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A N I N T E R N A T I O N A L J O U R N A L

Life Cycle Observations on *Amphilocus neapolitanus* (Della Valle, 1853) (Crustacea, Amphipoda) Associated with *Sargassum cymosum* C. Agardh, 1820 in Ubatuba, (SP), Brazil

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ABSTRACT

Day and night densities, reproductive biology and life cycle of Amphilocus neapolitanus *(Della Valle, 1853), associated with* Sargassum cymosum *C. Agardh, 1820, were studied during 13 months. The density of juveniles, males and females was higher in night samples than in day samples. Sex ratio was strongly favorable toward the females. Ovigerous females were present in similar densities in both the periods while the non-ovigerous females were predominant at night. The egg number was not correlated with the size of the females. Breeding occurred throughout the year.*

Keywords: Population structure, reproductive biology, life cycle, Amphipoda, *Sargassum*

INTRODUCTION

Amphilocus neapolitanus (Della Valle, 1853) is a pantropical, warm temperate species common on macroalgae (Tararam et al., 1986), seagrass (Scipione, 1999) and sponges (Serejo, 1998). This species was one of the most abundant within those associated to *Sargassum cymosum* C. Agardh, 1820 at Lamberto Beach, Ubatuba, (Leite, 1981; 1996a,b; Tararam and Wakabara, 1981) and other sites along the São Paulo coast, Brazil (Wakabara et al., 1983; Corbisier et al., 1991; Güth and Leite, 1998). Although, recent alteration of its density was observed in Lamberto Beach, probably related to an increase in human activities (Leite et al., subm.).

The aim of this study was to present same aspects of the reproductive biology and life history of this amphilochid gammarid since there is not much information about this species and to compare that with future studies.

MATERIALS AND METHODS

Sampling was performed in Lamberto Beach at Ubatuba, a low energy beach, situated in the innermost part of the Flamengo Bay (23°29'11"S-48°08'30"W) (Fig.1). Samples were collected monthly at night and day time during low tides, from January l972 up to January 1973, in the infralittoral fringe. The macroalgae *S. cymosum* was quickly placed in plastic bags at the collection

site. In the laboratory, the macro algae fronds were shacked in water buckets containing a few drops of formaldehyde. Subsequently the water was filtrated through a 285µm mesh sieve in order to retain all stages of amphipods. That residue was preserved into 70% ethanol and the animals were sorted out under a stereomicroscope.
The presence of oostegites in females and a pair of penis in males determined sex. Specimens that could not be sexed were assigned to the juvenile category.
The determination of the reproductive status of the females was based on the appearance, presence and condition of oostegites. Immature or non-ovigerous females had oostegites lacking setae. Ovigerous females had fully developed setose oostegites and eggs in the brood pouch.

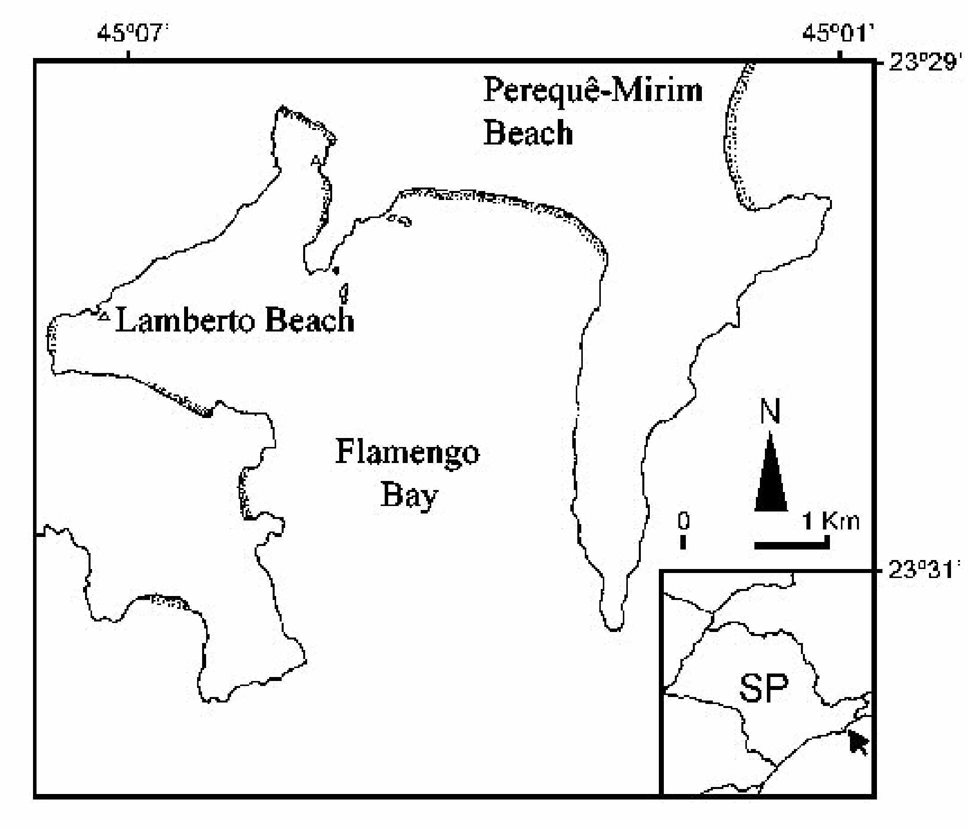

Figure 1 - Map of Flamengo Bay with the Lamberto Beach (São Paulo State)

Contents of the brood pouch were removed, counted, measured and separated in four arbitrary stages. Egg size was determined for the first stage (Leite and Wakabara, 1989). Regression analysis of eggs number (y) on head length (x) of ovigerous females was carried out. In order to minimize as far as possible the effect of egg loss and egg mortality within the brood pouch, only data on female carrying a closed marsupium have been included in this analysis.
Size was determined using a stereomicroscope fitted with camera lucida. Head length was measured from the anterior margin to the posterior edge and the total length from the anterior margin of the head to the anterior border of telson utilizing a lamina with a millimeter scale. As the relationship between total body length and head length was positively correlated ($y=0.029+0.092x$; $R^2=0.84$; $F_{1,56}=294.3$; $p<0.001$; $N=58$) head length was used instead of the total length, in view of the difficulty of measuring the amphipod's recurved body (Sutcliffe, 1965; Sheader and Chia, 1970; Wildish, 1972; Leite and Wakabara, 1989).

RESULTS

Total density of *Amphilocus neapolitanus*, and the densities of males, females and juveniles were higher at night (Wilcoxon paired-samples test; Z=2.551, p=0.001; Z=2.201, p=0.028; Z=2.551, p=0.001; Z=2.341; p=0.019), respectively (Fig.2). Density of non-ovigerous females was also higher at night (Z=2.587; p=0.010), differing from ovigerous females that showed similar density between sampling times periods (Z=1.293; p=0.96) (Fig.3).

Sex-ratio

A skewed sex ratio highly favorable to the females was encountered ($\chi 2$=1674.14; df=1; p<0.001). The proportion of males was variable over the sampling periods (months and day/night time) (Fig.4).
The mutual independence hypothesis among sexes, months and day/night time was tested using a multidimensional contingency table.

A high dependence was verified for these three variables ($\chi 2$=133.51; df=87; p<0.001). Partial independence (Zar, 1984) was tested between each variable and a combination of the two others. All comparisons revealed dependence among the tested pairs ($\chi 2$=1368.52; df=36, p<0.001; $\chi 2$=575.7; df=25; p<0.001; $\chi 2$=1093.0; df=25; p<0.001; respectively).

Life cycle observations

The analysis of life cycle of *Amphilocus neapolitanus* was based on the presence of juveniles, non-ovigerous and ovigerous females and males in the population (Figs.5, 6).

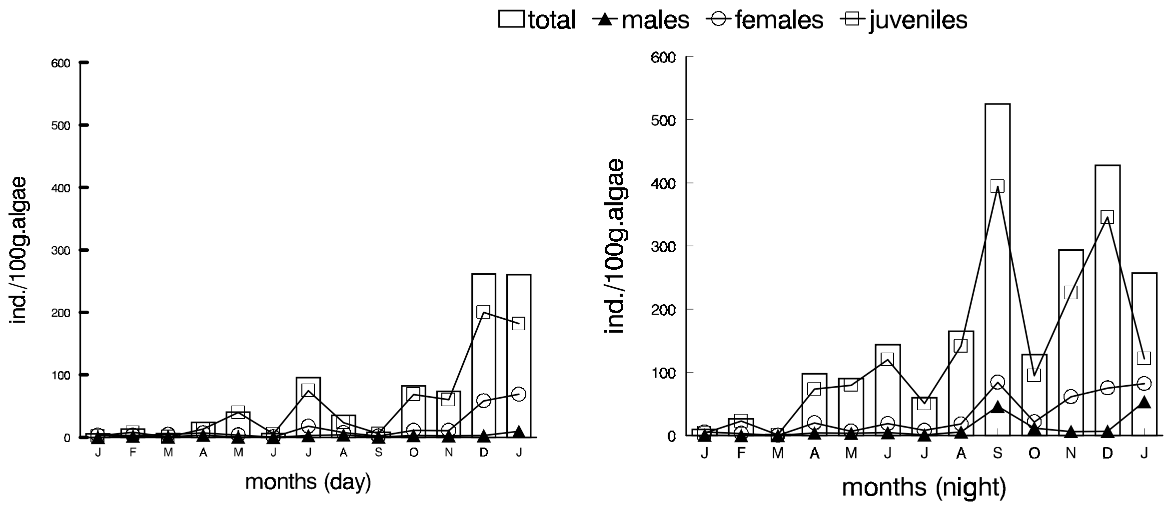

Figure 2 - *Amphilocus neapolitanus.* Day and night density (ind./100g wet algae) from January 1972 to January 1973.

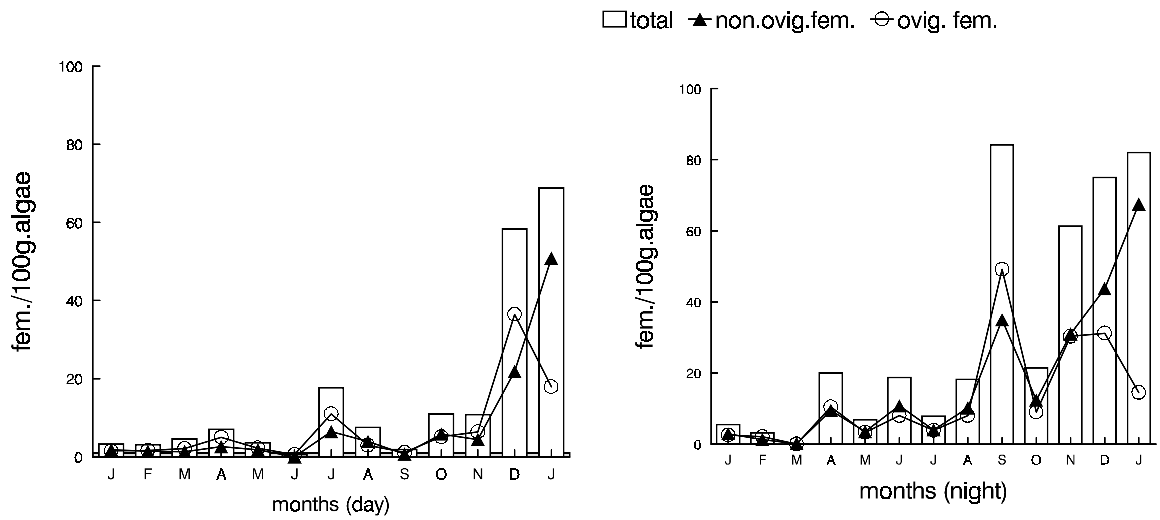

Figure 3 - *Amphilocus neapolitanus.* Day and night female density (ind./100g wet algae) from January 1972 to January 1973.

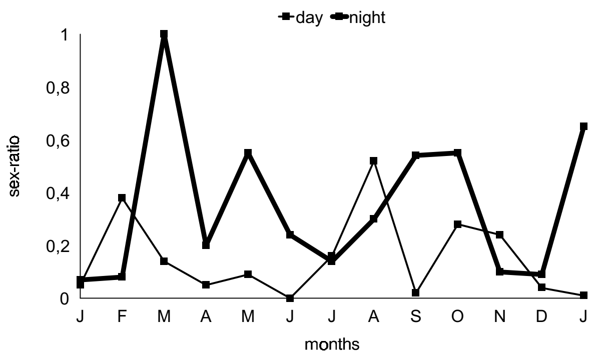

Figure 4 - *Amphilocus neapolitanus.* Day and night time sex ratio (♂:♀) from January 1972 to January 1973.

Juveniles (head length between 0.09 to 0.36mm) showed peaks of abundance in intermediate size classes. During February, June, August and December a bimodal size class distribution was observed.

The head length of non-ovigerous and ovigerous females ranged between 0.18 and 0.45mm. The unimodal peaks of the non-ovigerous females varied between the size classes of 0.24 and 0.33mm and those of the ovigerous females between 0.27 and 0.36mm. The males showed head lengths ranging between 0.21 and 0.42mm with unimodal peaks between the size classes of 0.27 and 0.36mm.

The juveniles were the most abundant group in the population throughout the year, with the exception of January and March 1972.

Breeding females occurred throughout the year and with percentages higher than 50% in the majority of months. Juveniles were present in higher percentages than ovigerous females. In January 1973, ovigerous females constituted only 20% of the females with a concomitant decrease in number of juveniles (Fig. 7).

The mean head size of ovigerous females was relatively constant through the year (Fig. 8) and ranged between 0.38 and 0.46 mm. The smallest mean lengths were obtained in January and March 1972.

Females between 0.27 and 0.37mm head length, carried in the brood pouch 1 to 7 eggs. The majority showed a number of 3 to 5 eggs. The clutch size was not a function of female's length (R^2=0.016; $F_{1,99}$=1.57; p<0.213; N=101). The equation obtained was y=0.948+5.017x.

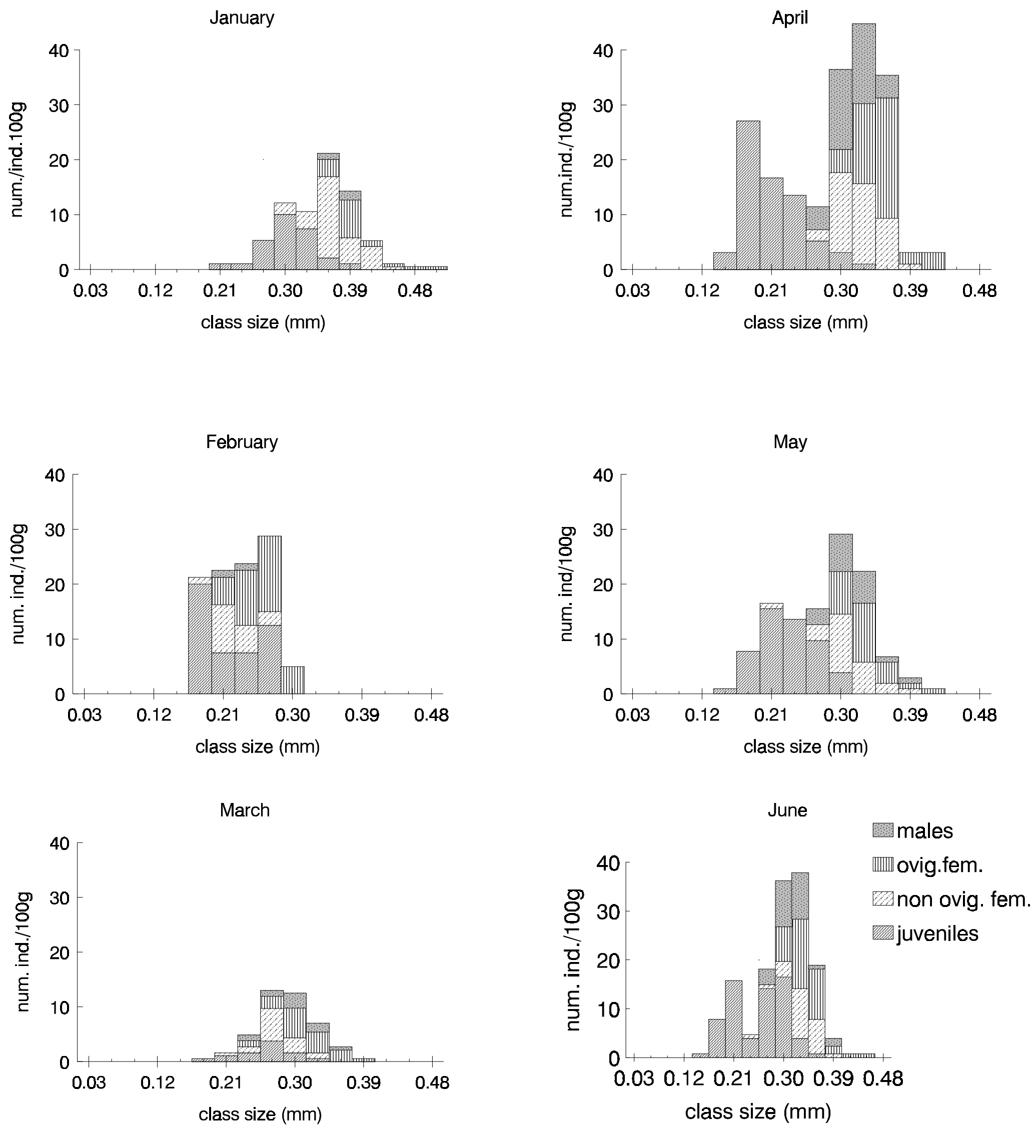

Figure 5 - *Amphilocus neapolitanus.* Size frequency histograms of juveniles, non-ovigerous female, ovigerous females and males (ind./100g wet algae) from January 1972 to January 1973.

DISCUSSION

The variation in the temporal abundance of vagile animals on macro algae is regulated mainly by seasonality in reproduction effort and by predation pressure. A minor role is played by physical factor (Stoner, 1980). Predation may be selective by sight feeding and is considered the principal factor promoting modifications in faunal assemblage and population decline (Stoner, 1980; Nelson, 1980; Van Dolah and Bird, 1980; Moore, 1981).
Allen (1978) hypothesized that the risk of predation is an increasing function of prey size and that the greater predation risk should occur in daylight. Therefore, larger benthic prey should be more constrained to nocturnal activity than smaller individuals (Newman and Waters, 1984). McBane and Croker (1984) noted that the relationship between a small body size with low fecundity in females of *Hyale nilssoni* may constitute an effective strategy of reducing predation by animals which tend to select larger prey (Nelson, 1980). In this way predation has an indirect effect on fecundity by reducing the mean size of the individuals of a given population (Sudo and Azeta, 1996).

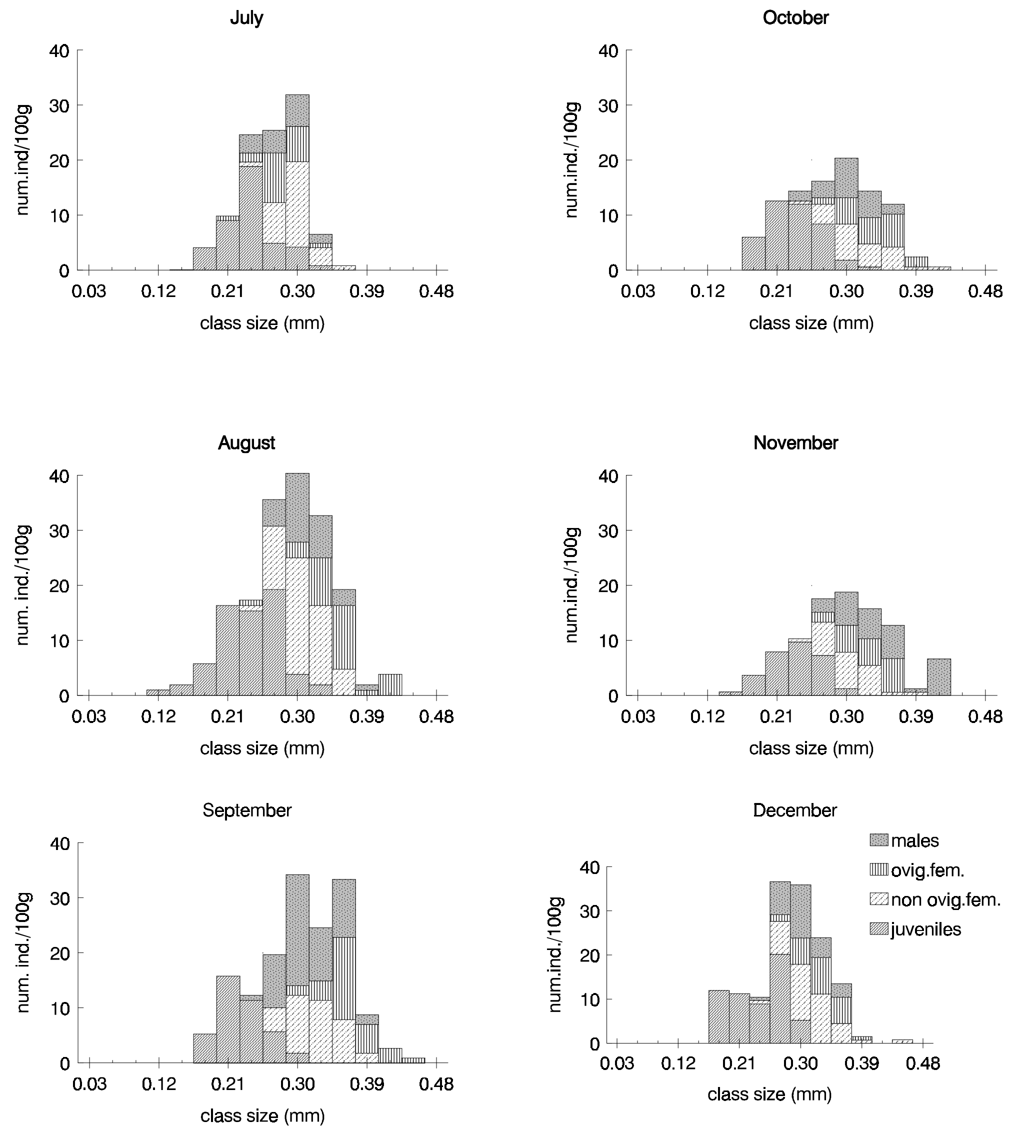

Figure 6 - *Amphilocus neapolitanus.* Size frequency histograms of juveniles, non-ovigerous female, ovigerous females and males (ind./100g wet algae) from January 1972 to January 1973.

The high density of *A. neapolitanus* among the gammarid species associated with *Sargassum cymosum* at Ubatuba (see also Leite, 1981) may be reflect avoidance from visual predators related to a small size. Size-selective predation on amphipods was demonstrated (Van Dolah, 1978; Nelson, 1979; Edgar; 1983) but the increase of habitat complexity of the macro algae, as *Sargassum* (e.g. Hacker and Steneck, 1990; Holmlund et al., 1990) enhanced protection for the prey and affect the foraging success of the predators (Holmlund et al., 1990; Sala, 1997).

Amphilocus neapolitanus, reproduces all over the year. More than one brood per female was expected since many ovigerous females of *A. neapolitanus* showed advanced ovarian development.

The small number of growth stages (Leite, 1996b) and small variations in the size of juveniles, males and females among monthly samples, suggest a short life cycle like other warm water species.

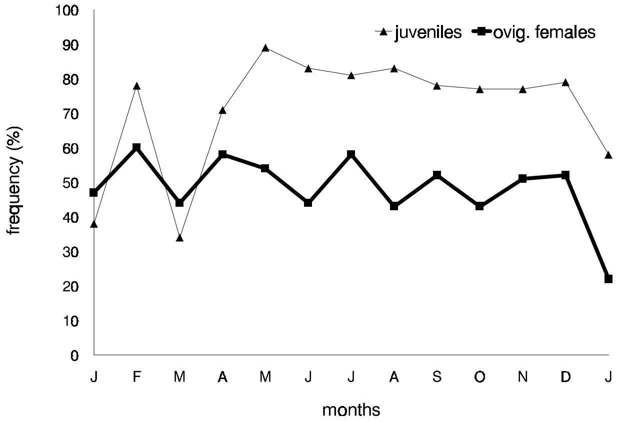

Figure 7 - *Amphilocus neapolitanus.* Frequency of ovigerous females to total females and frequency of juveniles to total population from January 1972 to January 1973.

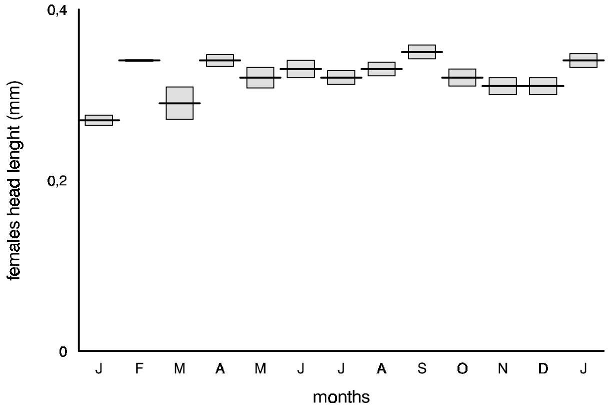

Figure 8 - *Amphilocus neapolitanus.* Mean head length size (mm) of ovigerous females from January 1972 to January 1973. Horizontal lines are the mean and vertical bars are standard deviations (SD); 95% CL.

Warm water species are generally characterized by multivoltinism, rapid growth, early maturity and short life span (e.g. Morino, 1978; Wildish, 1982; Sainte Marie, 1991). Such life history trait, often make it impossible for researchers to distinguish and track cohorts of amphipod species in warmer waters (Sudo and Azeta, 1996).

The lowest densities of *A. neapolitanus* were observed in the summer and early fall, especially in March, when males are absent and females showed the lower values of head mean size. Fluctuations in population density of gammarids are frequently observed and related to periods of intense reproduction (Marsden, 1991, Cardoso and Veloso, 1996). A similar pattern seems to occur with *A. neapolitanus* especially due to its associations with macroalgae and the stability of environmental conditions in tropical areas (Leite, 1981).

Usually, males and females show approximately equal numbers in populations. Skewed sex ratios may be affected by factors dependent on life cycle (Hamilton, 1967). Wenner (1972) referred to differential mortality, longevity and growth rate between sexes, while Vitagliano et al. (1996) attributed such differences to photoperiod or to microorganisms present in oocytes. Possibly one male of *A. neapolitanus* is able to fertilize several females as commonly observed in other gammarid species (Moore, 1981). There was no evidence of migration and sex reversal and the constant that monthly mean size of mature males may indicate males reproduce only once before dying (Kemp et al., 1985).

When the risk of adult mortality is low, the strategy to increase reproductive fitness would be to maximize energy expenditure per offspring to increase competitive fitness of species (Wildish, 1982). *Amphilocus neapolitanus* produced few and large eggs, probably resulting in a high number of surviving juveniles in the population. In an environment with relatively stable temperature and food availability breeding might be expected year round. *Amphilocus neapolitanus* matures quickly and many generations of reproductively active females can be produced in short periods of time.

The large egg size may indicate a longer incubation period for *A. neapolitanus* when compares to other gammaridean species with smaller egg size that occupy *S. cymosum*, (Leite, 1996a). This relationship between eggs size and ontogenetic development rate was demonstrated by Steele and Steele (1975) and by Marsden (1991). Cardoso and Veloso (1996) observed also a low fecundity of one sand hopper and related that to the decreased maturation size of the females. This, in turn, allows the production of more than one brood, therefore increasing their intrinsic population growth rate (Steele and Steele, 1975). The smallest ovigerous female of *A. neapolitanus* had a head length of 0.18mm (Leite, 1981). This small size and the observations about the presence of mature and colored ovaries, visible by transparency of the cuticle of ovigerous females and on the very low number of empty females in the population suggest continuous reproduction.

There was no correlation between the number of eggs and the size of the females, probably due to the narrow size range of the reproductive females (0.18-0.46mm of head length), to the large size and small number of eggs and, probably, to some egg loss.

It was not possible to determine the maximum life span of *A. neapolitanus* due the overlap of cohorts as explained by Sudo and Azeta (1996). It to supposed a short life span with a semi-annual life history in connection with tropical conditions (Morino, 1978; Wildish, 1982; Sainte-Marie, 1991) producing successive generations in a short period.

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RESUMO

A variação da densidade nos períodos diurno e noturno, aspectos da biologia da reprodução e do ciclo de vida de *Amphilocus neapolitanus* (Della Valle, 1853), espécie de anfípodo gamarídeo associada a *Sargassum cymosum* C. Agardh, 1820, foram estudados durante 13 meses. A razão sexual foi fortemente favorável para as fêmeas. Verificou-se que a densidade de jovens, machos e fêmeas foi significativamente mais alta nas amostras noturnas que nas diurnas. As fêmeas ovígeras estiveram presentes em densidades semelhantes tanto no período diurno como noturno enquanto que as não ovígeras foram mais numerosas no período noturno. Não foi obtida correlação entre o número de ovos com o tamanho das fêmeas ovígeras. A reprodução foi contínua ao longo do período estudado.

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