Brood size and larval length of *Paxyodon syrmatophorus* (Bivalvia, Hyriidae) from the Tocantins river, Brazil

by

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Abstract

Monthly estimates of brood size, and oocyte, embryo and glochidium length of the freshwater mussel *Paxyodon syrmatophorus* (MEUSCHEN, 1781) from the Tocantins river, were obtained during the breeding season (February to September 1998). A clear temporal pattern in these reproductive characteristics was observed during the breeding season such that small brood sizes, smaller oocyte, embryo and glochidium lengths are typical of the beginning of the breeding season. Brood size increases and oocyte, embryo and glochidium length increase as the breeding season progresses and maximum values of all reproductive characters occur between May, June and July. Brood size is smaller at the end of the incubation period. A significant positive correlation between adult female shell length and brood size was observed. The importance of determining the timing of reproductive events and peak periods of reproductive activity is discussed in the light of conservation and management measures such as direct protection, mussel stock enhancement, relocation and artificial culture of freshwater mussels.
Keywords: **Freshwater mussel, reproduction, Hyriidae, Amazonia.**

Resumo

Durante a estação de reprodução (fevereiro a setembro de 1998) foram obtidas estimativas mensais de número de larvas incubadas, tamanho do oócito, embrião e larva gloquídio do bivalve de água doce *Paxyodon syrmatophorus* (MEUSCHEN, 1781) do rio Tocantins. Um padrão claro temporal nestas características reprodutivas foi observado durante esta estação no qual menores números de larvas, menores oócitos, embriões e gloquídios foram típicos do início da estação de reprodução. Houve um aumento do número de larvas, assim como aumentaram o comprimento do oócito, do embrião e do gloquídio ao longo da estação de reprodução. Os valores máximos de todas as características reprodutivas foram alcançados entre maio e julho. O número de larvas incubadas é menor no final da estação reprodutiva. Foi observada uma correlação positiva e significativa entre o comprimento da concha da fêmea adulta e o número de larvas por fêmea. A importância da determinação da temporização de eventos reprodutivos e períodos picos de atividade reprodutiva é discutida na luz de medidas de conservação, tais como: proteção direta, aumento de populações de bivalves, relocalização e cultivo artificial de bivalves de água doce.

Introduction

Seasonal variation in the reproductive activity of freshwater bivalves in temperate (JIRKA & NEVES 1992; HAGGERTY et al. 1995; GARNER et al. 1999; HAGGERTY & GARNER 2000), subtropical (PEREDO & PARADA, 1986; JONES et al. 1986; PARADA et al. 1990; AVELAR & DE MENDONÇA 1998; BYRNE 1998; KENMUIR 1981), and tropical regions (GHOSH & GHOSE 1972; NAGABHUSHANAM & LOHGAONKER 1978; BEASLEY et al. 2000; VALE et al. 2004) may occur as a result of changes in water temperature, turbidity, light, composition of algal food sources, nutrient status and water chemistry, among other factors. Whatever the causes of seasonal variation, the fact that it occurs has implications for the conservation of freshwater bivalves, a group many of whose members and habitats are under diverse threats and/or facing extinction (BOGAN 1993; WILLIAMS et al. 1993; NEVES et al. 1997). Many freshwater mussel conservation measures are based on knowledge of their reproductive cycle and biology. For example, determining minimum size limits for a sustainable harvest depends on such biological information on a species-by-species basis (NEVES 1999a). Conservation measures, such as protection of mussels from harvesting, can be made to be more effective when enforced during the reproductive period when critical moments in mussel life history such as fertilization and glochidium release occur. Mussel stock enhancement using host fish (BAUER, 1987) and artificial rearing of glochidia (BUDDENSIEK 1995; UTHAIWAN et al. 2001) depend on a knowledge of the timing of reproduction of the species being enhanced. Relocation of mussels is common in many localities where habitat conditions are no longer appropriate for the continued survival and/or propagation of the mussel population (DUNN & SIETMAN 1997). Knowing if and when the mussels reproduce may prevent interruption of reproductive activity and may also may be useful in verifying mussel reproduction after relocation to better conditions (HEINRICHER & LAYZER 1999). *Paxyodon syrmatophoru* (MEUSCHEN, 1781) is a freshwater mussel common in the Amazon basin and collected for the production of buttons (BEASLEY 2001). The ultrastructure of spermatogenesis in *P. syrmatophorus* has been described by MATOS et al. (1998). A study of the seasonality of gametogenesis in *P. syrmatophorus* from the Tocantins river (BEASLEY et al. 2000), using numbers of gametes and gravid females during the year, showed that the most active reproductive period extended between February and September 1998. The present study, using the 1998 samples, includes further data on reproductive characteristics of *P. syrmatophorus* such as monthly estimates of brood size, oocyte, embryo and glochidium length obtained from gravid females during the reproductive period.

Materials and methods

Collection, transport and processing of samples of *P. syrmatophorus* from the Tocantins river between 1997 and 1998 is fully described in BEASLEY et al. (2000). Histological sections of the follicles of gravid females were examined under light microscopy and, for each individual mussel, 30 oocytes were measured (μm), using an ocular reticule, along their maximum length across a transect of the entire section. The demibranchs of each gravid female were opened along the posterior dorsal margins and all larvae were removed and counted using a gridded plate (BEASLEY et al. 2003). For each individual mussel, a sample of 30 larvae (glochidia or embryos) was measured (μm) under the light microscope. Shells of gravid females were measured for maximum anterior posterior length using the method described by MANSUR & CAMPOS-VELHO (1990).

Data were tested for adherence to the parametric assumptions of normality (Shapiro Wilk test) and

equality of variances (F_{max}test). These assumptions were met without the need for data transformation in the case of brood size and oocyte length. To reduce heterogeneity of variances, other variables were transformed as follows: embryo length ($x^{0.1875}$) and glochidium length ($x^{1.71}$). One-way analysis of variance was used to compare monthly means with use of the Welch correction where variances remained different even after data transformation (ZAR 1999). Tukey's Honestly Significant Difference (HSD) test was applied *post hoc* to determine between which months significant differences occurred (ZAR 1999). Correlations between shell length and length of larvae, oocytes and brood size were made using the Spearman rank correlation coefficient (r_s).

Results

Gravid females were found in February and between April and September (Fig. 1) and although there is a tendency for a peak in mean brood size around June, no statistically significant difference in mean brood size was found between months ($F_{6,24}$=1.917, n.s.). Mean brood size is low in February (13774 larvae per gravid female) but increases to a peak of 36779 larvae per gravid female in June. Thereafter brood size decreases until August when values are similar to those of February but a slight increase occurred in September. Over the entire incubation period, the smallest individual brood size of 7721 larvae was registered for April and the largest brood sizes occurred between May, June and July with 54648, 55085 and 55594 larvae, respectively (Table 1).

Mean oocyte length in gravid females was low (98.8 μm) in February, higher between April (101.7 μm) and July (115.3 μm) and low again in August (95.9 μm) (Fig. 2). There were significant differences between months ($F_{5,624}$=8.47, p<0.001) and the greatest mean oocyte length occurred in July (Table 1, Fig. 2). The July sample was significantly different from all other months (Tukey HSD, p<0.05).

Mean embryo length was initially low (207 μm) in February and rose to a maximum value (236 μm) in June (Fig. 3). Values decreased between July and August but rose again in September and there were significant differences in mean values between months ($F_{6,188.6}$=36.73, p<0.01). The largest embryo (278.4 μm) was found in June (Table 1).

Mature glochidia were not found in gravid females in February, March and August and mean glochidium length was significantly different between months ($F_{4,71.8}$=5.59, p<0.01). Mean glochidium length in April (241.3 μm) was significantly lower in comparison to other months except July (Tukey HSD, p<0.05) (Fig. 4). Variation in glochidium length was lower in May, June and September and glochidia reached their maximum size in May and June. The smallest individual glochidium (185.6 μm) was found in July whereas the largest glochidia (278.4 μm) were found in May and June (Table 1).

No significant correlations were found between shell length and length of oocytes (r_s=0.026, n.s.) or length of larvae (embryos and glochidia) (r_s=0.182, n.s.). However, there was a significant positive correlation between shell length and brood size (r_s=0.4-03, p<0.05). A simple linear regression of brood size on shell length gives the following significant ($F_{1,29}$=4.37, p<0.05) relationship: *y*=609.4*x*-11277.2 for *P. syrmatophorus* in the Tocantins river (Fig. 5). The mean shell length of gravid female *P. syrmatophorus* examined during the study was 58.1 mm.

Discussion

There is a distinct seasonal pattern to be found in several different aspects of the reproductive activity of *P. syrmatophorus* during the incubation period in the Tocantins

river. Larval brooding begins early in the wet season (February) and ends during the middle of the dry season (September) (BEASLEY et al. 2000). For *P. syrmatophorus* from the Tocantins river, the beginning of the incubation period is typically characterised by small brood sizes, smaller oocyte length, predominance of embryos and, if present, small glochidia. As the incubation period progresses, brood size increases and oocyte, embryo and glochidium length increase such that the presence of the largest oocytes, embryos and glochidia coincides with the largest brood sizes. The lack of a significant statistical difference in monthly mean brood size may be due to large within-month variation. On a given collection date, there are differences in brood size between individuals due to intraspecific variation (HEARD 1975) but on average it appears that peak numbers of larvae occur between May and July. The timing of glochidium development and release may be synchronized with host fish availability (KAT 1984). For example, glochidial discharge in *Potamilus alatus* (SAY, 1817) from the Tennesse River coincides with the spawning movements of its host fish (HAGGERTY & GARNER 2000). However, nothing is yet known about the host fishes of *P. syrmatophorus*, although host fish identity and the culture of juvenile mussels of this species has begun to be investigated by us.

The developmental sequence involving an increase in oocyte size, appearance of, and increase in size of embryos and glochidia, may also be seen in other species. In *Cucumerunio novaehollandiae* (GRAY, 1847) from Northern New South Wales (Australia) growth of oocytes increased rapidly between November and April after which a sharp decline in mean oocyte size indicated the movement of oocytes into the gills (JONES et al. 1986). In June, immature glochida appeared and in July mature glochidia were being released. HAGGERTY & GARNER (2000) found that oocyte size in *Potamilus alatus* was greatest just before movement into the gills in September. Immature glochidia appeared in early October and mature glochidia were found from late October to April. Likewise, oocyte size in *Cyclonaias tuberculata* (RAFINESQUE, 1820) was greatest in spring when numbers per follicle where highest (HAGGERTY et al. 1995). Embryos appeared in marsupia in May whereas mature glochida began to appear in July. Oocyte size in *Quadrula metanerva* (RAFINESQUE, 1820) increased during the autumn and late winter before fertilization and the appearance of embryos and glochidia in the spring and summer (GARNER et al. 1999). Similarly, in *Diplodon rotundus gratus* (WAGNER, 1827), a hermaphroditic species from subtropical Brazil, the appearance of individuals with empty marsupia was followed by those with embryos and subsequently mature glochidia (AVELAR & DE MENDONÇA 1998).

Mean fecundity (defined as the full complement of developing eggs produced by a female during a single reproductive period) varied widely among eight species of North American freshwater mussels (HAAG & STATON 2003). Three species had fecundities above 100,000 larvae with the greatest mean fecundity recorded for *Amblema plicata* (SAY, 1817) (326,000 larvae). The fecundity of four species varied between 25,000 and 40,000, whereas a single species, *Quadrula asperata* (LEA, 1861), had a mean fecundity of 10,000 (HAAG & STATON 2003).

Paxyodon syrmatophorus has a mean brood size that varies from 14,000 to 37,000 larvae although our estimates do not distinguish between undeveloped eggs and larvae. In the Pará river, mean brood size of *P. syrmatophorus* was 8200 larvae and gravid females were smaller (48.4 mm) than those from the Tocantins river (MIRANDA unpubl.). Brood size in other Amazonian freshwater mussels varies among species and

between rivers. Mean numbers of larvae in incubating females of *Triplodon corrugatus* (LAMARCK, 1819) varied between 25,000 in the Tocantins river and 4,000 in the Irituia river (MIRANDA unpubl.). *Castalia ambigua* (LAMARCK, 1819) from the Irituia river has an annual mean brood size of 725 larvae but mean brood size in July, the peak reproductive period, is 1500 larvae (VALE et al. 2004). However, in the Tocantins river, the mean brood size of *C. ambigua* is 2100 larvae (MIRANDA unpubl.). For both these species, mean shell size is significantly larger in the Tocantins river (BEASLEY 2001; VALE et al. 2003; MIRANDA unpubl.). Variation in brood size is common in both Amazonian and North American freshwater mussels and HAAG & STATON (2003) found that inter-site variation within species was also related to shell length, which explained between 43-88% of variation in fecundity. The relationship between length and fecundity was exponential in six species studied by HAAG & STATON (2003) but in two species the rate of increase in fecundity declined in larger mussels suggesting senescence with age. With *Paxyodon syrmatophorus*, mussels between 20 and 81 mm were sampled (BEASLEY et al. 2000) and, despite inclusion of presumably young and old mussels, a linear relationship between brood size and length was found. Shell length was also linearly related to the number of glochida in the Australian *Hyridella depressa* (LINNAEUS, 1758) (JUPITER & BYRNE 1997) and the mean number of glochidia per female ranged from 9,700 to 94,000 at the sites studied (BYRNE 1998).

Knowledge of the timing of reproduction is important in the conservation of freshwater mussels. Recommendations for the management of *P. syrmatophorus* (BEASLEY et al. 2000) include reducing or halting harvesting of mussels during the incubation period.

Reproduction may be related to the trophic status of the habitat such as with *Hyridella depressa* from New South Wales, Australia, where higher brood sizes and several brooding periods per year may occur at eutrophic sites in contrast to lower output and more seasonal reproduction at oligotrophic sites (BYRNE 1998). Thus it is important to evaluate reproductive status as an indicator of the potential of a population to persist at the locality.

Non-reproducing populations of freshwater mussels such as *Megalonaias nervosa* (RAFINESQUE, 1820) from the Cumberland river (Tennesse, USA) relocated to better quality habitat began reproductive activity again, although this took up to 2 years following relocation (HEINRICHER & LAYZER 1999). After 6 to 9 months following relocation to a eutrophic site, individuals of *H. depressa* showed significant increases in shell length, weight and fecundity as well as a doubling of brood size in response to the greater phytoplanktonic productivity (BYRNE 1998). Therefore, it is important to know reproductive output beforehand in order to assess output after relocation. On the other hand, the relocation of mussels to refuges, such as fish hatchery ponds, has been found to reduce survival and physiological condition in the long-term (36-40 months) (NEWTON et al. 2001) and probably also a decline in reproductive output. Survival of unionids after relocation from zebra mussel (*Dreissena polymorpha*) infested sites to a fish hatchery pond was very variable (35 to >80 %) after 35 days (NEVES 1999b).

Relocation is considered a high-risk conservation measure (NEWTON et al. 2001), appropriate perhaps for rare species either under immediate threat or to be relocated into restored historic habitat and the techniques are still inadequately developed for wide-scale use (National Native Mussel Conservation Committee, 1998). However, high rates

of recovery after relocation of unionoids involves careful handling and choice of relocation area, although avoiding dessication, crowding and extreme temperatures are also key factors in post-relocation survival (DUNN & SIETMAN 1997). Knowledge of the reproductive cycle may be important for the success of relocation. It make sense to avoid relocating species during the reproductive period so as to minimize stress in incubating females that may, for example, abort glochidia during handling (GARNER et al. 1999).

The *in vitro* culture of freshwater bivalves (ISOM & HUDSON 1982; HUDSON & ISOM 1984) and their subsequent release into their natural habitat is seen as conservation measure with considerable potential (O'BEIRN et al. 1998; UTHAIWAN et al. 2001). Mussel culture may also be a means of reducing the impact of shell collection on natural populations (NEVES 1999c). The first step in the production of juvenile mussels is the capture of gravid females from wild populations (NEVES 1999c). Knowledge of the reproductive period and the evaluation of reproductive output are essential for the eventual selection of sites from which gravid females of a given species are to be obtained. Finally, freshwater mussels are being recognized as useful organisms with which to monitor environmental pollution. The use of glochidia and juvenile mussels in toxicity bioassays (KELLER & ZAM 1990; KELLER & ZAM 1991) requires that the timing of reproduction be known. One application is the investigation of the effect of environmental factors on the timing of maturation of females, for example, the manipulation of temperature to allow the production of glochidia and juveniles all year round for toxicity testing (VAN VREEDE et al. 1999). The sensitivity of freshwater mussels to heavy metals (KELLER & ZAM 1991) may be one of the reasons for their disappearance in many habitats and the effect of such pollution on reproductive output and juvenile survival merit investigation. It is important that species-specific information on the reproductive cycle is obtained since interspecific differences in the timing of reproduction may occur, even in the same habitat and locality (ALDRIDGE 1999).

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Table 1: Descriptive statistics of reproductive characteristics of gravid females of *P. syrmatophorus* from the Tocantins river, between February and September 1998.

|	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.
Brood size								
Minimum	11371	0	7721	8114	21358	18442	10922	14851
Median	13774	0	17108	28230	33895	27900	14492	19373
Mean	13774	0	18756	29197	36779	32459	14169	18011
Maximum	16176	0	46282	54648	55085	55594	17093	19810
No. gravid females	2	0	8	8	3	4	3	3
Oocyte length								
Minimum	45	0	45	40	50	60	55	0
Median	100	0	105	105	102.5	115	100	0
Mean	98.8	0	101.7	100.7	102.5	115.3	95.9	0
Maximum	140	0	175	200	140	180	130	0
Embryo length								
Minimum	162.4	0	162.4	162.4	185.6	162.4	162.4	185.6
Median	208.8	0	208.8	232	232	208.8	185.6	232
Mean	206.8	0	211.1	220.6	236.3	209.8	196.4	226.2
Maximum	232	0	255.2	255.2	278.4	232	232	255.2
Glochidium length								
Minimum	0	0	208.8	232	232	185.6	0	232
Median	0	0	232	255.2	255.2	255.2	0	255.2
Mean	0	0	241.3	255.2	253.7	248.2	0	252.5
Maximum	0	0	255.2	278.4	278.4	255.2	0	255.5
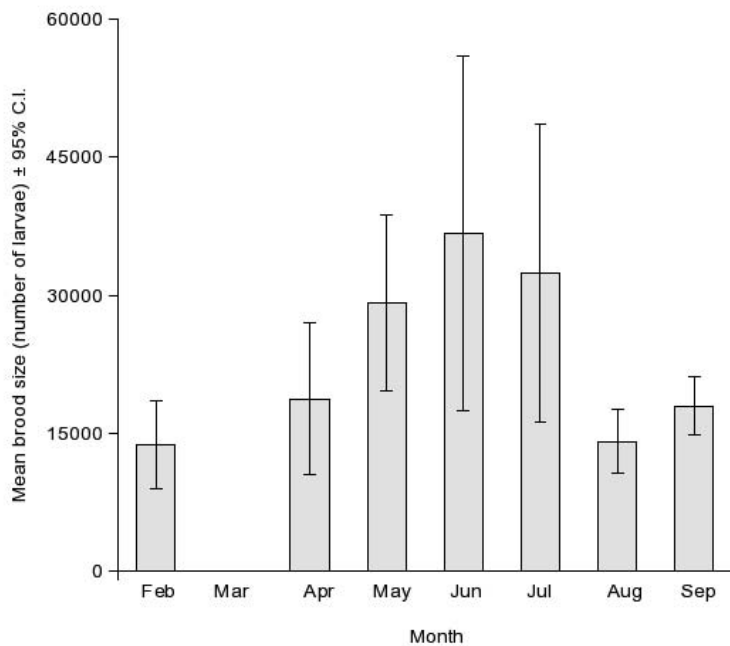
Fig. 1:
Monthly variation in mean brood size (number of larvae) ± 95% confidence interval (C.I.) of *P. syrmato-phorus* during the reproductive period.
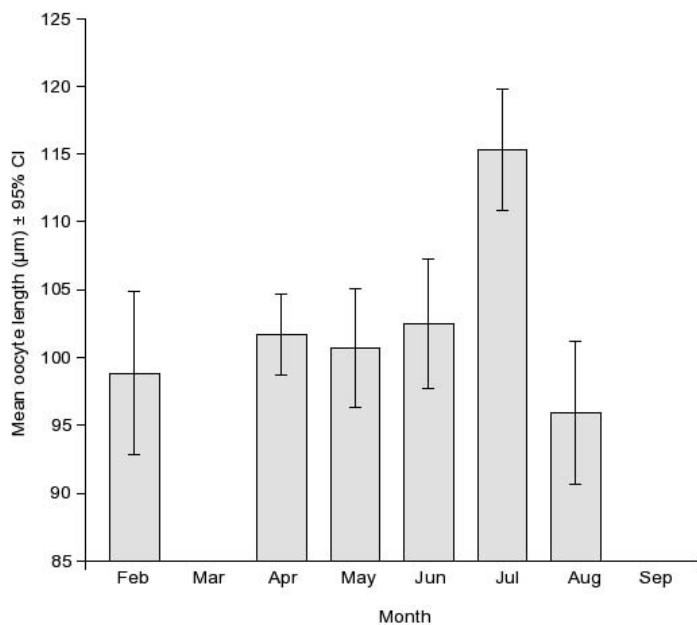

Fig. 2:
Monthly variation in mean oocyte length (µm) ± 95% confidence interval (C.I.) of *P. syrmatophorus* during the reproductive period.
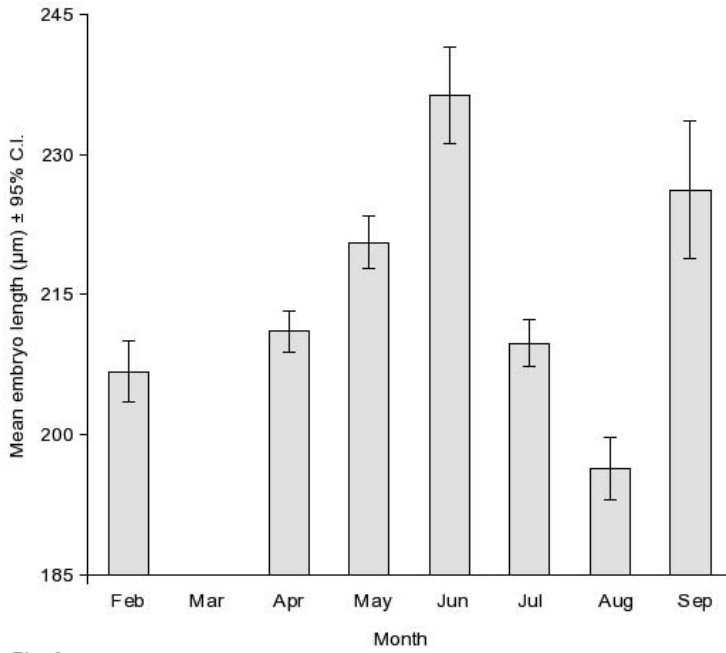
Fig. 3:
Differences in mean embryo length (μm) ± 95% confidence interval (C.I.) of *P. syrmatophorus* during the months of the reproductive period.
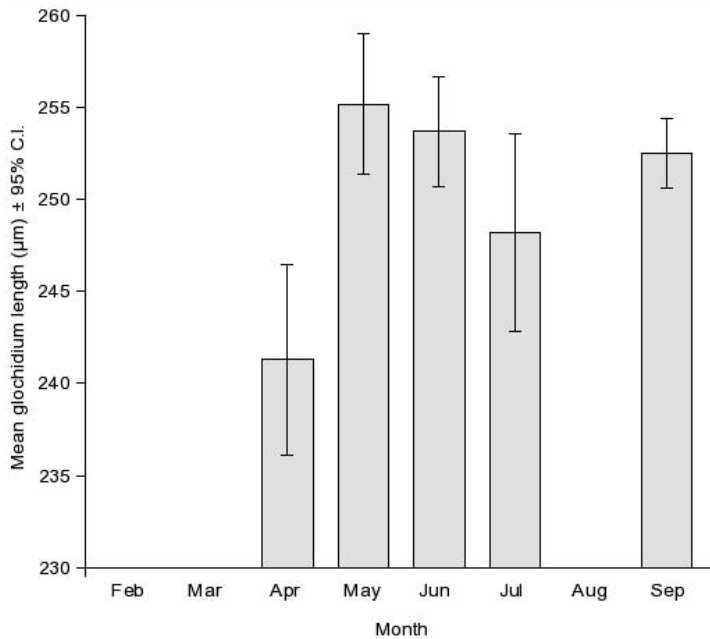

Fig. 4:
Differences in mean glochidium length (μm) ± 95% confidence interval (C.I.) of *P. syrmatophorus* during the months of the reproductive period.
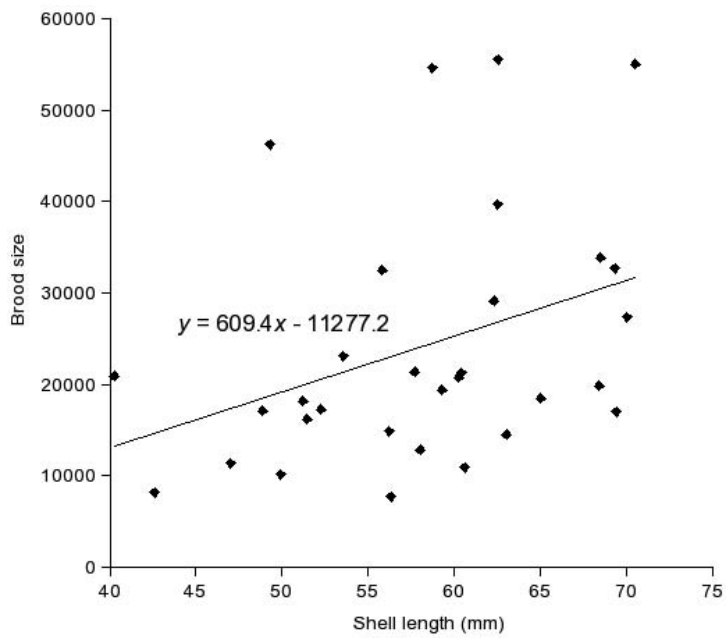
Fig. 5:
Relationship between female shell length (mm) and brood size (number of larvae) in *P. syrmatophorus*.