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Research Article

Biological parameters of the burrowing crayfish, *Parastacus pugnax* (Poeppig, 1835), in Tiuquilemu, Bío-Bío Region, Chile

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ABSTRACT. In order to determine the biological parameters of the burrowing crayfish (*Parastacus pugnax*), we fenced in 900 m^2 of a humid, low-lying sector in Tiuquilemu, Bío-Bío Region, Chile. Monthly samples were taken from August 2007 to August 2008, and 3,512 specimens were caught. Records were made of their carapace length (CL), total weight (TW), sex, and number of eggs (for females). The monthly size-structures showed few juveniles between 20 and 30 mm CL in nearly all months. *P. pugnax* was found to carry eggs from mid-spring through late autumn; size at first maturity for females was 38.1 mm CL; and fecundity varied between three and 45 eggs per female. In terms of the global sexual proportion, males were predominant in 11 of the 12 months, although the ratio was as expected (1:1). We estimated the parameters of the growth curve using modal progression analysis, obtaining CL_∞, K, and t_0 values of 55.3 mm, 0.23 mm year^{-1}, and -0.58 years, respectively. The length-weight relationship was calculated separately for males and females, and no significant differences were found between sexes. The common parameters defining the potential function of a = 0.00052 and b = 2.98 ($P < 0.05$) indicated isometric increments in weight.

Keywords: *Parastacus pugnax*, burrowing crayfish, growth, reproduction, Chile.

Parámetros biológicos del camarón excavador, *Parastacus pugnax* (Poeppig, 1835) en Tiuquilemu, Región del Bío-Bío, Chile

RESUMEN. Para determinar parámetros biológicos del camarón excavador (*Parastacus pugnax*), se cercó un área de 900 m² de un sector de vega en Tiuquilemu, Región del Bío-Bío Chile. En dicho lugar se efectuaron muestreos mensuales entre agosto de 2007 y agosto de 2008. A un total de 3.512 especímenes se les registró la longitud cefalotorácica (CL), peso total (TW), sexo y en hembras se determinó el número total de huevos. Se construyeron las estructuras de longitudes mensuales con escasa representación de juveniles entre 20 y 30 mm de CL, en casi la mayoría de los meses. Se determinó que el período de incubación en *P. pugnax* se extiende entre mediados de primavera y finales de otoño, la longitud de primera madurez en las hembras se calculó en 38,1 mm de CL y la fecundidad varió entre tres y 45 huevos por hembra. En cuanto a la proporción sexual global, hubo predominio de machos en 11 de los 12 meses; no obstante, estas no difieren de la razón esperada 1:1. Se estimaron los parámetros de la curva de crecimiento mediante el método de análisis de progresión modal, obteniéndose valores de CL_∞, K y t_0, correspondientes a 55,3 mm, 0,23 mm·año^{-1} y -0,58 años, respectivamente. La relación longitud-peso se calculó en machos y hembras separadamente, no existiendo diferencias significativas entre sexos, con parámetros comunes que definen la función potencial de a = 0,00052 y b = 2,98 ($P < 0,05$), estableciéndose que el crecimiento en peso es de tipo isométrico.

Palabras clave: *Parastacus pugnax*, camarón excavador, crecimiento, reproducción, Chile.

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INTRODUCTION

Six of the species of malacostracas inhabiting Chilean freshwaters belong to the family Parastacidae (Rudolph & Crandall, 2005, 2007; Jara *et al*., 2006): *Parastacus pugnax* (Poeppig, 1835), *P. nicoleti* (Philippi, 1882), *Samastacus spinifrons* (Philippi, 1882), *Virilastacus araucanius* (Faxon, 1914), *V. rucapihuelensis* (Rudolph & Crandall, 2005), and *V. retamali* (Rudolph & Crandall, 2007). Of these, *P.*

pugnax and *S. spinifrons* are intensely exploited for human consumption (Porter, 1904; Rudolph, 2002). Although the situation of *P. pugnax* is “vulnerable” throughout most of its distribution area (Bahamonde *et al*., 1998), few works have studied the biological aspects of this species; such studies are necessary to regulate its exploitation. Most research on *P. pugnax* concentrates on the study of the first postembryonic stages (Rudolph & Ríos, 1987), intersex (Rudolph, 1997; Martínez & Rudolph, 2011), proximate composition of the meat (Rudolph *et al*., 1991), and ecological aspects of the species (Arias & Muñoz, 1991; del Valle, 2002).

The burrowing crayfish (*P. pugnax*) is endemic to central-southern Chile (Jara *et al.,* 2006), where it is distributed from Río Aconcagua (32º50'S, 70º59'W) to Carahue (38º40'S, 73º09'W) (Rudolph, 1997). This crayfish builds underground galleries reaching up to 2 m depth in low-lying, semi-boggy ground known locally as *vegas* or *gualves* (Kilian, 1959; Bahamonde & López, 1963). These galleries may have from one to 14 entrances (del Valle, 2002). In winter, the artisanal exploitation of this crustacean provides an alternative income for local farmers (Arias & Muñoz, 1991; Rudolph *et al.,* 1991). At present, no official records track the exploited volume of *P. pugnax*. However, Silva & Spoerer (2006) report that each year some 43.5 million specimens are extracted and, if we consider that the average weight of the specimens sold is 30 g, then the approximate level of annual catches would be 1,260 ton, nearly one-third the volume of the nylon shrimp (*Heterocarpus reedi*) catches recorded in the same period (SERNAPESCA, 2005); *H. reedi* is one of the most important marine crustacean resources in terms of extraction volume in Chile.

The present work was developed given the scant information available on the population parameters for this crayfish. In order to determine the variations produced during an annual cycle, we isolated a section of low-lying ground in central-southern Chile inhabited by *P. pugnax*, as this allowed us to monitor a population undisturbed by human intervention. The research was aimed mainly at determining the growth, size structure, and reproductive aspects of *P. pugnax*. This information is necessary for the extractive management of the species and for determining its possibilities for culturing.

MATERIALS AND METHODS

The study was done in Tiuquilemu (36°22’S, 71°38’W), in the far north of the Bío Bío Region, Chile, where locals extract crayfish from the low-lying lands. The study area consisted of 900 m² enclosed with a fence made up of smooth and barbed wire and a nylon mesh sunk into the ground. This prevented the escape or entrance of crayfish during the study period.

Systematic monthly samples were taken from the fenced area between August 2007 and August 2008. During this period, 3,512 individuals were caught, pulling them from their tunnels with an artisanal vacuum pump. Females carrying eggs and adult specimens accompanied by offspring were examined in the field and returned immediately to their tunnels so as to keep the juveniles from their habitat for as short a time as possible, minimizing the impact on the studied population. For each specimen, we recorded the carapace length (CL) measured from the eye orbit to the furca of the carapace (Arias & Muñoz, 1991), total weight (TW), sex, and the total number of eggs carried by the females were recorded. Individuals fewer than 26 mm CL were categorized as undetermined, since it was not possible to distinguish the sex of these specimens with the naked eye (Rudolph, 1997).

Monthly size structures were constructed, grouping the measurements of CL in intervals of 1 mm. The size structures were done separately for males, total females, ovigerous females, undetermined, and total specimens (males, total females, and undetermined). In each case, the average length and standard deviation were estimated.

The length-weight ratio was determined separately for *P. pugnax* males and females, using the power function: $TW_i = a\ CL_i^b$, where TW_i and CL_i are the total weight and cephalothoracic length of the i-th individual, and *a* and *b* are the parameters of the potential function. Student’s t-test ($t_{\alpha/2,\ n-2}$) was used to establish the type of relative growth (allometric–isometric) presented by this species (Dixon & Massey, 1957). Moreover, an F-test$_{(1-\alpha;\ 2;\ n1+n2-4)}$ was used to compare the curves of the length-weight ratio for the two sexes (Neter & Wasserman, 1974).

The global sexual proportion and sexual proportion with respect to length were calculated monthly. In the latter case, a null hypothesis (H_0) that the proportion of the sample is equals to the theoretical proportion expected of 50% of the males and an equal proportion of females was cpnsidered. The test statistic was defined by:

$$Z_c = \frac{\hat{p} - P_0}{\sqrt{\frac{P(1-P_0)}{n}}}$$

Thus, the null hypothesis was rejected if $Z_c > Z_t$ with α = 5% and one degree of freedom.

The egg-carrying period was determined according to the monthly percentage of ovigerous females over the total females in the sample. To define the period of maximum incidence of ovigerous females (females carrying eggs + females with embryos + juveniles 1 and 2), we considered the periods in which we found percentages equal to or greater than 25% of females incubating eggs (Arana *et al.,* 1976). Later, we established the relationship existing between the number of eggs and the cephalothoracic length of the carrying females (Arana & Tiffou, 1970; Arana *et al.,* 1985; Sánchez *et al*., 2008) was established. In order to determine the expression that best represents the ratio between CL and fecundity, five mathematical models used previously by Palma & Arana (1997), were tested.

To determine the size at first maturity ($SFM_{50\%}$), we calculated the proportion of ovigerous females (*OF*) with respect to the total number of females in each range of CL of 1 mm was calculated. These data were fit to a sigmoid curve given by the expression:

$$\%OF_t = \frac{100}{1 + e^{(a - b \cdot CL_t)}}$$

where *%OF* corresponds to the percentage of ovigerous females at each length, and *a* and *b* are the parameters of the adjusted model.

The Von Bertalanffy growth curve and its parameters (CL_∞, K, t_0) were determined using modal progression analysis. The modal groups were identified using the Bhattacharya method (1967). Once the parameter CL_∞ was determined, the estimate of t_0 was done through the graph and the equation proposed by Von Bertalanffy (1934), whose expression corresponds to:

$$-\ln\left(1 - \frac{CL(t)}{CL_\infty}\right) = -K \cdot t_0 + K \cdot t$$

RESULTS

Undetermined specimens of *P. pugnax* ranged in size from 5.5 to 26 mm CL. It was possible to sex individuals between 25.5 and 53.7 mm (males) and 26.7 and 52.8 mm (females). The average carapace length varied monthly between 38.8 and 42.2 mm (males) and between 36.2 and 44.4 mm (females) (Table 1).

The size-frequency distributions showed poly-modal structures in most months. Bi-modal structures were observed for both males and females in December 2007. In the period analyzed, specimens between 20 and 30 mm CL were scarce, and between August and October 2007 and February and June 2008, no specimens were recorded between 20 and 25 mm CL (Fig. 1).

The potential fit of the length-weight relationship revealed values of 0.00053 and 2.97 in males and 0.00050 and 2.99 in females, corresponding to the parameters *a* and *b*, respectively ($P < 0.05$) (Figs. 2a-2b). No significant differences were found between sexes in this ratio ($F_c = 0.29 < F_{t(0.05;\ 2;\ 1391)} = 3.002$). Thus, the common equation was defined by the parameters $a = 0.00052$ and $b = 2.98$ ($P < 0.05$) (Fig. 2c). The isometric test determined isometric growth in *P. pugnax* ($t_c = 1.54 < t_t = 1.96$).

In general, the global monthly sexual proportion showed a slight predominance of males; the proportion of females was only greater in November 2007. Nonetheless, no significant differences were found in most samples with respect to the expected relationship of 1:1 ($P < 0.05$); the only exceptions occurred in March and May 2008. As for the sexual proportion with respect to the length, males were relatively predominant in all months for most of the sizes considered in the samples (Fig. 3).

The presence of ovigerous females was recorded for five consecutive months, with the exception of January, when no sampling was carried out. Thus, the greater number of ovigerous females in summer was noteworthy. On the contrary, no ovigerous females were recorded from August to October 2007 or from April to August 2008. The maximum period of egg-carrying occurred between December and April 2008, and the percentage of ovigerous females was highest in February of this same year (Fig. 4).

The fecundity of *P. pugnax* was estimated by analyzing 145 ovigerous females whose length ranged from 27.9 to 52.4 mm CL. Of these, individual fecundity varied between five and 46 eggs. According to the different mathematical models used to define the relationship between cephalothoracic length and the total number of eggs (*TNE*) carried by the females, it was determined that the data best fit to the linear model $TNE = -26.76 + 1.31\ CL$ ($R^2 = 0.8$, $P < 0.05$) (Fig. 5).

The presence of mature females was determined as of 28 mm CL, and as of 47 mm CL, 100% of the females were found in this condition. Therefore, it was not necessary to re-scale the data in order to fit them to the maturity ogive. Thus, the length was determined at first maturity to be 38.1 mm CL (Fig. 6).

The parameters of the Von Bertalanffy growth curve, estimated using modal progression analysis (Fig. 7), were $CL_\infty = 55.3$ mm, $K = 0.23$ mm year^{-1}, and $t_0 = -0.58$ (Fig. 8).

Table 1. Average monthly carapace length calculated separately for *P. pugnax* males and females.

Tabla 1. Longitud cefalotorácica promedio mensual calculada separadamente en machos y hembras de *P. pugnax*.

Month/year	Mean carapace length (mm)							
	Males	Standard deviation	Females	Standard deviation	Undetermined	Standard deviation	Total	Standard deviation
August 2007	39.1	5.3	36.6	4.9	8.7	2.2	42.0	5.7
September	39.5	4.2	36.2	5.5	9.1	1.9	38.0	5.1
October	40.1	5.5	41.0	3.0	10.1	1.3	40.5	4.5
November	39.9	4.4	39.8	4.7	11.2	2.0	39.9	4.5
December	41.5	5.2	42.0	4.4	14.9	2.3	41.7	4.9
February 2008	40.8	5.6	40.9	4.7	15.3	2.7	40.8	5.2
March	38.8	5.5	40.2	5.7	7.4	4.1	39.3	5.6
April	42.1	5.8	40.0	5.9	9.0	4.3	41.2	5.9
Mayo	42.2	7.8	44.4	5.4	9.5	4.9	43.0	7.0
June	40.0	4.7	40.7	5.1	11.7	3.6	40.3	4.9
July	40.0	4.4	41.1	4.8	11.3	3.6	40.6	4.6
August	39.0	5.9	38.9	5.0	12.4	4.3	39.0	5.4

DISCUSSION

In this study, *P. pugnax* ranged in length from 5.5 to 53.7 mm CL. This was similar to the lengths reported by del Valle (2002) of 4.7 to 52 mm CL, but different from those found by Arias & Muñoz (1991) and Rudolph (1997) of 8 to 49 mm and 6.6 to 47.4 mm CL, respectively.

In general, the average carapace length of *P. pugnax* females was greater than that of the males; the average CL was greater for males in only three of the 12 samplings (Table 1). This could be associated with the fact that the females are prepared physically to carry eggs and care for offspring, as shown by Rudolph (1997), who determined that females have abdomens that are, on average, wider and longer than the males.

The size structure revealed, in most months, at least three modal groups. In March 2008, it was observed the appearance of a new group, due possibly to a prior cohort. The size structures of adult specimens were identified as of December 2007, which could possibly be associated with the larger sample size taken on this date. The low proportion of specimens between 20 and 30 mm CL determined in most of the samplings is similar to that found by Arias & Muñoz (1991), who recorded a similar decrease in the percentage frequencies, especially in the size range between 16 and 25 mm CL. Rudolph (1997) did not register the presence of specimens between 20 and 25 mm CL in November 1994, or June and October 1995, but did detect specimens –albeit a low quantity– in this length range in most of the remaining months. Likewise, the *P. pugnax* size structures presented by del Valle (2002) reveal a low amount of specimens between 21 and 31 mm CL.

This particular length structure observed in *P. pugnax,* still has no clear explanation. Nonetheless, a first hypothesis is that the specimens reach between 12 and 18 mm CL in winter and begin to dig small holes off the parental tunnels or galleries (Del Valle, 2002). This would hinder their extraction and at the same time reduce their probability of being caught until reaching a larger size.

The estimated length-weight ratio indicated no statistically significant differences between the ratios established for the two sexes, further determining that the growth of this species is isometric in type ($b \approx 3$). This result differs from that obtained for *Samastacus spinifrons*, which showed isometric growth in the males and negative allometric growth in the females (Bocic *et al*., 1988). Thus, there is no common pattern in the length-weight ratio for species of a single family or sexes of a single species.

The global sexual proportion, in general, was slightly predominated by males in most months of this study. Nonetheless, the proportion of males and females did not differ from the expected ratio of 1:1, which agreed with the results obtained by Rudolph (1997) for specimens over 25 mm CL. Likewise, Rudolph (2002) studied the global sexual proportion

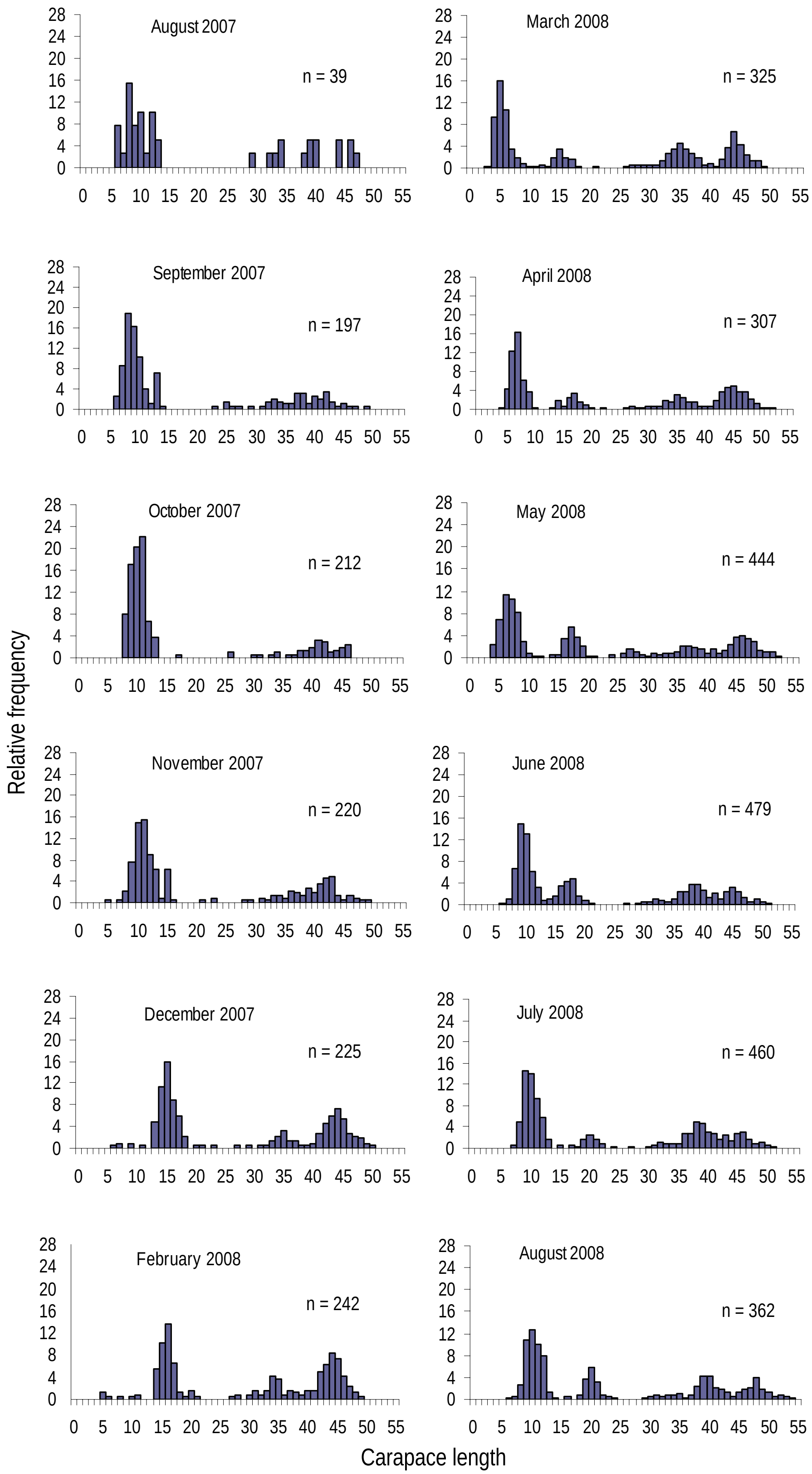

Figure 1. Distribution of size frequency in all *P. pugnax* specimens (males + females + undetermined).

Figura 1. Distribución de frecuencia de tallas en el total de ejemplares de *P. pugnax* (machos + hembras + indeterminados).

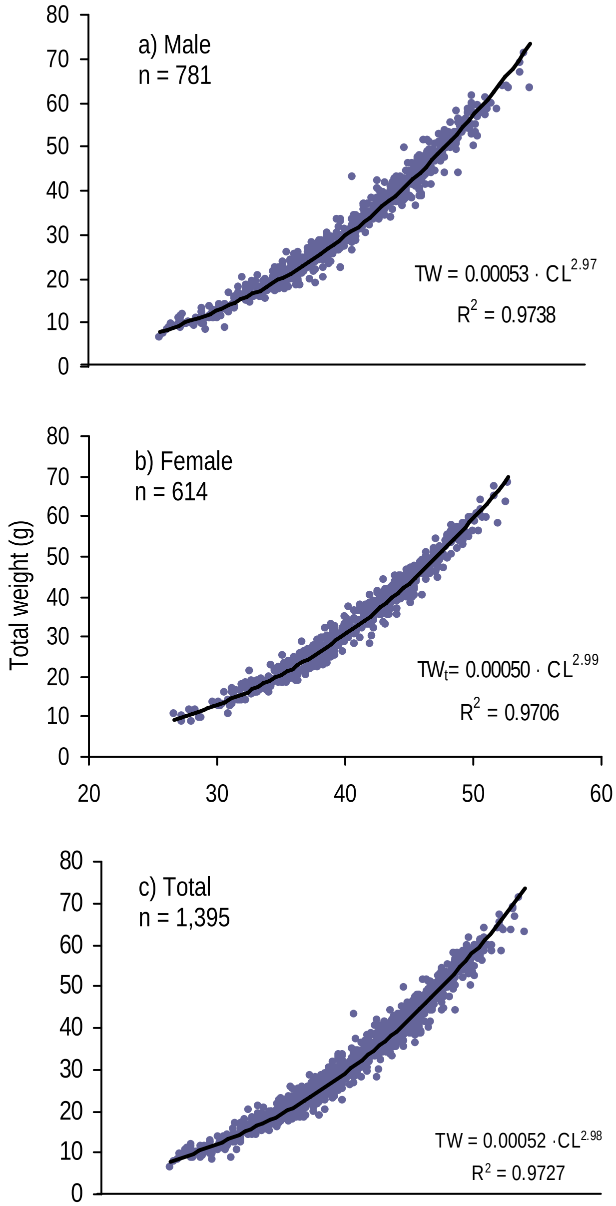

Figure 2. Length-weight relationship of *P. pugnax* in a) males, b) females, c) total.

Figura 2. Relación talla-peso de *P. pugnax* en a) machos, b) hembras, c) total.

in *S. spinifrons*, determining that, in general, males were predominant. However, these proportions were as expected (1:1) for the sexes. The same was true of *Parastacus defossus* (Noro & Buckup, 2009), *P. brasiliensis* (Fontoura & Buckup, 1989a, 1989b), and other Parastacidae species (Noro & Buckup, 2009).

The sexual proportions with respect to the length, in general, showed predominance of males in most length ranges. This coincided with that determined by Rudolph (1997) for some class-size intervals; that author found that males were predominant in length ranges < 26 mm CL and between 40 and 49.9 mm CL.

The results showed the presence of ovigerous females between November 2007 and May 2008, with a maximum proportion of specimens in this condition in February 2007. In another study, Del Valle (2002) caught one ovigerous female in December 1999 and two females in the post-embryonic incubation phase in January 2000. Moreover, Rudolph (1997) recorded the presence of ovigerous females in October, November, and January, similar to the period established in the present study. Thus, it is possible to deduce that the period of egg-carrying for *P. pugnax* lasts from mid-spring to the end of autumn. Our study differs from those of the aforementioned authors in terms of the proportion of females with eggs: whereas it was found a maximum of 67.2% of the females were ovigerous (February 2008), Del Valle (2002) recorded one ovigerous female and only 10% of the females in the study by Rudolph (1997) were carrying eggs.

The size at first maturity of *P. pugnax* was estimated to be 38.1 mm CL, similar to that presented by Rudolph (1997), who did not record the presence of ovigerous females under 30 mm CL. The same author indicated that the maturity of the females begat at approximately 26 mm CL, the size at which the pubertal molt occurs, agreeing with that registered in the present investigation, in which ovigerous females under 28 mm CL were not recorded.

The fecundity determined herein was lower than the maximum found previously for this species. Low fecundity is characteristic of crayfish with semi-terrestrial lives, as compared with others such as *S. spinifrons*; this fact is related to the direct development that characterizes these freshwater crustaceans (Rudolph, 2002) and the extensive care of their offspring (Del Valle, 2002). Histological analyses have shown this species to have a maximum of 100 oocytes (E. Rudolph, *pers. comm.*), allowing us to suppose that it is possible to increase their fecundity under special culturing conditions.

In this study, females with a minimum of three and a maximum of 43 eggs were found. These results coincide with those presented by other authors on *P. pugnax*, whose range of fecundity varied between 6 and 71 eggs (Dalannais, 1984; Rudolph & Ríos, 1987; Rudolph, 1997; Del Valle, 2002). As in studies of other freshwater shrimp species, this relationship was best represented by a linear function (Sanz, 1986; Rudolph, 2002; Alvarez & Rangel, 2007).

In terms of the parameters of the estimated growth curve, the value of CL_∞ was similar to the maximum values of CL found in the studies of this species done

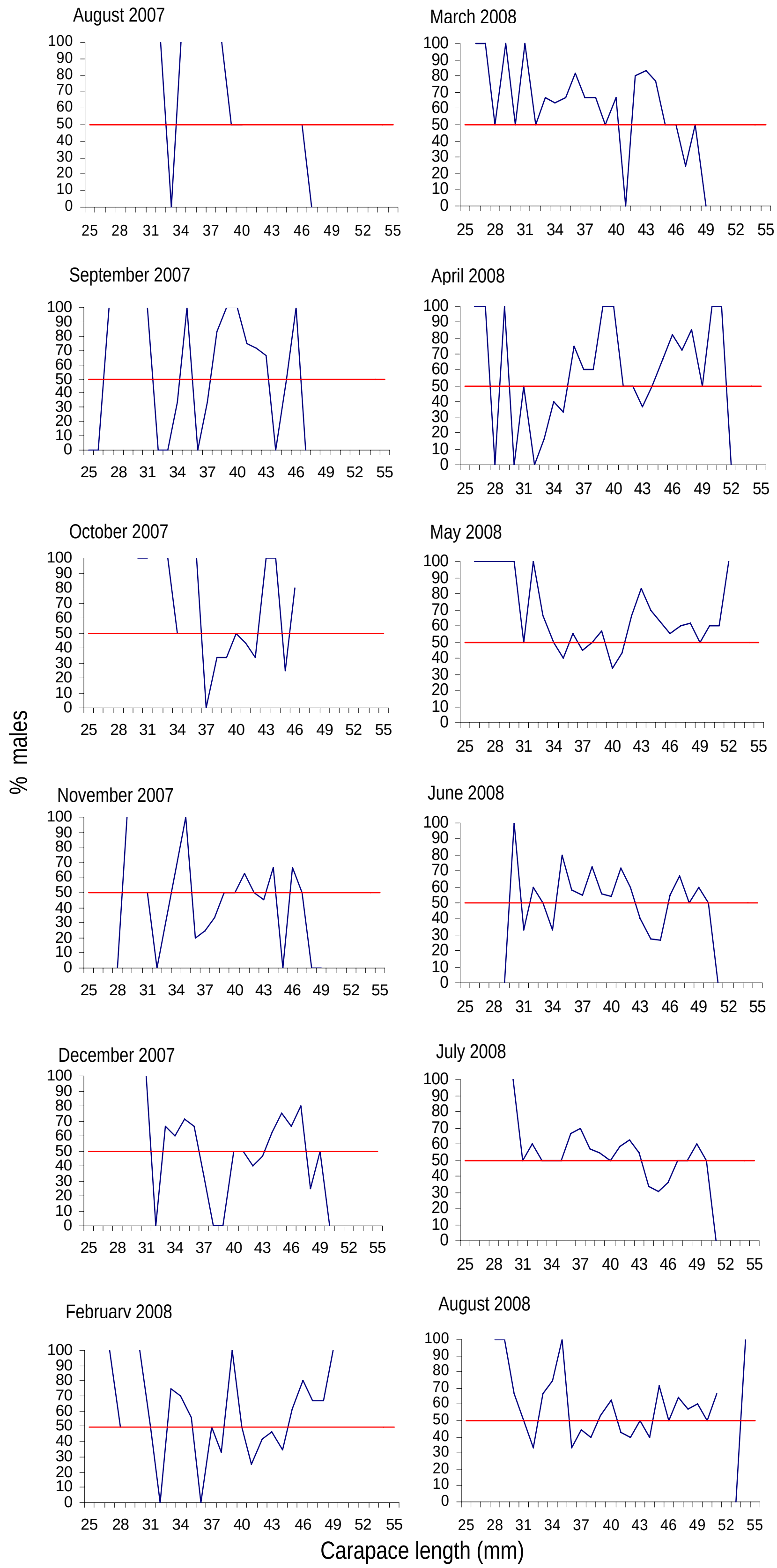

Figure 3. Sexual proportion at size per month in *P. pugnax*.

Figura 3. Proporción sexual a la talla por mes, en *P. pugnax*.

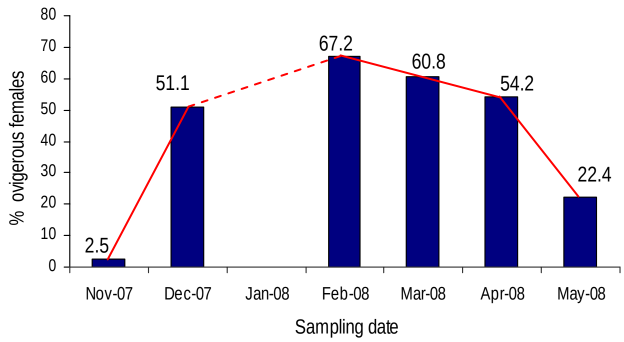

Figure 4. Monthly percentage of ovigerous females of *P. pugnax*.

Figura 4. Porcentaje mensual de hembras ovíferas de *P. pugnax*.

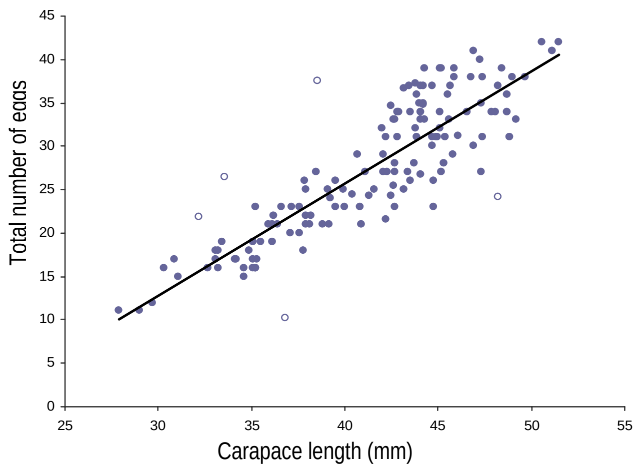

Figure 5. Relationship between carapace length and number of eggs carried by *P. pugnax* females.

Figura 5. Relación entre longitud cefalotorácica y número de huevos portados por hembras de *P. pugnax*.

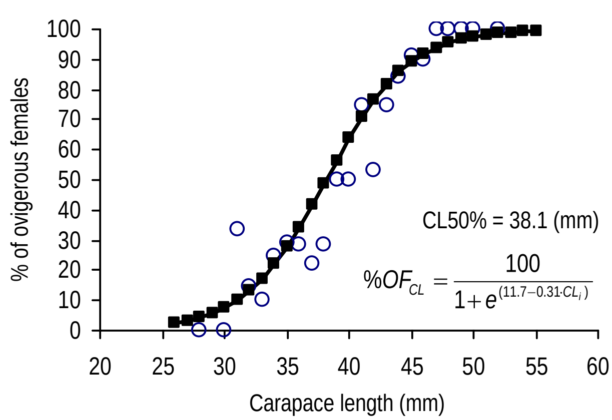

Figure 6. Adjusted sigmoid curve to determine the size at first sexual maturity in burrowing crayfish *P. pugnax*.
Figura 6. Curva sigmoidea ajustada, para determinar la talla de primera madurez sexual en camarón excavador *P. pugnax*.

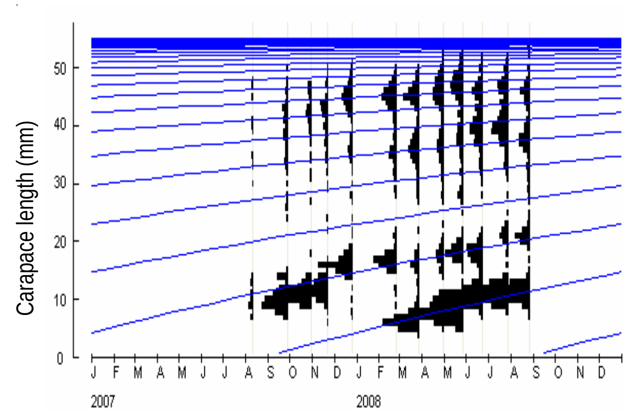

Figure 7. Modal progression analysis with the distributions of size frequencies determined in *P. pugnax,* between August 2007 and August 2008.

Figura 7. Análisis de progresión modal con las distribuciones de frecuencias de tallas en *P. pugnax,* determinadas entre agosto 2007 y agosto 2008.

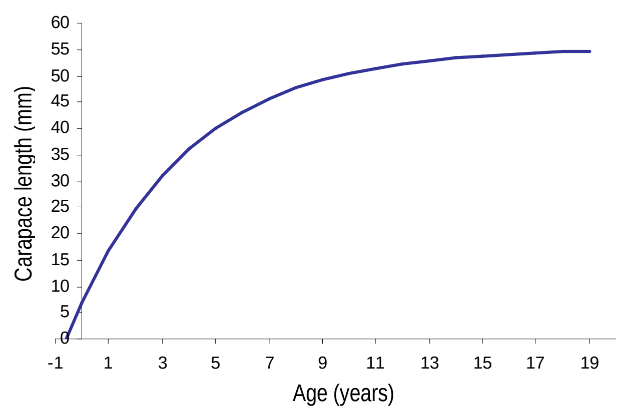

Figure 8. Growth curve of *P. pugnax*.

Figura 8. Curva de crecimiento de *P. pugnax*.

by Arias & Muñoz (1991), Rudolph (1997), and Del Valle (2002). The differences between the growth curves found in this study and those estimated by Del Valle (2002) may have been influenced by the different sampling designs applied in the two studies: in the former, individuals were collected from different arbitrarily selected zones in low-lying lands whereas in the latter, a systematic sampling design was applied over a single population.

Moreover, it was not possible to differentiate the size structure of the adult specimens presented by Del Valle (2002), which could affect the modal progression analysis, which is based on the differences of the average sizes or modal lengths of the cohorts born in different years or seasons (Sparre & Venema, 1997). It should be noted that the low-lying lands sampled in the Del Valle (2002) study were used for agricultural activities, modifying the environment of the resource. This factor could have determined the differences found between the growths curves of these two studies.

The value of CL_∞ estimated in this study (55.3 mm) was similar to that determined by Ibarra & Arana (2011) (55.9 mm) through the technique of marking and re-capturing. Nonetheless, certain differences were noticed between the growth rates (*K*) estimated in both studies, which caused discrepancies in the time required by these species to reach the salable size of 30 mm CL that has been established for *S. spinifrons* (Rudolph *et al*., 2010). According to Ibarra & Arana (2011), this would be reached in 3.1 years and according to the present study, in 1.9 years. This discrepancy could be attributed to a possible negative effect in growth when using tags adhered to the body of the organisms or rather to an overestimate of the age when using the modal progression method, as noted by different authors (*e.g*. Morales-Nin, 1989; Keyl *et al*., 2011).

According to our results, the growth, maximum length, and reproduction of *P. pugnax* are similar to those of *S. spinifrons*, a Parastacidae known to have a high potential for farming (Rudolph *et al*., 2010). *S. spinifrons* can reach a maximum length of 62.2 mm CL (Rudolph, 2002) and has fecundity of up to 192 eggs per female, post-embryonic development, and extensive parental care (Rudolph *et al*., 2010), among other characteristics. Thus, *P. pugnax* could also be considered to be in this category, and the data generated and gathered in this study could be used to create proposals intending to diversify Chilean aquaculture to include astaciculture and, specifically, *P. pugnax*.

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