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Research Article

Population size of *Aegla paulensis* (Decapoda: Anomura: Aeglidae)

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ABSTRACT. We used the Schumacher-Eschmeyer method for closed populations to estimate and compare the population size of adults of *Aegla paulensis*, from Jaraguá State Park (São Paulo, Brazil), in two periods of the year with contrasting climatic conditions (late winter and late summer). The calculated density of adult individuals was considerably higher in the summer (11.5 ind m^{-2}) than in the winter (6.7 ind m^{-2}). This density difference of adult individuals was attributed to variation in the population structure of coexisting cohorts of adults at each sampling season of the year, due to dissimilarities in the cumulative abundance of recruits that effectively become adults after puberty molt, and difference in longevity between sexes.

Keywords: freshwater decapods, Aeglidae, population structure, mark and recapture, close population, Brazil.

Tamaño poblacional de *Aegla paulensis* (Decapoda: Anomura: Aeglidae)

RESUMEN. Se utilizó el método Schumacher-Eschmeyer para poblaciones cerradas, para estimar y comparar el tamaño poblacional de adultos de *Aegla paulensis*, de Jaraguá State Park (São Paulo, Brasil), durante dos períodos del año, con condiciones climáticas contrastantes (fines de invierno y fines de verano). La densidad de adultos fue considerablemente más alta en verano (11,5 ind m^{-2}) que en el invierno (6,7 ind m^{-2}). Esta diferencia en la densidad de individuos adultos fue atribuido a variaciones en la estructura poblacional de cohortes coexistentes durante cada una de las estaciones del año. Estas variaciones fueron debido a disimilaridades en la abundancia acumulativa de reclutas que efectivamente serán adultos, después de la muda de pubertad, y a diferencias en la longevidad entre los sexos.

Palabras clave: decápodos de agua dulce, Aeglidae, estructura poblacional, marcaje y recaptura, población cerrada, Brasil.

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INTRODUCTION

Mark-recapture technique has been frequently used in population studies of several freshwater decapods species. This technique proved to be a convenient tool for monitoring locomotor rhythm, use of space, dispersal, growth, and estimation of population size of several crayfish species (Acosta & Perry, 2000; Gherardi *et al.*, 2000; Robinson *et al.*, 2000; Parkyn *et al.*, 2002; Silva & Bueno, 2005; Nowicki *et al.*, 2008).

Mark-recapture technique has also been employed to estimate the population size of two aeglid species, *Aegla platensis* and *A. franca* (Bueno & Bond-Buckup, 2000; Bueno *et al.*, 2007). Regarding the latter species, Bueno *et al.* (2007) estimated the population size during two contrasting seasons of the year (summer and winter), and reported similar densities regardless of the season considered.

The freshwater anomuran decapod *Aegla paulensis* Schmitt, 1942 is endemic to Brazil. Distributional reports of the species include three distinct hydrographic basins (Bond-Buckup & Buckup, 1994). Biological information concerning this species is limited to two field studies (López, 1965; Cohen *et al.*, 2011). Both studies together provide a substantial amount of biological information related to reproductive period, fecundity and size of eggs, absolute and temporal variation of sex ratio, temporal size-class frequency distribution, growth, longevity, recruitment, and migration. The present study used the Schumacher-Eschmeyer method for closed populations (see Krebs, 1999 for details) to estimate and compare the population size of *Aegla paulensis* from a Conservation Unit (Jaraguá State Park, São Paulo, Brazil) sampled in two periods of the year with contrasting climatic conditions (winter and summer) to verify whether the results observed by Bueno *et al.* (2007) can be extended to other aeglid species.

MATERIALS AND METHODS

Field work

The study site is located at Pai Zé stream (23º27' 27.9"S, 46º45'32.3"W) in Jaraguá State Park, a Conservation Unit Area near the city of São Paulo, Brazil. Detailed description of the sampling site can be found in Cohen *et al.* (2011).

The Schumacher-Eschmeyer estimation method for closed populations was employed to estimate the population size in two different occasions: September 2009 (late winter) and in March 2010 (late summer), each one consisting of seven consecutive days of fieldwork.

To ensure the required working condition of a closed population, a section of the stream was isolated with 4 mm mesh fishing nets, installed across opposite margins in the upstream and downstream limits, thus preventing migration of aeglids during the experiment. The total length of the isolated working area was 77 m in September 2009 and 68 m in March 2010. In both periods, the stream had an average width of two meters in the isolated working area.

All fieldwork procedures and techniques followed those described by Bueno *et al.* (2007), and are here briefly described. Aeglid specimens were sampled with the aid of twenty baited plastic traps that were randomly distributed in the isolated working area. Bait consisted on fish flavored dried cat feed, placed inside a small perforated plastic canister in each trap, favoring dispersion of odor and preventing contact with food (Bueno *et al.*, 2007). To verify the integrity of the isolated area during each 7-day estimation period, additional traps were placed up and downstream beyond the boundary of the working area; the marked specimens caught by these traps would indicate isolation breach in the net, which would require immediate correction. All traps were set late in the afternoon and checked for caught specimens in the following morning. Used baits were discarded on dry land and replaced by new ones for the following sampling event. The total area required for the density calculations of the delimited section of the stream, was estimated as the sum of areas of successive contiguous trapezoids (length of margins = sides; widths of the stream = bases). These estimated areas were 141 m² and 125 m² in September 2009 and March 2010, respectively.

All captured aeglids were sexed according to the presence (females) or absence (males) of pleopods, and by the position of gonopores, located on the coxa of the third pair of pereopods in females (Martin & Abele, 1988). Carapace length (CL) of each specimen was measured with the aid of a digital caliper to the nearest 0.01 mm, from the orbital sinus to the mid-posterior border of the carapace.

The estimation of population size was based on adult individuals only. For practical reasons during field working conditions, the minimum size of adults for both sexes was arbitrarily fixed as 9.00 mm CL, based on the average size at the onset of morphometric maturity (ASOMM) in females (9.08 mm CL), as mentioned by Cohen *et al.* (2011). These specimens were marked with a mixture of super glue (cyano-acrylate) and commercial dye (silver fine powder) applied as a thin round spot on the dorsal region of the carapace (see Bueno *et al.*, 2007 for details). The use of this non-permanent marking in preference to wound inflicting semi-permanent ones, such as hot cauterization (Abrahamsson, 1965), or making incision or punching holes on external structures (López, 1965; Guan, 1997), is based on Bueno *et al.* (2007). These authors demonstrated that the impact of losing marks due to ecdysis may be considered negligible as long as (1) the sequential period of samplings is kept short (Seber, 1982), and (2) the calculation of population size is restricted to adults, which molt less frequently than juveniles. The population size estimation based on adults is in accordance to one of the criteria for the evaluation of the conservation status of endangered species, recommended by the IUCN (2010).

Soon after all measurements and observations were completed, all marked individuals were randomly, and as evenly as possible, released back into the isolated working area. A minimum time lapse of three hours was maintained between the release of marked animals and the installation of traps late in the evening. Animals with CL <9.00 mm were not marked and were released downstream and outside the boundaries of the working area.

Data analysis

All terms used in the Schumacher-Eschmeyer method are according to Krebs (1999). The estimation of population size was calculated by the formula:

$$\hat{N} = \frac{\sum_{t=1}^{s}(C_t M_t^2)}{\sum_{t=1}^{s}(R_t M_t)},$$

where $\hat{N}$ is the estimated population size; C_t is the total number of individuals captured in the t-th sampling; M_t is the accumulated number of marked animals by the time of the t-th sampling; R_t is the number of marked individuals captured in the t-th sampling; and s is the total number of samplings. The adequacy of the Schumacher-Eschmeyer method for each estimate was verified by applying through-the-origin regression (Zar, 1996) on the data points of the proportion of marked animals in samples ($Rt\ Ct^{-1}$ = X), and the accumulated number of marked animals (Mt = Y) (Krebs, 1999). A positive linear relationship between these variables is expected when the assumptions required to validate the method are met (Seber, 1982; Krebs, 1999). The 95% confidence limits were calculated from the normal approximation by using the critical value of the t distribution for s -2 degrees of freedom (see Krebs, 1999 for details).

Since ASOMM differed between sexes (9.08 mm of CL for females and 9.92 mm of CL for males; Cohen *et al.*, 2011), some individuals that could have been considered as juveniles were marked as adults and included in the calculations of the population size. The corrected population size estimate was calculated by deducting the total percentage of marked juveniles individuals of both sexes altogether (9.0 mm ≤ CL < ASOMM according to sex) from the general estimate value (CL ≥ 9 mm).

Analysis of body size structure of males and females were based on CL-class frequency distribution. For this purpose, all data were included, even from those aeglids with CL <9 mm. Cohort identification was based on a previous growth study of this population (see Cohen *et al.*, 2011 for details). The Bhattacharya-method routine of the FISAT II computer program (Version 1.2.0, Gayanillo *et al.*, 2005) was used to separate the cohorts-related normal components of the CL-class frequency distributions and to estimate their respective means.

Sex-ratio deviation from 1:1 was checked with the Yates-corrected goodness-of-fit chi-square test on data from adult specimens (Zar, 1996). All statistical analyses ($\alpha = 0.05$) followed Zar (1996).

RESULTS

Field data for late winter (September 2009) and late summer (March 2010) estimates are presented in Table 1. A total of 552 and 842 adults were marked in late winter and late summer, respectively. In late winter, females were captured in higher numbers (1.44 females per male, $\chi^2 = 17.76$; $P < 0.05$), while in late summer males and females were captured in similar rates ($\chi^2 = 1.81$; $P > 0.05$). No ovigerous females were sampled in both estimation periods. Only two marked aeglids (less than 0.2% of the total sampled), for each estimate period, were caught by the traps placed outside the working area. No newly-molted aeglids were captured. For both samplings, the Mt *vs* $Rt\ Ct^{-1}$ regression analyses provided significant results (late winter: $y = 0.0009x$, $r^2 = 0.692$, $P = 0.0002$; late summer: $y = 0.0855x$, $r^2 = 0.919$, $P = 0.000003$).

Estimated population size and density for adults can be found in Table 2. The highest density was observed in late summer. Both sampling seasons showed narrow confidence intervals around the estimated population size.

Overall size-class frequency distributions of males and females for both periods are shown in Figure 1. A total of 1,321 and 887 aeglids were sampled in late winter and late summer, respectively. In late winter, the majority of aeglids were juveniles (58.2%), whereas in late summer most sampled individuals were adults (94.9%). Two cohorts for males and three cohorts for females were distinguished in the late winter sample (Fig. 1). Except for a small cohort poorly represented (arrows; Fig. 1), the cohorts sampled in late summer (March 2010) were the same as those sampled in late winter (September 2009), but six months older. The mean CL of cohort-related normal components (CLnc) for males was 8.22 mm and 12.50 mm in late winter, and 11.52 mm and 15.29 mm in late summer. The CLnc for females was 8.16 mm, 11.57 mm, and 14.48 mm in late winter; and 10.58 mm, 12.80 mm, and 15.07 mm in late summer.

DISCUSSION

The Schumacher-Eschmeyer mark-recapture method for closed population is considered a very robust and useful estimator when multiple sequential samplings are involved (Krebs, 1999). In the present study, the highly significant Mt *vs* $Rt\ Ct^{-1}$ linear regression and the narrow range of the 95% confidence interval around the estimate obtained for each season studied (Table 2) are strong indicators that the required assumptions to validate the application of this method were fulfilled (see Krebs, 1999, for list of assumptions).

This population size estimation method has been previously employed to estimate the population size of

Table 1. Field data for the estimation of population density of *Aegla paulensis*, during late winter (September 2009) and late summer (March 2010). C_t: total number of aeglids caught in sample *t*; R_t: number of recaptures (previously marked aeglids) when caught in sample t; U_t: number of marked aeglids in sample *t*; M_t: accumulated number of marked aeglids before sample *t* is taken.

Sampling events	C_t	R_t	U_t	M_t	R_t/C_t
Late winter					
August 31st	223	0	223	0	0
September 1st	147	32	115	223	0.22
September 2nd	117	62	55	338	0.53
September 3rd	119	56	63	393	0.47
September 4th	143	55	88	456	0.38
September 5th	167	75	92	544	0.45
September 6th	160	82	-	636	0.51
Sum	1076	362	636		
Late summer					
March 13th	165	0	165	0	0
March 14th	235	41	194	165	0.17
March 15th	251	52	199	359	0.21
March 16th	201	86	115	558	0.43
March 17th	202	96	106	673	0.48
March 18th	174	82	92	779	0.47
March 19th	163	95	-	871	0.58
Sum	1391	452	871		

Table 2. *Aegla paulensis* population size and density in the isolated working area (Jaraguá State Park, São Paulo, Brazil). Estimations based on data of aeglids with CL ≥9 mm. (*) Estimated population size of adults calculated using their proportions in the samples.

Season of the year	Estimated population size	95% confidence interval	Proportion of adults (%)	Estimated population size of adults*	Area of the stream section (m^2)	Density of adults ($ind.m^{-2}$)
Late winter (Sept. 2009)	1088	857-1490	86,66	943	141	6,7
Late summer (March 2010)	1483	1314-1701	96,67	1434	125	11,5

Aegla franca from Claraval, state of Minas Gerais, Brazil. The reported densities for that species in summer and in winter were lower than those observed for *A. paulensis* in this study. Most important, however, is that the estimated densities showed a small variation between seasons of the year for *A. franca* (2.4 ind m^{-2} in the winter and 2.7 ind m^{-2} in the summer) (Bueno *et al*., 2007), but nearly doubled for *A. paulensis* (6.7 ind m^{-2} in late winter and 11.5 ind m^{-2} in late summer). Differences in the life cycle pattern and population structure between these species may account for the variation in density, or lack of it, as observed within a short lapse of time of approximately six months.

Both species have a marked seasonal reproductive period (austral autumn through mid- winter), that yields a single brooding and, therefore, a single recruitment pulse per year (Bueno & Shimizu, 2008; Cohen *et al*., 2011). However, upon reaching functional maturity, females of *A. franca* reproduce only once during lifetime at 21 months of age, in an estimated lifespan of less than 29 months (Bueno & Shimizu, 2008), whereas *A. paulensis* outlives *A. franca* by approximately one year. The estimated longevity of approximately 40 months of *A. paulensis* females secures that this species has full potential to reproduce twice during its lifetime (Cohen *et al*., 2011). Thus, the population structure of each species

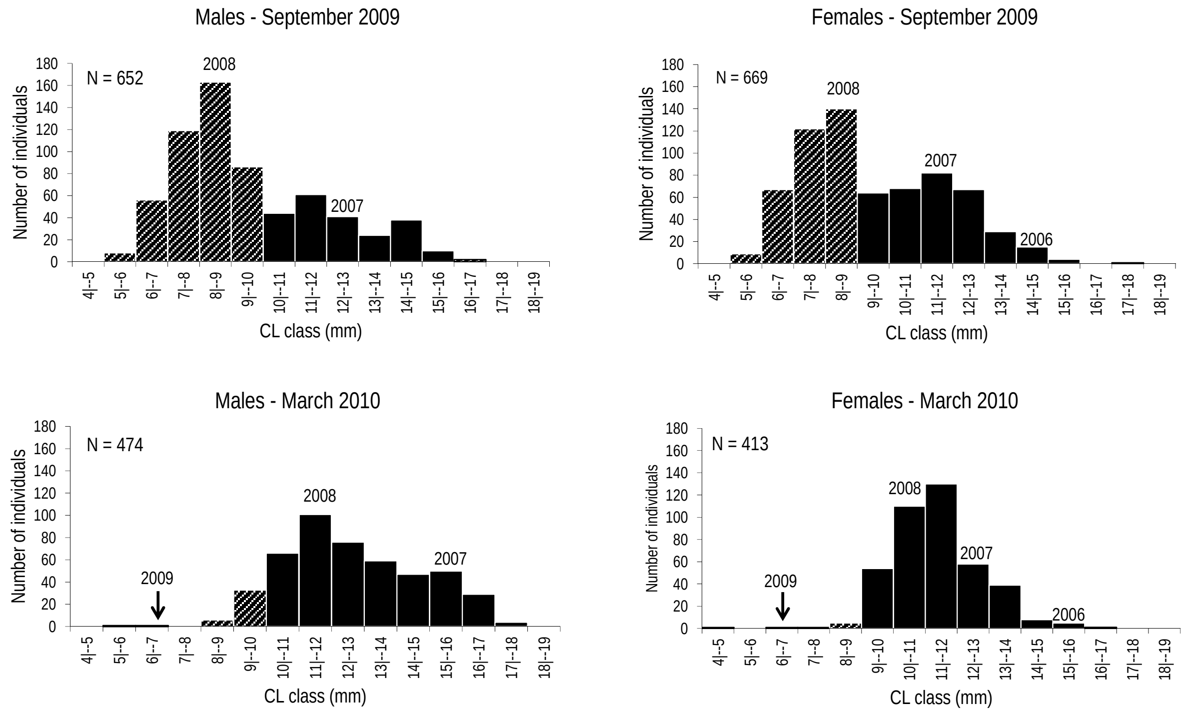

Figure 1. Size-class frequency distributions of males and females of *Aegla paulensis* sampled in Jaragua State Park, São Paulo (Brazil). Shaded bars correspond to adult size classes. Depicted numbers indicate the hatching years for each cohort sampled, and are placed above the size-class that contains the mean CL of the cohort-related normal components (Cohen *et al.*, 2011).

will also differ in terms of the number of coexisting cohorts of adults, regardless of the season of the year considered.

When the size-class frequency distribution of *Aegla franca* (Fig. 5 in Bueno *et al.*, 2007) is analyzed, it becomes clear that only a single adult cohort (males and females shown separately) is distinguishable, and it may explain the narrow temporal variation of adult densities. This variation may be attributed to differences in the cumulative abundance of recruits that effectively become adults after puberty molt, when each season sampling was carried out. The higher density value observed in the summer season, results from the pronounced shift in the proportion of adults in the population structure.

The size-class frequency distribution of *Aegla paulensis* differs from that of *A. franca* because it depicts two distinctly pronounced adult cohorts with a difference of one year of age between them (Fig. 1). Number of detected coexisting adult cohort at a given month may vary as a result of differences in longevity between sexes (Cohen *et al.*, 2011 and Figure 4 in this publication), which explains why females had an additional adult cohort per season, when compared to males (Fig. 1). Nevertheless, the same pattern regarding cumulative life stage transition at puberty molt described for *A. franca* (Bueno & Shimizu, 2009), can also be found in *A. paulensis*. In both sexes, most of the juveniles that bulked the sampled population in late winter 2009, presented itself as the youngest as well as the second most conspicuous and coexisting cohort of adults by late summer 2010. Therefore, the doubling of density observed in late summer of 2010 was caused by the sampling of individuals of both sexes from two well-pronounced coexisting adult cohorts, while only one conspicuous adult cohort was present (males) or well represented (females) previously in late winter of 2009.

Our results also suggest that adults of both sexes had an equal chance of being captured. In September (late winter), adult females were sampled in higher number than adult males (1.44:1). This observed predominance of females may be attributed to the presence of an older cohort of females (2006 in Fig. 1). Although this cohort was still present in March 2010, it was mostly formed by few senescent individuals belonging to a gradually dying off cohort (Cohen *et al.*, 2011), and had little or negligible effect on sex-ratio that did not depart from 1:1 (Fig. 1).

Future evaluations regarding the conservation status of aeglids should consider the temporal fluctuations of coexisting cohorts of adults, especially for species that exhibit a well-marked seasonal reproductive period, as has been demonstrated herein for *Aegla paulensis*. Therefore, we strongly recommend that field studies aiming at the determination of population size and density of aeglid populations should be preceded by previous and solid knowledge of the life cycle of the species, including patterns of reproductive period, recruitment, temporal variations in the population structure, observation of possible coexisting cohorts during the adult phase, growth and longevity, size at the onset of sexual maturity, and detection of possible differential behavior between sexes that might affect trap catchability (Cohen *et al.*, 2011). Previous knowledge of these pieces of information is important for planning experimental field studies and assisting data interpretations.

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