*Journal of Shellfish Research*, Vol. 18, No. 2, 361–365, 1999.

# THE REPRODUCTIVE CYCLE OF *LIMNOPERNA FORTUNEI* (DUNKER, 1857) (MYTILIDAE) FROM A NEOTROPICAL TEMPERATE LOCALITY

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***ABSTRACT*** *Limnoperna fortunei* is a dioecious, freshwater mytilid native to Asia. The reproductive biology of this invasive species is analyzed for the first time in its neotropical habitat. About 35 specimens were processed monthly using standard histological techniques (sections ~ 6 μm thick). The size of mussels at sexual maturity varied seasonally. From June to October, sex differentiated at 5–6 mm shell length (SL), and from March to May at 7–10 mm SL. Adult males contained mature sperm throughout the year. Mature oocytes measured 70–100 μm. Three main spawning events occurred in May to July 1993, April to May 1994, and October 1994. Two partial spawnings were observed in September to October 1992 and December 1992 to January 1993. No spawnings were recorded from October 1993 to February 1994 and from June to October 1994. Oocyte proliferation was continuous and immature oocytes were recorded throughout the year. The population appears to have continuous reproduction with peaks of maturing activity related to temperature changes.

***KEY WORDS:*** reproductive biology, bivalve, invasive species, freshwater, South America

## INTRODUCTION

*Limnoperna fortunei* (Dunker 1857) is a freshwater species, native of rivers and creeks of China and southeastern Asia (Morton 1977). It was first recorded in the Americas in Bagliardi Beach (lat 34°55′S, long 57°49′W), in the argentinian littoral zone of the Río de la Plata, in September 1991 (Pastorino et al. 1993), at a density of 4 to 5 specimens/m$^2$. During 1993, maximal densities of about 80,000 specimens/m$^2$ were recorded in this same locality (Darrigran 1995). Its current density reaches 150,000 specimens/m$^2$ (Darrigran et al. 1998b), similar to the densities of populations of the rocky shore marine mytilid *Brachidontes rodriguezi* d' Orb. found in Mar del Plata (lat 38°00′S, long 57°33′W) (Penchaszadeh 1973). *L. fortunei* invaded the Hong Kong area in the late 1960s (Morton 1977), and Japan in the 1970s (Kimura 1994). In the Americas, this species is found currently in the rivers Río de la Plata, Paraná, and Paraguay. It causes an important impact in the human environment (the principal problems caused by larval invasion, settlement, and maturity of *L. fortunei*, into water distribution systems, are: reduction of pipe diameter, blockage of the pipeline, decreased water velocity caused by friction, accumulation of empty shells, contamination of water pipelines by mass mortality and filters occlusion) and natural environment (affecting the taxocenosis of autochthonous molluscs, favoring the settlement of other macroinvertebrate fauna not common in the environment; Darrigran et al. 1998b).

Up to now, the reproductive cycle of *L. fortunei* is only known in subtropical habitats of Hong Kong (Morton 1982). The Hong Kong population of *L. fortunei* is dioecious and semelparous, and no cases of hermaphroditism have been reported (Morton 1982, Morton 1991). In this study, a description of the reproductive cycle of a Río de la Plata population of *L. fortunei* is presented.

## METHODS

Collections of *Limnoperna fortunei* from Bagliardi Beach, Río de la Plata, Argentina, were made from the rocky coast, during low tides (Table 1). This locality (lat 34°55′S, long 57°49′W) is characterized by temperate environmental conditions. Monthly mean air temperatures range between 7.8 °C (July) and 24.1 °C (January) (Fig. 1), and water temperatures between 14.0 °C (May) and 24.0 °C (February) (Guerrero et al. 1997).

The shell length (SL) of 638 specimens was measured. Specimens were then fixed in Zenker's fluid with added formalin (10%

TABLE 1.

**Dates and number of specimens histologically processed per sample.**

| Dates | Total n | Size range (cm) | Undifferentiated n | male n | female n |
|---|---|---|---|---|---|
| 11/07/92 | 29 | 0.5–1.6 | 6 | 11 | 12 |
| 29/09/92 | 30 | 0.5–1.7 | 3 | 13 | 14 |
| 19/10/92 | 24 | 0.5–1.9 | 3 | 14 | 7 |
| 13/12/92 | 41 | 0.3–1.9 | 12 | 17 | 12 |
| 26/01/93 | 23 | 0.6–1.4 | 0 | 12 | 11 |
| 27/02/93 | 36 | 0.3–1.8 | 9 | 11 | 16 |
| 28/03/93 | 35 | 0.4–2.1 | 13 | 11 | 11 |
| 16/05/93 | 35 | 0.3–1.0 | 9 | 9 | 17 |
| 14/08/93 | 28 | 0.5–1.5 | 5 | 13 | 10 |
| 7/10/93 | 25 | 0.6–2.2 | 1 | 12 | 12 |
| 3/01/94 | 28 | 0.9–1.5 | 3 | 10 | 15 |
| 31/01/94 | 31 | 1.0–1.9 | 0 | 19 | 12 |
| 28/02/94 | 27 | 1.1–1.8 | 0 | 13 | 14 |
| 27/03/94 | 33 | 0.5–2.1 | 4 | 15 | 14 |
| 25/04/94 | 29 | 0.7–2.3 | 7 | 15 | 7 |
| 30/05/94 | 37 | 0.6–1.9 | 9 | 13 | 15 |
| 24/06/94 | 42 | 0.6–2.0 | 0 | 22 | 20 |
| 28/09/94 | 37 | 0.6–1.8 | 1 | 20 | 16 |
| 25/10/94 | 38 | 0.5–1.6 | 4 | 23 | 11 |
| 25/11/94 | 30 | 0.5–2.4 | 4 | 16 | 10 |
| TOTAL | 638 | 0.3–2.4 | 93 | 289 | 256 |
| | % | | 14.57 | 45.29 | 40.12 |

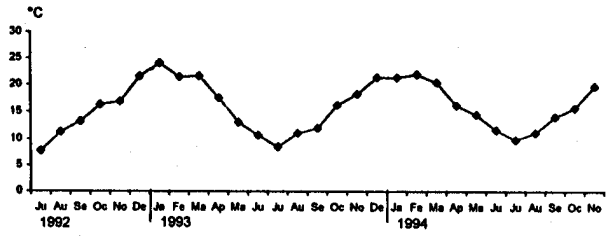


**Figure. 1. Temporal variation of air temperature.**

in mixture volume) for 4 h, washed in tap water for 24 h, dehydrated 3 times in ethanol 96% (each wash of 2 h), and washed 4 times in N-butanol (each wash of 24 h). Finally, specimens were embedded in paraffin. Sections of ~ 6 μm thick were stained with Mayer's hematoxylin and eosin.

Oocytes with conspicuous nucleoli were measured (10 to 25 oocytes from each gonad, 7 to 16 females from each sample), including those found free in the follicular lumen, as well as those smaller ones attached to the wall.

The percentage of males with mature spermatozoa was calculated for each month's sample.

The percentage of follicular occupation of the mantle for each mussel was calculated (200×) in three different sections of the mantle (upper, middle and lower sections) by visual estimation of the fields of view. The periods of lysis and oocyte reabsorption were determined by microscopic analysis.

## RESULTS

From a total of 638 specimens, 45% were males, 40% females, 15% undifferentiated. Figure 2 shows different morphological aspects that characterize the reproductive process of *L. fortunei*.

Female and male follicles differentiate at 5 mm (spring) to 9 mm (summer and fall).

The minimal size of sexual differentiation was found to be 5 mm SL, both for males and females, while gonadal maturity was attained at 6 mm SL, both in males and females. Size of sexual maturity varied through time.

Size-frequency histograms of oocytes showed (Fig. 3):

a) Five spawning events occurring: September to October 1992, December 1992 to January 1993, May to July 1993, April to June 1994, and October to November 1994. The first two events were less apparent than the last three.
b) No spawning events were recorded from October 1993 to February 1994, and from June to October 1994.
c) Oocyte proliferation was continuous throughout the sampling period. But from December 1993 to March 1994, small oocytes (<30 μm) were less numerous or were absent.
d) The total range of oocyte sizes was uniform in most of the samplings.

Figure 4a shows the temporal variation in the percentage of follicular occupation of the mantle for both sexes. There were periods of follicular repletion (June to August 1993; January to February 1993; December 1993 to January 1994), and periods of


**Figure. 2. Light micrographs of the mantle of adult females (Fig. 2a) and adult males (Fig. 2b) of *Limnoperna fortunei*. Fig. 2a: (1) Early development of the ovary with small isolated follicles. (2 and 3) Follicles invading the space previously occupied by connective tissue, a stage of oocyte proliferation and growth. (4 and 5) enlarged oocytes with new growing ones. (6 and 7) Ripe ovaries ready to spawn. (8) Spawned ovary with remnant oocytes and few new growing cells. Fig. 2b: (9) early development of testicular follicles. (10) growing follicles invading the space previously occupied by connective tissue. (11 and 12) mature males; Scale: (1–8) scale bar 225 μm; (9) scale bar 100 μm; (10, 11, 12) scale bar 150 μm.**

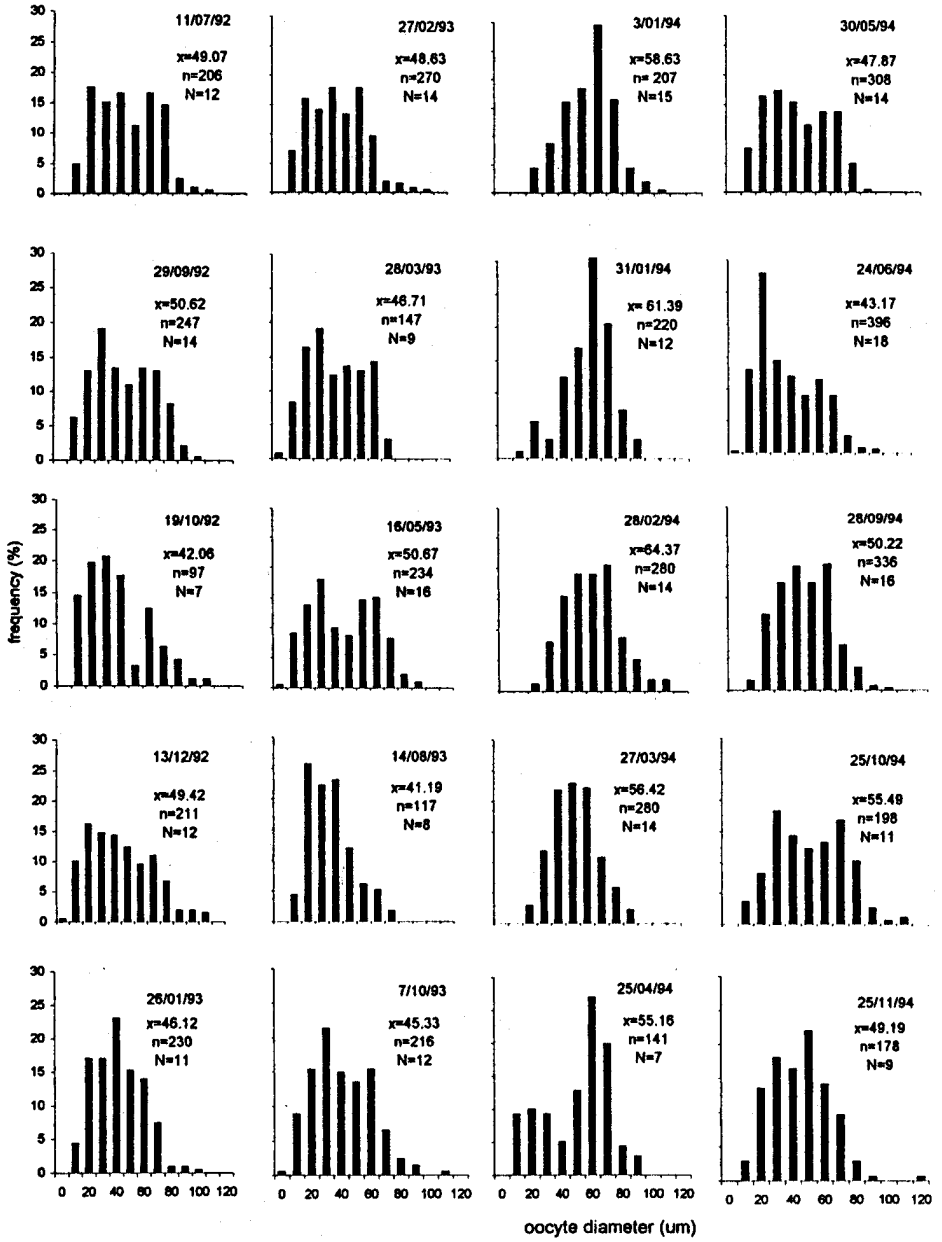


**Figure. 3. Frequency (%) of oocyte size (µm). x, mean oocyte size; n, number of oocytes; N, number of females.**

maximal gonadal follicle occupation of the mantle (January 1993; May 1993; October to December 1993; November 1994).

The analysis of the percentage of immature oocytes showed three times of maximal proliferation (Fig. 4b): July to November 1992 with a maximum in October; March to November 1993 with a maximum in August; and April to July 1994 with a maximum in June.

Likewise, two outstanding periods were found when examining the immature oocytes: a continuous and high proliferation (December 1992 to May 1993), and a scarce oocyte proliferation (December 1993 to March 1994). Figure 4c shows the percentage of mature oocytes, showing 2 main evacuation processes and 4 secondary evacuations with a quick recovery.

The main evacuations occurred in May to July 1993 and April to June 1994 and the secondary evacuations in September to October 1992, December 1992 to January 1993, October to November 1994, and February to May 1994. No spawnings were recorded between August and December 1993.

August 1993 was mainly a proliferation period. Between October 1993 and May 1994, there was clear oocyte growth, and energetic losses in maturation or recovery of small oocytes did not occur (Fig. 4b and 4c).

Figure 4d shows a long period of sperm accumulation in the follicles that lasted from August 1993 to January 1994. Two main periods of evacuation and 3 secondary periods may also be observed.

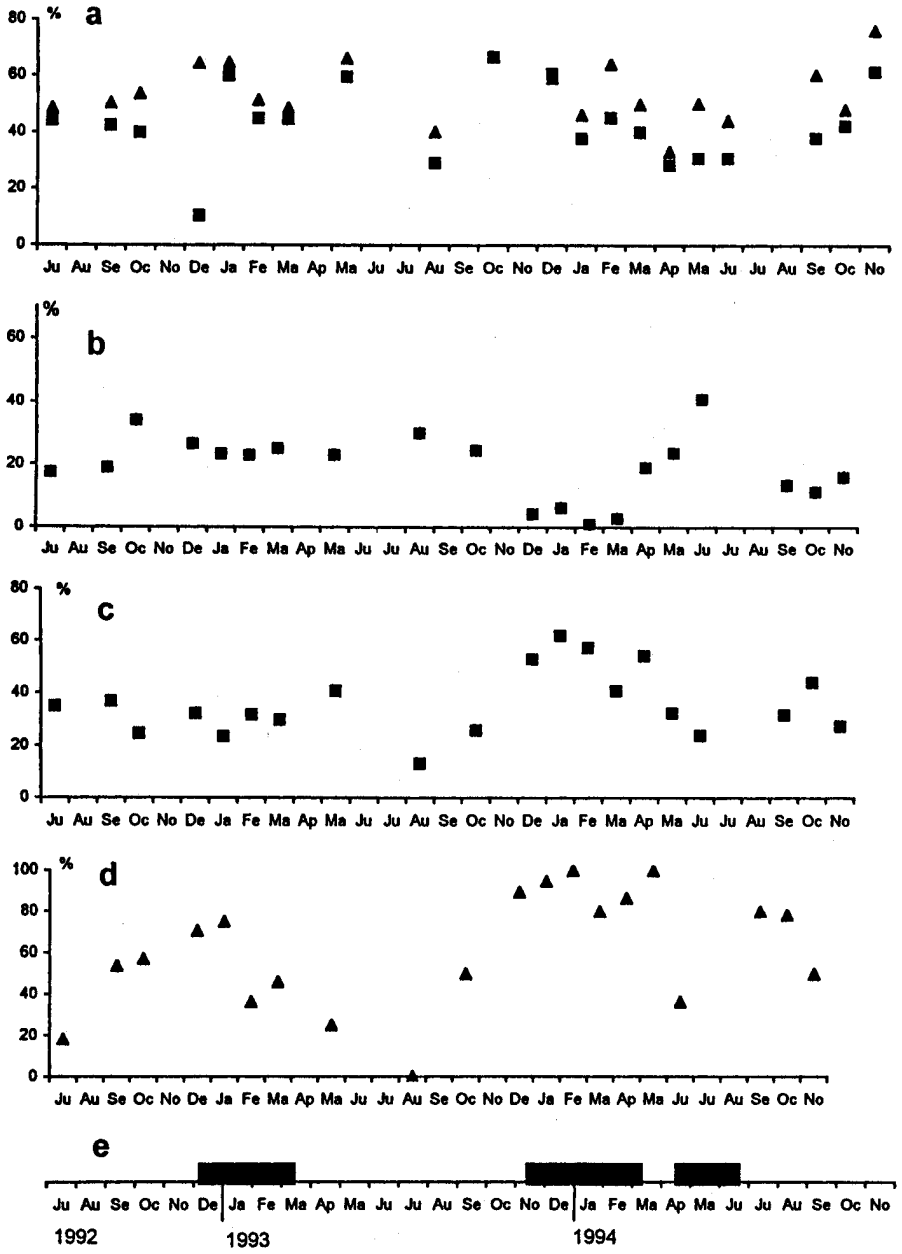


**Figure. 4. Temporal gonadal development. (a) percentage of occupation of the mantle in males (▲) and females (■). (b) percentage of oocytes smaller than 20 µm. (c) percentage of oocytes larger than 60 µm. (d) percentage of male follicles with sperm. (e) periods of oocyte lysis.**

Lysis phenomena were recorded from December 1992 to March 1993, from November 1993 to March 1994, and from April to June 1994 (Fig. 4e).

## DISCUSSION AND CONCLUSIONS

*Limnoperna fortunei* is a dioecious species. However hermaphrodite specimens occur frequently in other mytilid species (Lubet 1959), and unlike what Morton (1982) observed in Hong Kong, 0.55% of hermaphrodites (Darrigran et al. 1998a) were recorded.

Gametogenic cycles are generally ruled by external environmental factors that may trigger and synchronize the "timing" of the different stages (Lubet 1983). The synchronization of gonadal cycles of a population is probably also the result of some kind of exogenous regulation (Gallardo 1989).

The reproductive pattern of this population, examined during 1992 and early 1993, differs from the period recorded later. As *Limnoperna fortunei* is an invasive species introduced in 1991 in the Americas, and the study began in the middle of 1992, the observed reproductive pattern probably resulted from the lack of fitness and synchronization of the reproductive cycle of this population to the new environment.

Short evacuations of low intensity were recorded from September 1992 to January 1993 (3 events). These events were associated with a partially stable stage of the follicular occupation of the mantle. These short evacuations were preceded by a period of proliferation of oocytes smaller than 20 µm. The proliferation was continuous and, in males, the amount of spermatozoa first increased and then decreased in January to February.

After February 1993, two main spawning events could be recognized in the reproductive process, in agreement with Morton (1982). The first event was May to July 1993, and the second April to May 1994. During May 1993, there was a high occupation of the mantle; and the abundance of oocytes larger than 60 μm reached 40%. Sperm decreased sharply until no sperm was recorded in August 1993. From June to August 1993, oocytes smaller than 20 μm increased and those of 60 μm decreased. However, unlike what Morton (1982) recorded in Hong Kong, no clear periods of gonadal inactivity were recorded after either of the two evacuation processes.

After a long period of increasing oocyte size and scarce gonadal proliferation, two evacuation events were recorded: February to March 1994 and a larger one April to June 1994. During this latter event, there was an increase in oocytes smaller than 20 μm coincident with a decrease in the sperm percentage. The percentage of occupation of the mantle shows a low coincidence with the first evacuation event (February to March), and a slight recovery during the following event. Thus, gametogenesis was continuous in the study population and major and minor spawning events occurred during the study period.

Iwasaki and Uryu (1998) found that *L. fortunei* from Kyoto reproduce only once a year, from June to September. Like the observations of Morton (1982) in Hong Kong, the larger spawnings in the Río de la Plata develop during temperature changes, starting with both the maximums and the minimums.

The results of this study demonstrate a correlation between environmental temperatures and the reproductive cycle of *Limnoperna fortunei* in Bagliardi Beach. The processes of gametogenesis and spawning in bivalves are related to temperature changes (Lubet 1983). Temperature must be considered as the main factor responsible for the variations in the reproductive cycle (Kimura and Sekiguchi 1996). However, changes in water quality (pH, salinity, dissolved oxygen) may also affect this cycle. Consequently, multiple factors could trigger this biological process (Morton 1982).

The combination of early sexual maturity, high fecundity, semelparity, and wide environmental toleration permits this species to be transported and easily introduced into a new environment. Likewise, it rapidly colonizes the new environment, eventually becoming dominant (Morton 1989).

## ACKNOWLEDGMENTS

The authors wish to thank the Antorchas Foundation; the CONICET PIA N°6139 Project, the PEI N°0548/97, the Facultad de Ciencias Naturales y Musco (UNLP) and the Agencia Nacional de Promoción de Ciencia y tecnologia No. 01-03453 Project partially funded this research.

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