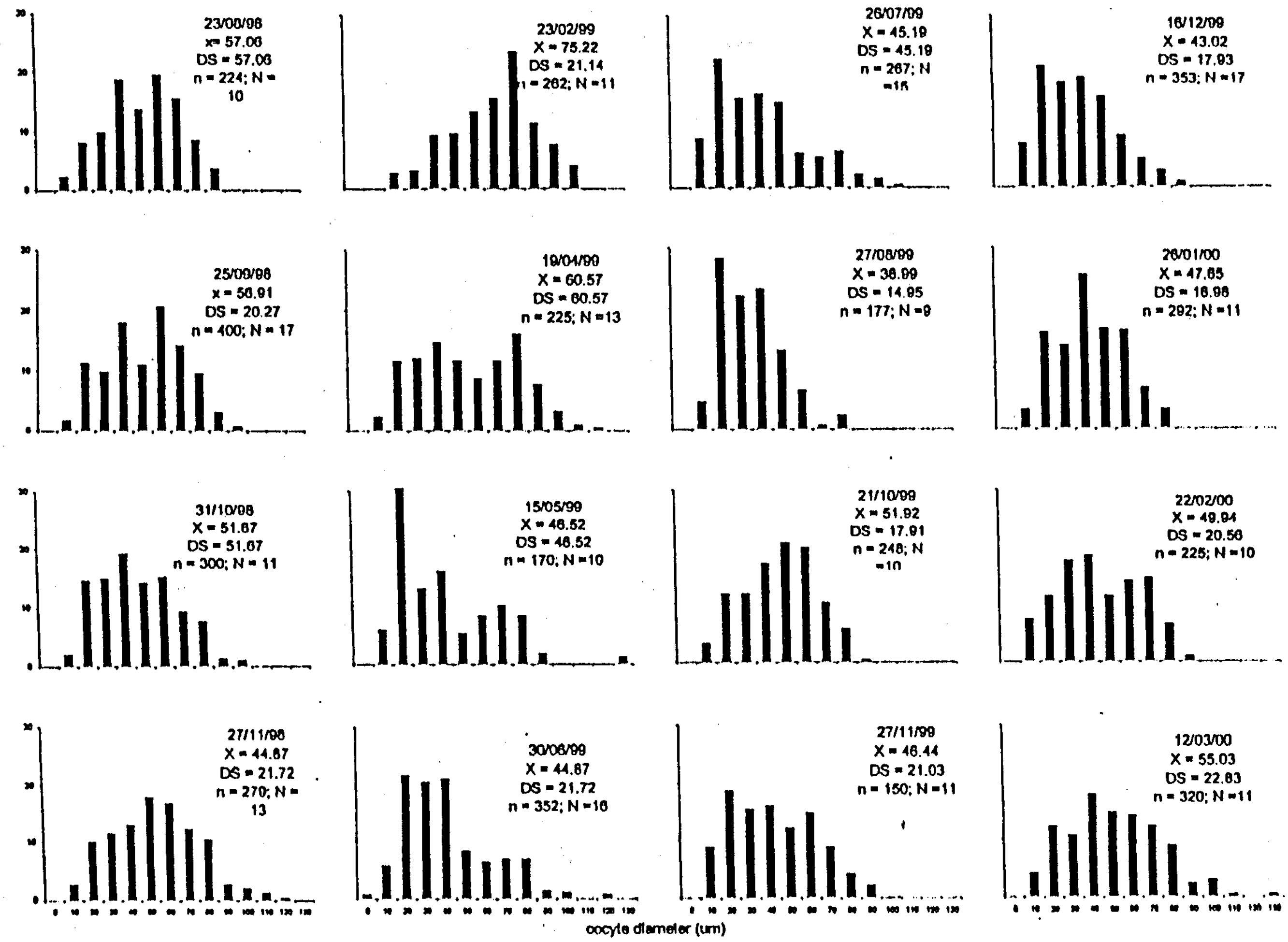

Figure 5. Frequency (in percentage) of oocyte sizes (μ) in different samplings. x, mean oocyte size; s, standard deviation; n, number of oocytes; N, number of females.

In males spermatozoids and spermatocites are observed (Fig. 4F). Partly developed oocytes, oogonies, and young oocytes are retained on the female follicle walls (Fig. 4C). Oocitary lysis phenomena (Fig. 4B), with yellow bodies are evident for a short time after spawning is completed.

The body length at which the follicle, either female or male, development is completed, varies seasonally. The smallest shell length at which follicles differentiate is 5.5 mm, for both males and females (Fig. 5). During this study (August 1998 to March 2000), oocyte growth was always recorded. From May 1999 until August 1999, the oocytes smaller than 20 μ were 30% of the total oocytes examined.

The change in frequency of oocytes <20 μ and >60 μ indicates two reproductive peaks each year. The first peak occurs at the end

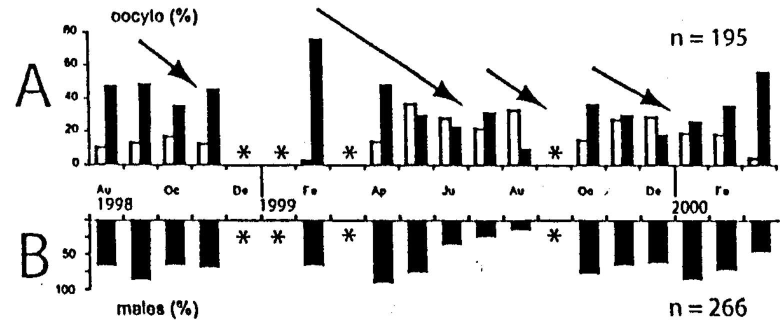

Figure 6. Temporal variation. (A) Percentage of oocytes bigger than 60 μ (full bars) and smaller 20 μ (empty bars). The arrows indicate moments of gamete liberation. (B) Percentage of males with sperm. *Without data. n, number of male individuals.

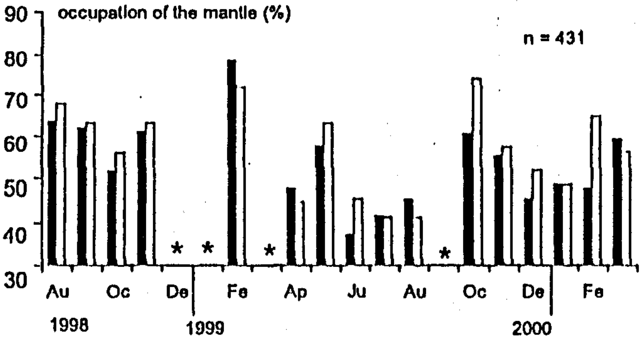

Figure 7. Temporal variation of mantle occupation. Female follicles (full bars) and males (empty bars). n, total number of considered males and females. *Without data.

of winter or beginning of spring (August to September of 1998, October to November of 1999) and the second peak is recorded during the summer (February of 1999, March of 2000). During these periods in the female follicles the oocytes bigger than 60 μ dominate, while smaller oocytes are scarce (<20%). During the period of study gonad recuperation were observed (October 1998 and May to June of 1999). Through the analysis of oocyte percentages present in the gonad, four spawning events were observed (Fig. 6 A):

(1) From September to October 1998.

(2) February 1999 to May 1999. It is the most important for its duration and magnitude.

(3) in July to August 1999, the least important.

(4) between October and December 1999,

Figure 6B shows the percentage of males with sperm throughout the period considered. The pattern agrees in general with that observed for females.

The spawning pattern mentioned is similar to the follicular occupation of the mantle (Fig. 7). The percentage of occupation decreases during the spawning periods and stays low during the recuperation period (June, July, and August 1999).

Lysis phenomena were observed in several samples (Fig. 8). They are more important during May to August 1999, and coincide with recuperating follicles or in partial evacuation.

DISCUSSION

The bivalve sexual processes are generally related to ambient temperature (Lubet 1983). The results presented here for a population of L. *fortunei*, as well as those observed in the first study (Darrigran et al. 1999), those performed for a Hong Kong population (Morton, 1982), and the analysis of larvae density in the Río de la Plata (Cataldo & Boltovskoy 2000) show the strong relationship between ambient water temperature and the reproductive cycle. The spawning events are regulated by changes in temperature, and increases and decreases of temperature rule the gametogenesis in this species.

During the initial study (Darrigran et al. 1999), we found that oocytes were always present in the mussels even during the resting period. Periods of scarce proliferation were recorded from December 1993 to May 1994. This study was performed a short time after the first record of *L. fortunei* in the Americas (Pastorino et al. 1993). The analysis of reproductive biology at that time differentiated numerous spawning events (five were recorded), many of them of low magnitude. Between September 1992 and January 1993 (the first period), two spawnings of reduced intensity were recorded and between February 1993 and November 1994 (the second period) three spawnings were recorded (two of these of higher magnitude). During the first period, the oocytes bigger than 60 μm and those smaller than 20 μm are always present and their proportion is similar (about 30%), the spawnings are low in magnitude but the proportion of oocytes bigger than 60 μm is always larger than 20%. During the second period, the spawnings are more intense and result in a diminution of the bigger oocytes proportion (by 10%). In contrast to the first period, the oocytes bigger than 60 μm reach more than 60% (Darrigran et al. 1999).

The population analyzed here shows a predictable reproductive pattern. Only two major spawnings are observed throughout the year, one when summer temperatures are recorded and the other with spring temperatures. A small winter spawning is also observed. This pattern, after 10 years of settlement in America, is similar to that described by Morton (1982) for the population of Hong Kong where the spawnings take place between May to June and November to December. The pattern shown during the first study [only after a year of settlement in the location considered (Darrigran et al. 1999)] could be due to the recent invasion.

Morton (1982) describes short spawnings for a month in spring and a month in autumn. In this study in South America, mainly in autumn, the evacuation continues from April 1999 to May 1999. The presence of larvae in the Río de la Plata, between August and April (Cataldo & Boltovskoy 2000), also indicates that the spawning periods are longer than those described by Morton (1982).

Similar to what was found in the first study of the golden mussel reproductive cycle (Darrigran et al. 1998a) 0.25% of the population was hermaphrodite.

According to the variation of population density, this species, at the beginning of the invasion in temperate climate, presents a noticeable increase of density. Then, it decreases its density to a third part and stabilizes. At the same time, it presents an age structure with most class intervals represented. These facts would indicate a stable settlement of the population to the environment.

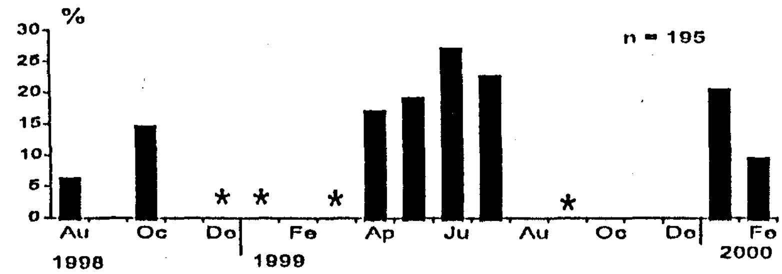

Figure 8. Percentage of females with follicles where lysis phenomenon occur. *Without data. n, number of considered females.

The initial increase recorded in a temperate climate could also be observed in a subtropical climate. Despite the preliminary studies of this species invasion in the south of Brazil, subtropical climate (Mansur et al. 1999), the golden mussel presents an increase in its population density similar to that observed in this study. Two years after its first record (Mansur et al. 2001a, Mansur et al. 2001b), the maximum density is 62,100 ind/m^2.

The golden mussel, like other invasive species, is opportunistic. This fact makes it difficult to relate the reproductive pattern with environmental variables and to determine the different facts that might be modified in the reproductive cycle. *L. fortunei*, for its great adaptability and reproductive capacity, increases its distribution permanently by occupying environments of particular features.

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