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REPRODUCTIVE CYCLE, MANAGEMENT AND CONSERVATION OF *PAXYODON SYRMATOPHORUS* (BIVALVIA: HYRIIDAE) FROM THE TOCANTINS RIVER, BRAZIL

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ABSTRACT

The reproductive cycle of a population of *Paxyodon syrmatophorus* (Meuschen, 1781), a mussel exploited for its shells in the lower Tocantins River, Brazil, was studied between September 1997 and August 1998. Monthly examination of gonad sections and inspection of the demibranchs of females showed that gametogenesis takes place all year round and that spawning occurs during the months of the dry season. Gravid females were found throughout the period between February and September. Sexually mature mussels as small as 23 mm in length were found and the smallest gravid female was 32 mm in length. The implications of the findings are discussed in relation to the conservation and management of exploited freshwater mussel populations in the region.

INTRODUCTION

Freshwater mussels (Unionoidea, Hyriidae) from the lower Tocantins River in northern Brazil are hand collected by fishermen who dive without the use of any special equipment, from a small boat to the river bed. The mussels are cleaned, dried and sold to a nearby factory that uses material from the nacreous mother-of-pearl layer to manufacture buttons for the clothing industry. Recently however, fewer mussels are being harvested and in some populations many shells are too small to be commercially useful. Shell harvesters are concerned for the future of their activities and have expressed a desire to manage the mussel beds in a sustainable way.

The present study reports the results of an investigation into the reproductive biology of *Paxydon syrmatophorus* (Meuschen, 1781), the predominantly exploited species (Beasley, in press). *P. syrmatophorus* belongs to the tribe Prisodontini (Parodiz & Bonetto, 1963) and is common in the lower Amazon basin (Bonetto, 1967; Mansur & Valer, 1992). Determination of the timing and duration of gametogenesis and larval brooding can be valuable in the elaboration of management strategies for exploited freshwater mussel populations. Sustainable harvesting of wild populations of organisms entails taking an optimal harvest, or one that takes as many individuals as possible without causing the populations to decline. Harvesting of mussels can be reduced or suspended during peak periods of these activities in order to maximise the number of fertilisations and thus the subsequent number of glochidia being available to host fish. Information on size at sexual maturity may be used to prescribe limits to the size of individual mussels being caught. The objectives of the study were to determine the timing of maturation of the gonads of *P. syrmatophorus*, the length of the glochidial brooding period as well as establish at what size mussels first reach sexual maturity.

MATERIALS AND METHODS

Monthly samples of 20 mussels from a range of shell sizes (20 mm to 81 mm) were collected between September 1997 and August 1998. The animals were transported over ice in a cooler box and were processed within 24 hours. After fixing the visceral mass in 10% formalin, transverse sections of the central portion were dehydrated, cleared, infiltrated and

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embedded in paraffin. Sections were cut at 7 µm, mounted on glass slides, stained with Haematoxylin and Eosin and later examined under light microscopy. Several different stages of male and female gamete cells were identified according to descriptions given by Peredo & Parada (1984) (including multinucleated dark bodies or sperm morulae) but these stages were summarised as either developing or mature gametes. Gametogenesis was quantitatively evaluated according to the methods of Haggerty, Garner, Patterson & Jones (1995). In the case of males, a transect was made across the centre of each of ten randomly chosen acini and developing or mature sperm cells touching the eyepiece pointer along the transect were identified and tallied. Mean numbers of these cells were determined for each individual. For females, a transect was made across the entire section along which developing and mature ova were identified and tallied in each of the first thirty follicles encountered. Mean numbers of ova were determined for each individual. The demibranchs of females were dissected and stored in 70% alcohol and subsequently examined for the presence of glochidia under a stereo microscope. The G-test was carried out to determine if there was a difference from 1:1 in the sex ratio. The Kruskall-Wallis test was carried out to check for seasonal differences in the median numbers of gametes produced and to compare median numbers of gametes among each of three size classes of mussel. Spawning in males and ovum release in females were inferred from declines in mature gametes. Monthly values of water temperature (°C, sampled from near river bottom), transparency (m, Secchi disc) and depth (m) were obtained at the site where mussels were sampled.

RESULTS

Sex ratio

Of the 240 mussels collected over the year, 132 were male and 93 female. The ratio of males to females differed significantly from a 1:1 sex ratio ($G_{adjusted} = 6.78$, d.f. = 1, $p < 0{,}01$). Only 2 hermaphrodites were found and sex was undetermined in the remaining 13 mussels.

Male reproductive cycle

Spermatogenesis occurs throughout the year (Fig. 1a,b). Significant differences among monthly collection dates were found for developing sperm cells ($K = 99.09$, d.f. = 11, $p < 0.01$) numbers of which were relatively constant between September 1997 and January 1998 (Fig. 1a). Thereafter numbers fluctuated widely with peaks in February, June and August (Figure 1a). Numbers of mature sperm differed significantly among monthly collection dates ($K = 100.04$, d.f. = 11, $p < 0.01$). In September 1997 numbers of mature sperm were high and decreased until December with little change until January 1998 when numbers increased and continued to rise until May 1998 (Fig. 1b). Between May and July numbers of mature sperm decreased but rose again slightly in August (Fig. 1b).

Female reproductive cycle

Oocyte production occurred throughout the year (Fig. 2a,b). Significant differences in numbers of developing ova were found among monthly collection dates ($K = 384.7$, d.f. = 11, $p < 0.01$). From high numbers in September 1997 there was a decrease until December (particularly marked between November–December) with little change until February 1998 (Fig. 2a). Thereafter numbers of developing ova increased and, with the exception of a slight decrease in April, continued to rise until July. Numbers declined markedly in August (Fig. 2a). Mature ova differed significantly in numbers among monthly collection dates ($K = 266.7$, d.f. = 11, $p < 0.01$). Two minor peaks in numbers occurred in October 1997 and April 1998 (Fig. 2b). Between May and August numbers rose markedly with a major peak in July-August 1998 (Fig. 2b).

Synchrony between male and female reproductive cycles

Males appeared to spawn between September and December 1997 (Fig. 3) when numbers of mature sperm declined rapidly (Fig. 1b). It is likely that movement of ova into the gill chambers occurs between August and September (Fig. 2a,b) when the number of mature ova per follicle decline. Fertilisation probably takes place at the end of the dry season, between September and December, when the release of mature sperm and eggs coincide (Fig. 3). A second spawning period for males occurred between May and July 1998 (Fig. 3) when mature sperm declined in numbers (Fig. 1b). Numbers of mature ova are low and relatively similar between September and May and there is little evidence of movement of ova into the gills between April and May (Fig. 2b). In December 1997 there is a slight decline in mature ova (Fig. 2b) indicating possible movement of ova into gills and coincides with male spawning in the same period (Fig. 3).

Glochidial brooding

Gravid females containing embryos with a vitelline membrane were first found in February 1998. Both embryos and mature glochidia were recorded between April and September 1998. Peak numbers of gravid females were found in April (Fig. 4). Between April and August gravid females predominated over non-gravid females (Fig. 4). In most samples males predominated over females, particularly during June, July and August (Fig. 4). Embryos measured approximately 200 μm and glochidia measured 250 μm along the maximum valve diameter in the plane of the hinge. Mature glochidia were characterised by the presence of

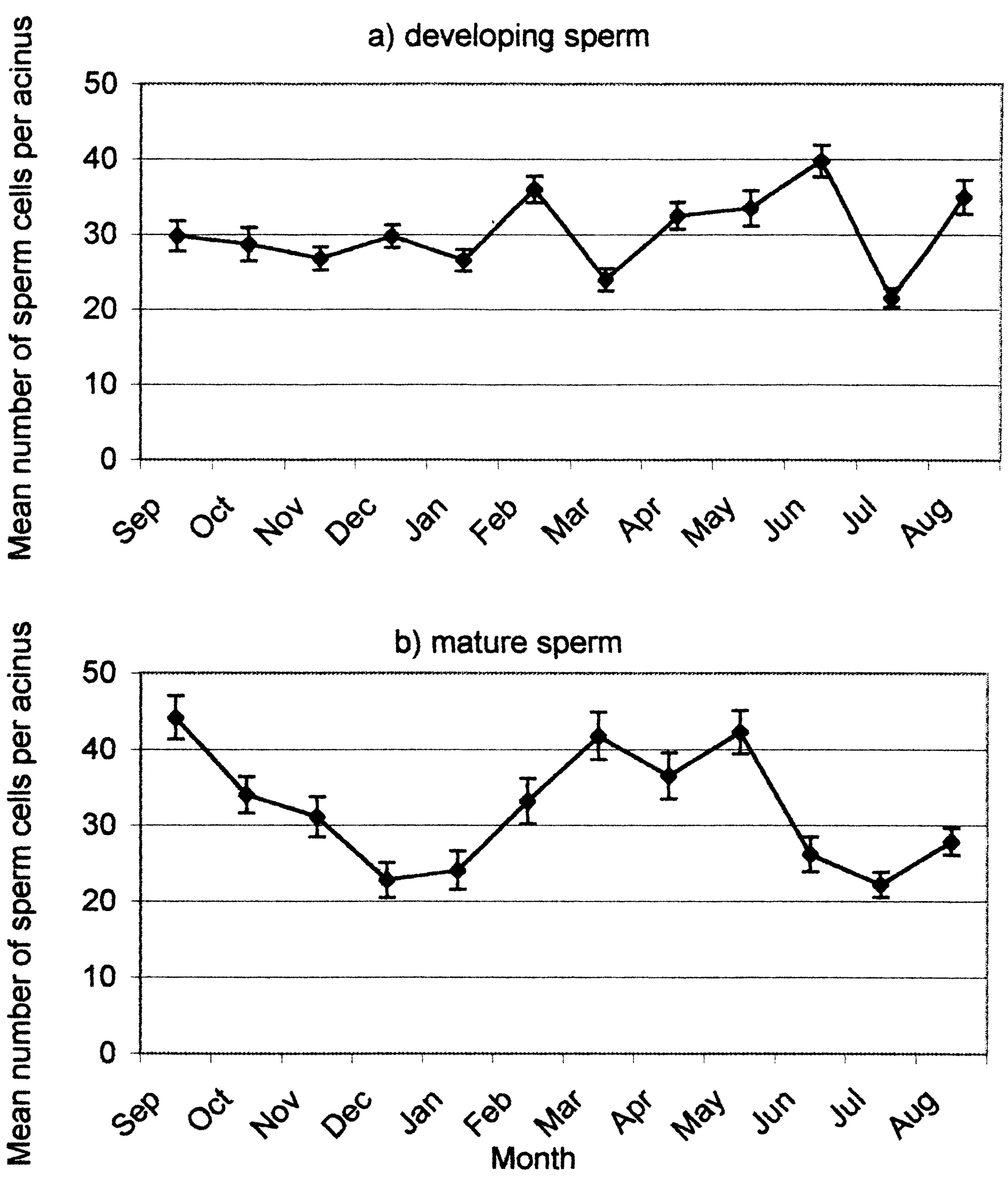

Figure 1. Variation in the mean number of a) developing and b) mature sperm cells per acinus (± s.e.) during the study period.

teeth and a large adductor muscle (Bonetto, 1963). In September 1998 five gravid females were found.

Mussel size and gametogenesis

The numbers of each sex in different size categories of mussel is shown in Figure 5. Although males predominated over females in the 21–30 mm and 61–70 mm categories, it appears that both have a similar size frequency distribution. The mean number of gamete cells counted per follicle for mussels in each of three size categories is shown in Figure 6. Significant differences in the median number of gametes were found among size categories of both male ($K = 48.71$, d.f. $= 2$, $p < 0.01$) and female ($K = 81.23$, d.f. $= 2$, $p < 0.01$) mussels. The median number

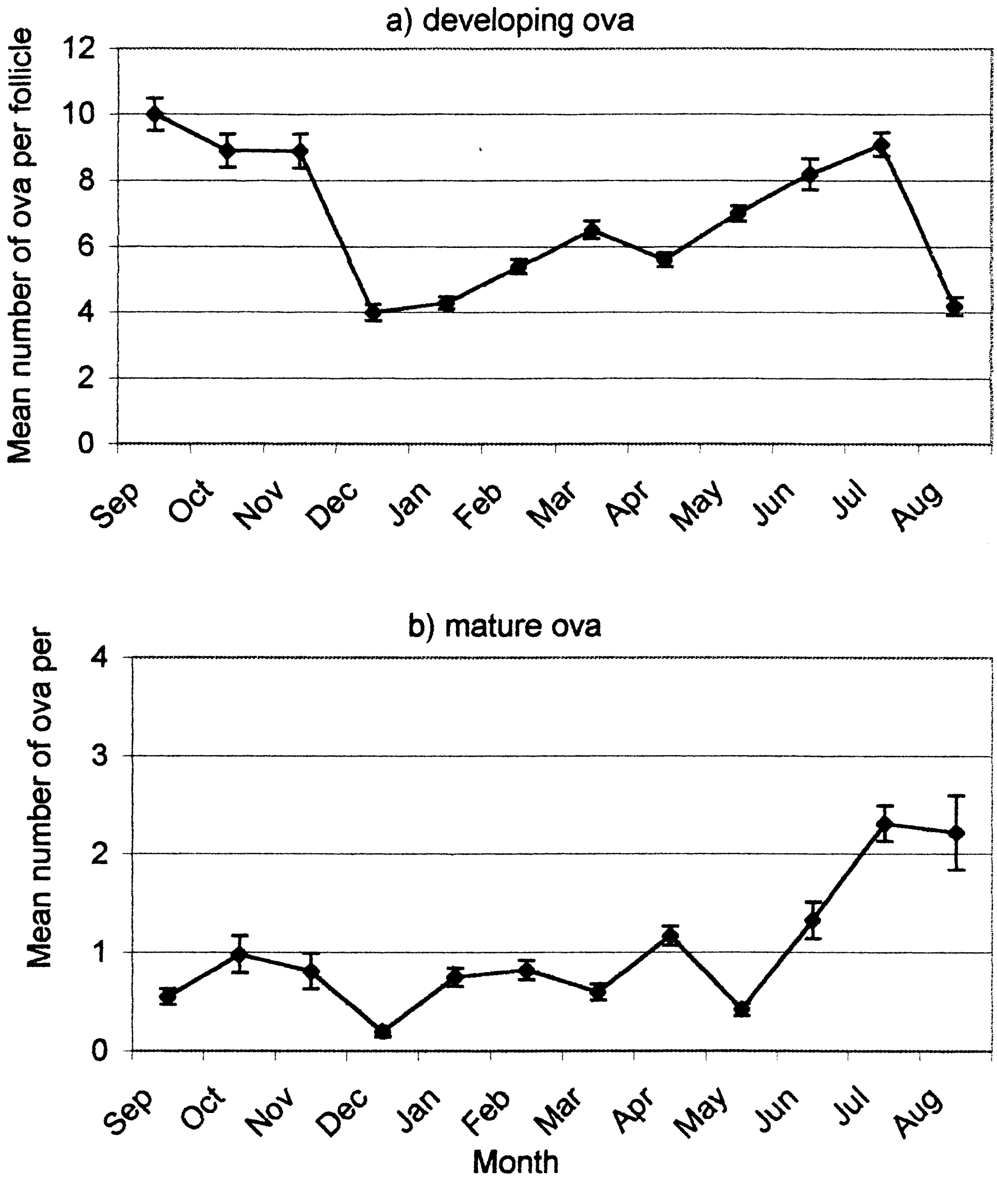

Figure 2. Variation in the mean number of a) developing and b) mature ova per follicle (± s.e.) during the study period.

of gamete cells for the 0–30 mm category is significantly lower than that of the other two categories (Fig. 6). The smallest mussels sampled in this study were 20 mm in length for males and 21 mm for female mussels. The size of the smallest mussels containing mature gametes was 23 mm for males and 31 mm for females. The smallest gravid female was 32 mm.

Environmental parameters

Little variation in water temperature occurs during the year (Fig. 7). Transparency was lowest in February and the highest value was recorded in August (Fig. 7). Water depth was greatest in November but was relatively similar throughout the year (Fig. 7).

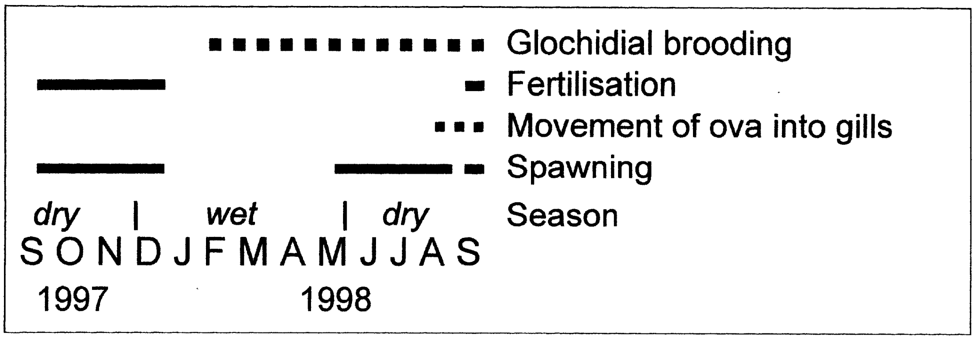

Figure 3. The timing of reproductive events in *P. syrmatophorus* in relation to season. Spawning of males and movement of ova into gills by females were inferred from declines in mature gametes in gonad sections.

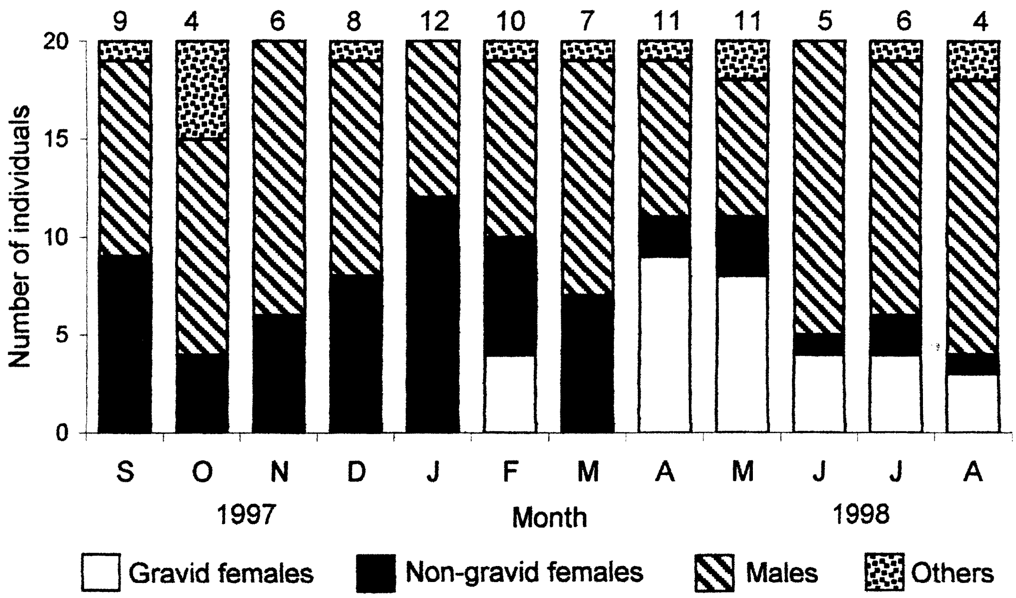

Figure 4. Numbers of individuals in each monthly sample according to sex. Females are further grouped as gravid or non-gravid. Hermaphrodites and individuals of unknown sex are grouped as Others. The number above each column gives the total number of females examined.

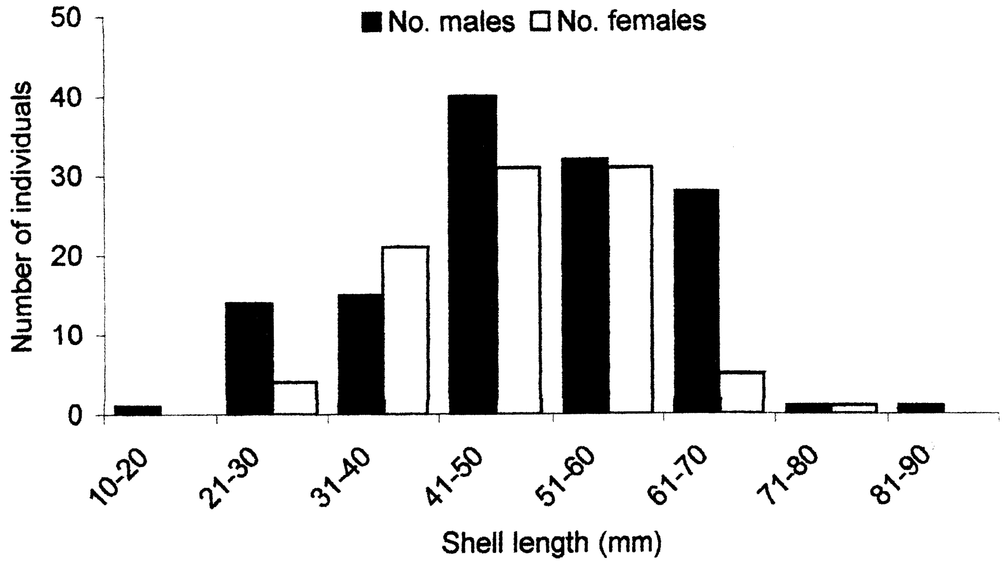

Figure 5. Numbers of males and females of *P. syrmatophorus* in relation to shell length. Hermaphrodites and individuals of unknown sex are not included.

DISCUSSION

In the population studied, *P. syrmatophorus* appears to be a dioecious species in which hermaphrodites are rare and males predominate. The reason for the latter phenomenon is not clear since there are no obvious differences between males and females in terms of size or shell shape that could cause sampling bias. Studies of other South American freshwater mussels show that hermaphrodites are present in both the Mycetopodidadae (Hebling, 1976; Veitenheimer & Mansur, 1978; Avelar, 1993) and the Hyriidae (Hebling & Penteado, 1974; Avelar & Mendonça, 1998). Populations of the Australian hyriid *Hyridella depressa* (L.) were found to be predominantly females, some of which had small amounts of male gonadal tissue, i.e. microhermaphrodites (Byrne, 1998). Other studies of the Hyriidae indicate that separate sexes are the rule and that hermaphrodites are rare or absent (Peredo & Parado, 1984; Jones, Simpson & Humphrey, 1986; Avelar & Santos, 1991). Jirka & Neves (1992) found that males predominated in samples of two North American freshwater mussel species whereas Young & Williams (1984) found that up to 70% of individuals were female in some populations of *Margaritifera margaritifera* L. in Scotland.

Gametogenesis has been found to occur throughout the year in members of the Hyriidae from subtropical Australia (Jones *et al.*, 1986), temperate Chile (Peredo & Parada, 1986) and in Unionidae from tropical India (Nagabhushanam & Lohgaonker, 1978; Ghosh & Ghose, 1972). The study of Australian populations of *H. depressa* (Bryne, 1998) showed gametogenesis to be continuous, particularly at eutrophic sites where reproductive output was high. However, at oligothrophic sites, gametogenesis was more seasonal and reproductive failure more common (Byrne, 1998). In a study of the South American subtropical hyriid *Diplodon rotundus gratus* (Wagner, 1827), Avelar & Mendonça (1998) found that gametogenesis occurs only during the summer months with a single synchronised peak in production of mature male and female gametes.

Spawning of male *P. syrmatophorus* began before, and overlapped with, the movement of eggs into the gills in females and such synchronisation may help maximise the chances of fertilisation. Similarly, partial spawning may reduce the risk of mistiming the release of gametes by the sexes and has been reported in *Cucumerunio novaehollandiae* (Gray, 1834) (Jones *et al.*, 1986) and *Diplodon chilenis chilensis* (Peredo & Parada, 1986).

Although two spawning periods for males were found during the study, it appears that a single reproductive period for females (movement of ova into gills) occurs during the dry

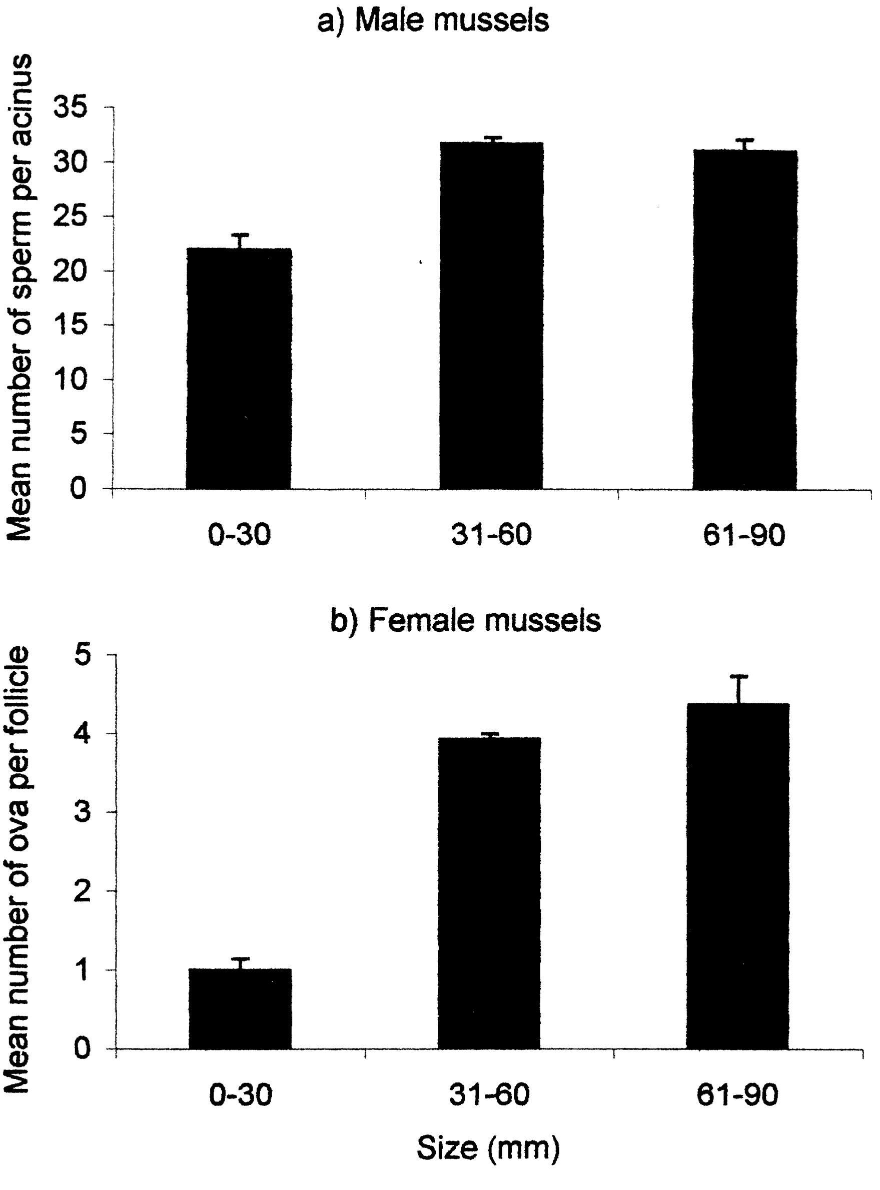

Figure 6. Gamete production [number of cells per follicle (± s.e.)] in relation to shell length of a) male and b) female mussels.

season, when water levels are low. Whether there is another movement of eggs in April–May, during the wet season, is not clear. Repetitive breeding has been reported in subtropical Hyriidae (Jones *et al.*, 1986, Byrne, 1998) and tropical unionaceans (Kenmuir, 1981). A study lasting several seasons is necessary to determine whether breeding is repetitive in *P. syrmatophorus*.

It has been suggested that spawning of freshwater mussels during low water periods may help concentrate spermatozoa in the water column and thus increase the rate of fertilisation (Ross, 1992; Haggerty *et al.*, 1995). In subtropical freshwater mussels spawning has been associated with the decline in water temperature (Jones *et al.*, 1986; Avelar & Mendonça, 1998). Spawning of *P. syrmatophorus* occurred

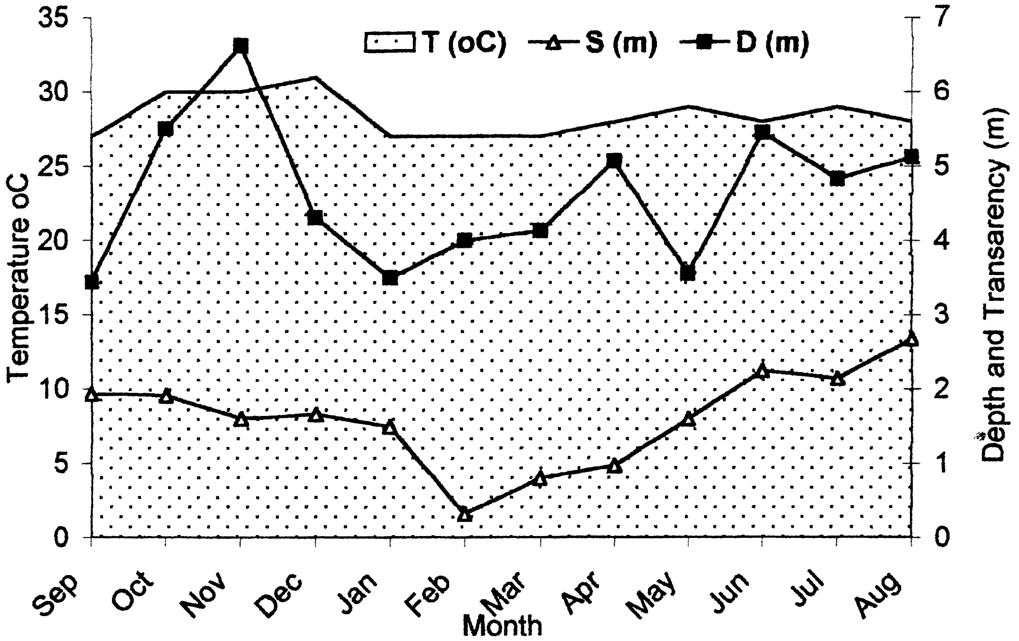

Figure 7. Seasonal variation in environmental parameters measured during the study: (T) water temperature (°C), (S) transparency (m) and (D) water depth (m).

in the dry season and corresponds to the period with the highest transparency values or, in other words, lowest suspended solids loads. In equatorial areas such as the Amazon region where water temperature is more constant, fluctuations in water level, current speed and suspended solids load may act as alternative environmental cues for spawning in freshwater mussels. Our water level data does not correspond well to the high water period (March) but appears to coincide with the low water period (September). Local people report that periodic releases of dammed water from the Tucuruí hydroelectric plant may occur from time to time. To what extent artificial variation in the water levels is occurring and what its influence is on spawning is unknown and merits investigation.

Brooding in *P. syrmatophorus* occurs over a long period since gravid females were found between February and September. Similarly, Bryne (1998) reported that *H. depressa* is a long-term brooder with glochidia present in the marsupia for up to eight months. Much of the brooding period of *P. syrmatophorus* spans the wet season and the low level of shell collecting during this time (Beasley, in press) may help to maximise the number of glochidia available for uptake by fish hosts. There may be a wider distribution and a greater abundance of host fish during the wet season therefore the release of glochidia during this time may increase the chances of a larva finding a host. Between September 1997 and January 1998 no glochidia were found and a possible reason for this is a poor rate of fertilisation during this period. That glochidia were first found in February 1998 may be the result of fertilisation occurring around December 1997 when there was a slight decline in mature ova.

The identity of the host fish(es) of *P. syrmatophorus* is unknown and lack of this information represents a considerable barrier to any conservation or management scheme since mussels are unable to complete their life cycle in the absence of their host fish. Such a gap in our knowledge is not just limited to Amazonian freshwater mussels. In North America, where the mussel fauna is relatively well known, host fishes have been identified for only about 25% of unionoid species (Bogan, 1998). The recent report of a population of *P. syrmatophorus* from a central Amazonian reservoir (Mansur, Volkmer-Ribeiro & Lopes de Carvalho, 1997) highlights the need to investigate host fish populations that may be adversely affected by dam construction (Watters, 1996).

The reproductive period occurs during the dry season, which is also the period in which most shell collecting is carried out (Beasley, in press). To recommend a reduction in collecting activity during this time would be unacceptable to the fishermen, particularly should there be a heavy demand for shells from the factory.

Shell collectors typically select the largest shells (>50 mm) as these are preferred by the button manufacturing industry. Individuals of *P. syrmatophorus* as small as 23 mm may attain sexual maturity and those females as small as 32 mm can be gravid. No age at length data for *P. syrmatophorus* is available yet but considering that the largest specimens may reach over 90 mm (Beasley, in press) then sexual maturity appears to be attained quite early. Jirka & Neves (1992) found that the age at sexual maturity of six North American unionid species varied between 4 and 6 years. Sexually mature individuals of *Diplodon chilensis chilensis* may be as small as 16 mm (Peredo & Parada, 1984) which corresponds to between 2 and 3 years of age (Parada, Peredo, Lara & Valdebenito, 1989).

In terms of a management strategy, it is desirable that a proportion of reproductively mature individuals be left in the population to allow breeding to take place. Thus it appears that since even small individuals are capable of reproducing, there will be sufficient adults to ensure some breeding success because fishermen do not normally take small mussels. A useful size limit to catches would be 50 mm in length and mussels below this size should be left undisturbed in order to guarantee recruitment in the population. As shell-collecting activities in this region are rather variable in intensity due to fluctuations in demand from the factory, the relaxation of harvesting during slack periods may allow over-exploited mussel populations to recover. During the study period, shell collecting by locals had ceased between September 1997 and June 1998, after which collecting only resumed in small quantities until the present time (September 1999). Currently, the most appropriate management recommendation that can be made, based on information from the reproductive cycle of *P. syrmatophorus*, is that concerning the size limit to catches. Other recommendations based on ecological data can be found in Beasley (in press).

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