Comparison of three quick methods to estimate crab size in the land crabs *Cardisoma guanhumi* Latreille, 1825 and *Ucides cordatus* (Crustacea: Brachyura: Gecarcinidae and Ucididae)

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Abstract: Quick, reliable and non destructive methods are necessary to estimate size structure on commercial land crabs, in order to acquire relevant information concerning the health of their populations. *Cardisoma guanhumi* and *Ucides cordatus* are two land crabs that are exploited at a high scale and also in an artisan way in the Caribbean area and in the coasts of Brazil, which populations are endangered due to uncontrolled exploitation. The purpose of this work is to provide various methods to estimate indirectly crab body size. Sampling was carried out in Carenero (*C. guanhumi*) and Cumaná (*U. cordatus*) (Venezuela). For each species, three methods were used to measure burrow diameter (Vernier, internal spring caliper and photograph), and these were correlated with real body size of the crabs. Model II linear regression analyzes, i.e. Ordinary Least Squares and Mayor Axis, were used to build and test the performance of forecasting models. *Cardisoma guanhumi* showed a high bivariate data dispersion using Vernier and photo measuring methods, increasing these towards larger animals. Less dispersion was achieved with the spring caliper method; this resulted in the most accurate measurements of indirectly estimated body size in *C. guanhumi* (r^2= 0.61), whereas Vernier measurements were the least precise. On the other hand, all three methods gave reliable estimates for *U. cordatus*, being the Vernier method the most accurate (r^2= 0.71). However, in both species, all forecasting equations overestimated the size of smaller crabs (those below the mean) but underestimated the size of larger crabs. Nevertheless, all three methods were statistically significant for each of the species, and looking at the above mentioned under- and overestimations, they can serve as reliable and fast non-destructive tools to be used by resource managers and field biologists to acquire size structure information concerning these two species. Vernier and internal spring caliper methods are recommended for relative small sampling areas, while photo method is suggested to be used in very extensive sampling regions. Rev. Biol. Trop. 60 (Suppl. 1): 139-149. Epub 2012 March 01.

Key words: Indirect body size measuring methods, *Cardisoma guanhumi*, *Ucides cordatus*, Vernier measurement, internal spring caliper measurement, photographic measurement.

Population structure and density estimates of commercial land crabs are a need of considerable importance, in order to establish their population status and define management politics. The land crab *Cardisoma guanhumi* Latreille 1825 has been the target of commercial exploitation and in an artisan way in several Caribbean countries and in Brazil. In Puerto Rico, there has been a decline of *C. guanhumi* since 1960 (Matos 1997). Moreover, in Cuba, there was a 75% drop and more in the fisheries of this land crab between the decades of 1980 and 1990 (Baisre 2000). Furthermore, in Brazil, *C. guanhumi* has also been under strong fishery pressure, also being endangered and overexploited (Amaral & Jablonski 2005). Specifically in Venezuela, *C. guanhumi* has been commercially captured and exported since the seventies (Taissoun 1974) without official harvesting statistics. The second largest land crab in the Caribbean and in Brazil, *Ucides cordatus*, has also been extensively exploited in this country (which exhibits the highest populations of this species in the American continent) (Nóbrega Alves & Nishida 2003, Jankowsky et al. 2006, Pimentel Rocha et al. 2008) and in the Orinoco Delta-Venezuela (Novoa 2000). In the latter, *C. guanhumi* and *U. cordatus* are illegally extracted and exported to Trinidad without any regulations (Novoa 2000).

Although there are several studies dealing with the population biology of these land crabs, population estimations have been mainly done through the extraction of the animals and destruction of their habitats (Warner 1969, Oliviera Botelho et al. 2001). Only in few cases population structure was determined using indirect methods to estimate the body size of *C. guanhumi* (Govender & Rodríguez-Fourquet 2000) and as well as of *U. cordatus* (Schmidt et al. 2008). Indirect crab size estimation has been recently applied with a higher frequency to several burrowing species, such as in the fiddler crabs *Uca spinicarpa* Rathbun, 1900 and *U. longisignalis* Salmon and Atsaides, 1968 (Mouton & Felder 1996), *U. tangeri* Leach, 1814 (Lourenço et al. 2000), and *U. annulipes* H. Milne Edwards, 1937 (Skov & Hartnoll 2001), in the ocypodid crabs *Dotilla myctiroides* Stimpson, 1858 (Lee & Lim 2004) and the soldier crab *Heloecius cordiformis* H. Milne-Edwards, 1837 (Macfarlane 2002). In all the works mentioned above, Vernier caliper was used to measure the diameter of the burrow entrance.

Direct capturing of animals and visual census methods for assessing population structure are difficult to perform, because it involves personnel and equipment costs, destruction of burrows and long observation times. On the other hand, methods for indirect estimation of body size are more efficient to achieve this goal and avoid the destruction of crab habitats. For these reasons, the purpose of this research is to test and compare three types of burrow measurements that can estimate carapace length (in mm) of the land crabs *C. guanhumi* and *U. cordatus*, and determine which is more suitable to estimate body size. Moreover, it is intended to prove that these methods can serve as reliable measurements to assess crab body size structure in a fast, cheap and non-destructive manner.

MATERIALS AND METHODS

Field Measurements: Sampling and measurement of crabs and its associate burrows were performed on two Venezuelan localities: Carenero (Miranda state) (10° 32’ 5.97”-N, 66° 7’ 45.78”-W) for *Cardisoma guanhumi*, and Cumaná (Sucre state) (10°27’ 43.11”-N, 64° 6’ 07.99” -W) for *Ucides cordatus*. Both sites are characterized by a predominant vegetation of *Avicennia germinans*. With the help of local fishermen, two different capture methods were applied. For *C. guanhumi*, wooden baited traps were used between May and October 2010; for *U. cordatus*, fishing net pieces were placed over the burrow entrances, between February and April 2011. Normally, burrows from *C. guanhumi* are mainly occupied by only one crab (Taissoun, 1974, Moreno, 1980). Similarly, burrows from *U. cordatus* are also occupied by solely one specimen (personal observations). After confirming that only one crab was captured from each burrow, these were sexed and their carapace length measured with a 0.05-mm-precision Vernier caliper. Nevertheless, due to the fact that the purpose of this work was to compare crab size with estimated burrow size through three different measuring methods, we considered unnecessary to separate male and female carapace measurements. Corresponding burrows of captured animals were measured using three different methods: 1.- Superficial crab burrow diameter (in mm) was mensurated with a 0.05-mm-precision Vernier caliper (from now on defined as “Vernier”), taking into account that crabs (*C. guanhumi*, as well as *U. cordatus*) entered and got out their burrows sideways and that their carapace length was well lined up with the diameter of the burrow; 2.- The internal diameter of the burrow was estimated with an inside spring caliper (from now on defined as “caliper”) (Fig. 1), taking the same considerations when

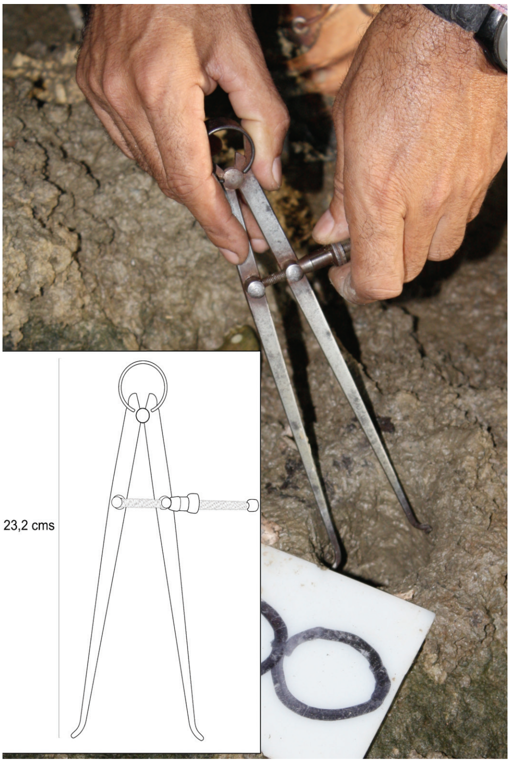

Fig. 1. Photograph and drawing detailing the internal spring caliper used to measure the diameter of the burrows of the crabs *Cardisoma guanhumi* and *Ucides cordatus*.

burrows were measured with the Vernier and being very cautious that the tips of the spring caliper touched the walls of the burrow without penetrating these. The length between the tips was measured with the Vernier; 3.- Each burrow was photographed with a measuring scale placed on its side, and photographs were analyzed with tpsDig v.2 software (Rohlf 2004) (a computer program for statistical analysis of morphometric data) to calculate burrow diameter. This software has been used successfully to quantify size and shape of spermathecae in the beetle *Onthophagus taurus* Schreber 1759 (Simons & Kotiaho 2007), as well as studying the geometric morphometry of two species of the beetle *Erodiontes* spp. (Taravati et al. 2009) and to determine body size preferences in the seahorse *Hippocampus abdominalis* Lesson 1827 (Mattle & Wilson 2009). A total of 118 *C. guanhumi* (73 males and 45 females) were trapped and their carapace length measured (in mm), showing a mean body size of 58.79±18.03 (max= 140.7; min= 32.8); furthermore, 118 vernier measurements, 89 spring caliper measurements and 112 photographs were taken, but only paired data (81) were considered for the analyzes. For *Ucides cordatus*, 105 crabs were captured (mean body size= 35.99±7.34; max= 54.2; min= 18.6) (52 males and 53 females), with the same numbers of Vernier measurements, spring caliper measurements and photographs taken.

Statistical Analyzes: Model II Linear regressions analyzes were calculated using a subset of random measurements and Ordinary Least Squares (OLS) in order to generate predictive models for size estimation for each crab species, in accordance to the suggestions given by Laws & Archie (1981) and Quinn & Keough (2002) to analyze bivariate data subjected both to sampling error. To validate the best forecasting model, Model II via Mayor Axis (MA) regression analyzes was applied using the remaining data. Before theses analyzes, data was first checked for normality with the Shapiro-Wilks test, and the homogeneity in the bivariate dispersion was approached with scatterplots (Quinn & Keough 2002). In both species, individuals and burrows with standardized residuals larger than 3.0 were considered outliers and excluded from the analyzes in order to generate more robust models (Sokal & Rohlf 1995, Quinn & Keough 2002). In the case of *C. guanhumi*, from 81 samples (individuals and their respective burrow sizes), 50 were randomly chosen to build the linear models. To evaluate the predictive capacity of the resulting models, the estimated crab body size of the remaining 31 samples was plotted and analyzed against the real size using MA analyzes. For *U. cordatus*, 29 samples were randomly chosen from a set of 58 to construct the linear model. Crab body size from the remaining 29 samples was estimated and compared with the real ones, using the MA linear regression. For both species, if the predictive models are good, then the regression lines with MA analyzes should show slopes= 1, intercepts= 0 and angle of 45º (Legendre 2001).

RESULTS

Statistical results for *C. guanhumi*: Graphs with all data included for each of the burrow measuring methods and carapace length of crabs are shown in Fig. 2. Both bivariate data between carapace length and measured burrows with the Vernier (Fig. 2A), and carapace length and measured burrows with the photo-method (Fig. 2C) showed a noticeable increase in the dispersion towards larger sizes. Bivariate data of carapace length and burrow measure with the spring caliper showed less dispersion (Fig. 2B).

For the construction of the predictive model, none of the sampled data (body size and burrow measurements with the different methods) adjusted to normality. Thus, tests based on permutations were applied (Legendre 2001). Estimation variability increased as body size increased, particularly data coming from the Vernier and photo measurements (Fig. 2A and 2C, respectively). After excluding the outliers and using the 50 random selected sample pairs for each of the measuring methods, carapace length adjusted to normality (Shapiro-Wilks test, p>0.05), but burrow size with different methods did not (Shapiro-Wilks test, p<0.05). Nevertheless, homogeneity was achieved in the bivariate distribution in the three data sets from each of the measuring methods (i.e. points around the line are equally distributed). Vernier method as well as photo method showed a reduced dispersion of the data (Fig. 3A and 3C, respectively), but the spring caliper method still evidences the smallest dispersion of all (Fig. 3B). The results of the linear regression analyzes are shown in Table 1A. The three models are statistically significant. The spring caliper measuring method is the best adjusted model (r^2= 0.61) and with the less unexplained variability.

Results of the evaluation of the predictive capacity of the regression models (MA) are shown in Table 2A. All regressions present intercepts that are significantly higher than 0 and slopes significantly lower than 1 (Table 2A). This indicates that all methods overestimate smaller crab body size compared to the real body size mean and underestimate larger size. The imprecision can be observed in Fig. 4, in which the MA linear regression lines show angles above the referential line of 45%. Nevertheless it apparently seems to be that the caliper method revealed a better performance,

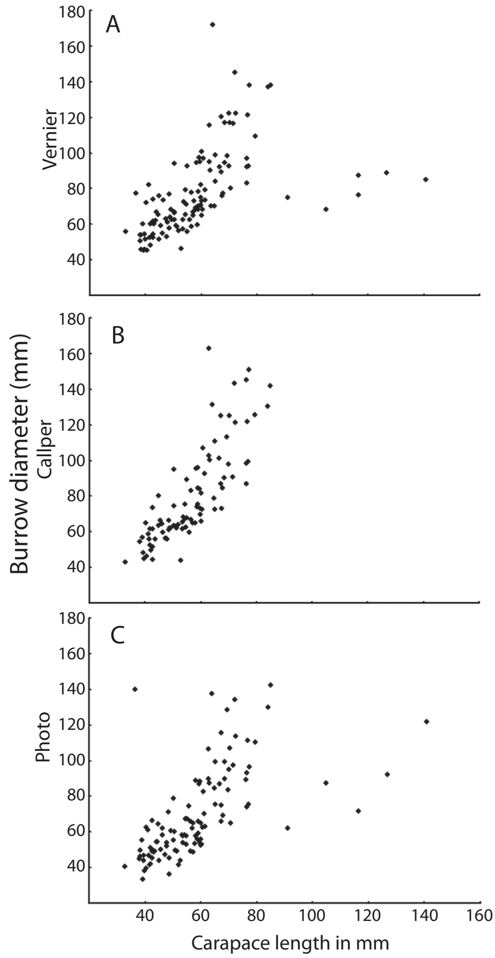

Fig. 2. Bivariate scatterplots of carapace length (mm) and burrow diameter (mm) of *Cardisoma guanhumi*. A: Burrow diameter measured with Vernier; B: Burrow diameter measured with spring caliper; C: Burrow diameter measured from digital photograph.

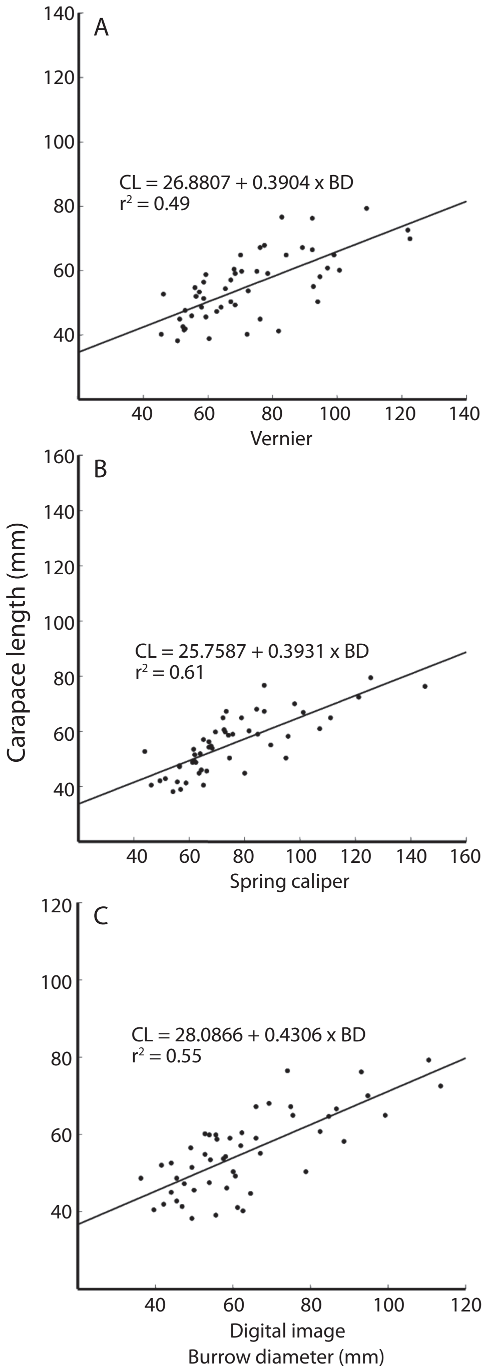

Fig. 3. Model II OLS linear regression method for carapace length (mm) and burrow diameter (mm) in *Cardisoma guanhumi*. A: Burrow diameter measured with Vernier; B: Burrow diameter measured with spring caliper; C: Burrow diameter measured from digital photograph.

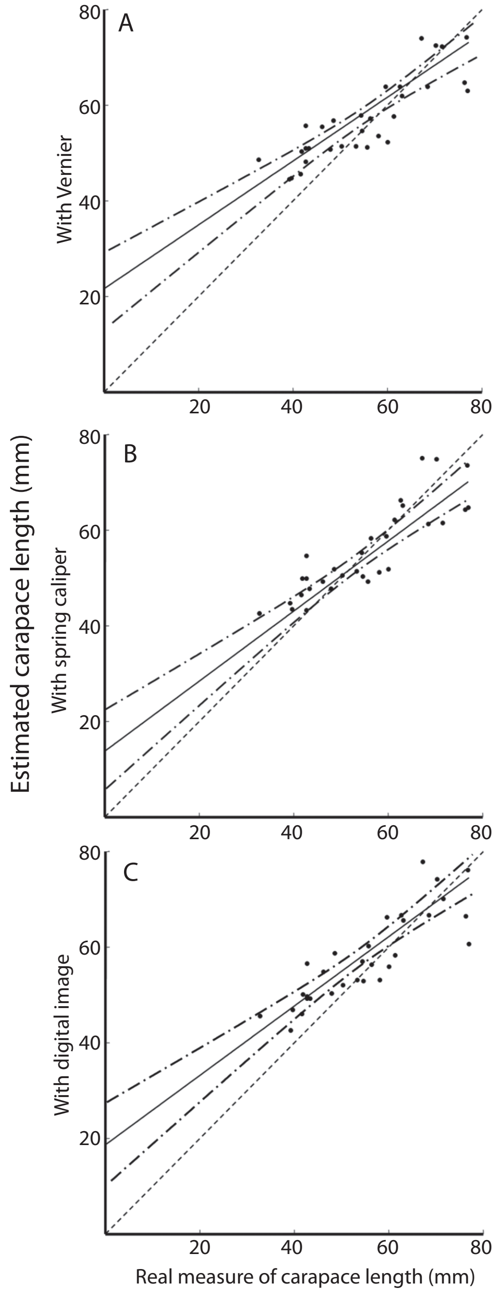

Fig. 4. Model II MA linear regression for the validation of the three measuring methods in *Cardisoma guanhumi*. Estimated carapace length (mm) vs. real carapace length (mm). A: Vernier method; B: Spring caliper method; C: Digital photograph method.

TABLE 1
Linear regression analysis between carapace length of *Cardisoma guanhumi* (A; n= 50) and *Ucides cordatus* (B; n= 29), and the three burrow measuring methods, using Model II (OLS). Significance of slope was tested using t-test and 4999 permutations

A

Method	Intercept	Slope	*t*-test	r^2
Vernier	26.88	0.39	p<0.05	0.49
Spring caliper	25.76	0.39	p<0.05	0.61
Photo	28.08	0.43	p<0.05	0.55

B

Method	Intercept	Slope	*t*-test	r^2
Vernier	2.38	0.71	p<0.05	0.71
Spring caliper	8.14	0.57	p<0.05	0.61
Photo	7.40	0.68	p<0.05	0.53

since the confidence intervals include the referential line along almost all the measures.

Statistical results for ***U. cordatus***: Graphs with all data included for each of the burrow measuring methods and carapace length of crabs are shown in Fig. 5. In each of the graphs, no strong dispersion was observed. Crab body size, as well as burrow measurements with the three methods adjusted to normality (all Shapiro-Wilks tests, p>0.05). This satisfies the assumption of distribution for the conventional tests, but tests were applied based on permutations. Similarly, homogeneity was maintained in the bivariate distribution in all the Vernier and caliper measuring methods, but variation increases with increasing body size in the photo method (Fig. 5). After excluding outliers and randomly choosing 29 measurements, body size as well as burrow measures from all estimating methods were still adjusted to normality (Shapiro-Wilks tests, p>0.05). Similarly, homogeneity was maintained in the bivariate distribution in the three methods (Fig. 6). All linear regressions built with Model II OLS method are statistically significant (Table 1B). The Vernier measuring method showed the best adjustment and the less unexplained variation (r^2= 0.71, Table 1B). MA linear regressions analyzes are shown in Table 2B. The three methods have an intercept significantly above zero and slopes that include 1 or approach it significantly. All three methods slightly overestimate smaller crab body size and underestimate the average sizes greater than the population average. Such imprecision can be seen in Figure 7, where the regression lines presented MA angles below the baseline of 45°. Estimates presented by the photo method show the greatest portion of the referential line outside the IC 95%, followed by the caliper and finally by the Vernier methods, the latter having apparently the lowest degree of imprecision in estimating sizes.

TABLE 2
Validation model between real and estimated carapace length for *Cardisoma guanhumi* (n= 31) and *Ucides cordatus* (n= 31), using Model II and MA method

A

Method	Intercept	I.C. 95 % Intercept		Slope	I.C. 95 % slope		Line angle
Vernier	20.89	12.06	28.60	0.65	0.51	0.81	33.20
Spring caliper	15.47	5.95	23.64	0.73	0.58	0.90	35.96
Photo	17.97	8.20	26.30	0.72	0.57	0.90	35.84

B

Method	Intercept	I.C. 95 % Intercept		Slope	I.C. 95 % Slope		Line angle
Vernier	6.26	-1.62	12.70	0.82	0.63	1.04	39.22
Spring caliper	9.35	0.37	16.54	0.73	0.51	0.99	35.98
Photo	11.84	2.42	19.37	0.65	0.43	0.93	33.14

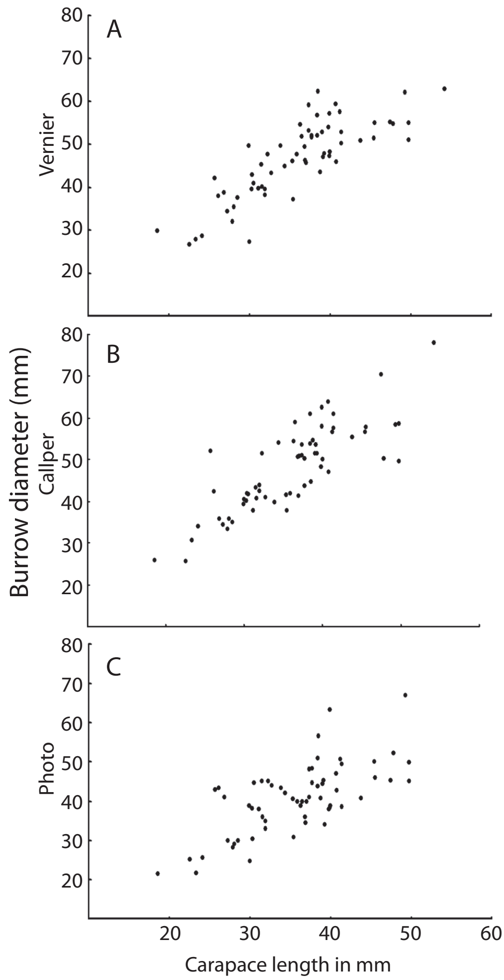

Fig. 5. Bivariate scatterplots of carapace length (mm) and burrow diameter (mm) of *Ucides cordatus*. A: Burrow diameter measured with Vernier; B: Burrow diameter measured with spring caliper; C: Burrow diameter measured from digital photograph.

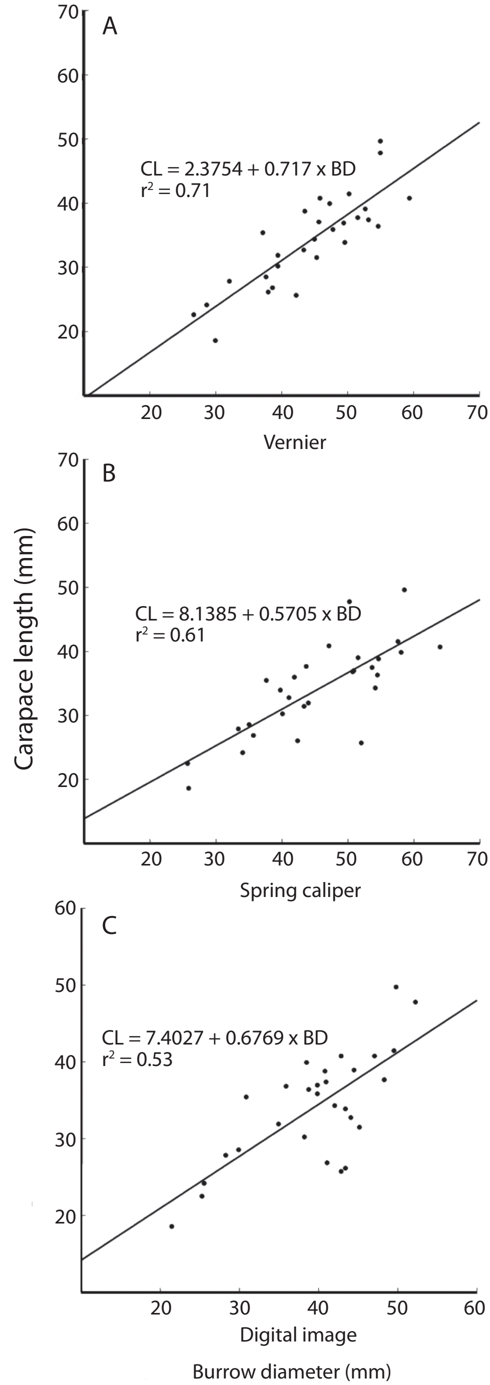

Fig. 6. Model II OLS linear regression method for carapace length (mm) and burrow diameter (mm) in *Ucides cordatus*. A: Burrow diameter measured with Vernier; B: Burrow diameter measured with spring caliper; C: Burrow diameter measured from digital photograph.

DISCUSSION

Although OLS linear regressions for each of the applied measuring methods in each species resulted statistically significant (see Tables 1A and 1B), the MA analyzes reflect the same bias in the estimations: these methods overestimate sizes of smaller crabs and underestimate larger ones. The spring caliper method proved

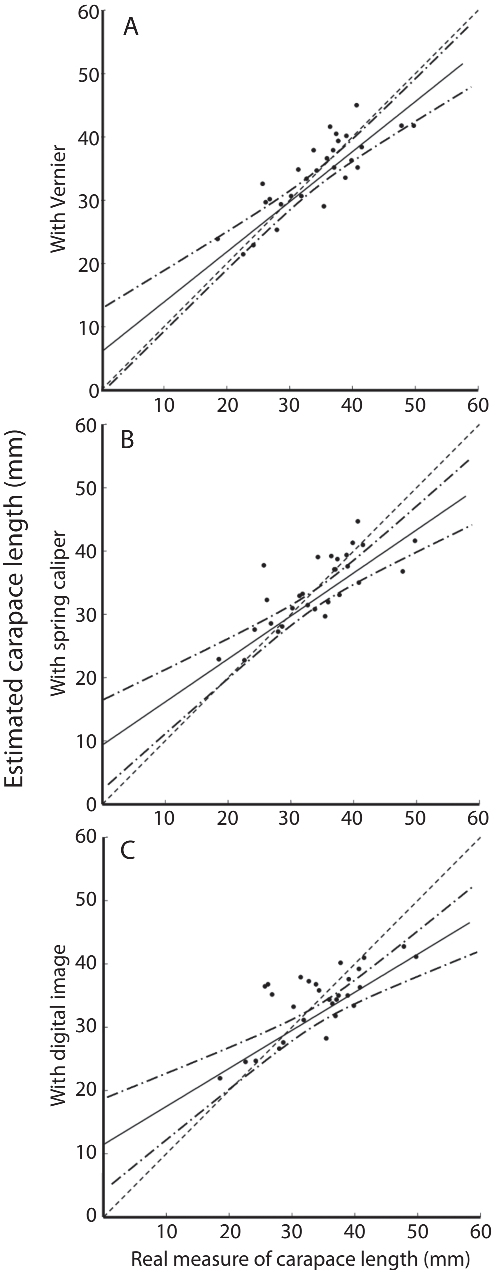

Fig. 7. Model II MA linear regression for the validation of the three measuring methods in *Ucides cordatus*. Estimated carapace length (mm) vs. real carapace length (mm). A: Vernier method; B: Spring caliper method; C: Digital photograph method.

to be the most accurate and with the lowest degree of bias to be applied in *Cardisoma guanhumi*, and the Vernier method in *Ucides cordatus*. In *C. guanhumi*, Vernier method showed a correlation r^2= 0.49, apparently being lower than Govender & Rodríguez-Fourquet (2008) results using the same measuring methodology, where they obtained a high correlation between carapace width and burrow diameter (r^2= 0.89). Moreover, body size of several burrowing crab species have also been estimated using values obtained with a Vernier, achieving high correlations, such as in *Heloecius cordiformis* (ln carapace length r^2= 0.83, MacFarlane 2002) and *Dotilla myctiroides* (carapaces length r^2= 0.89). Although we used carapace length as the reference size, and not carapace width, as used by Govender & Rodríguez-Fourquet (2008), we consider that this is not relevant to the discrepancy between the two correlations, but rather has to do with two aspects: the nature of the substrate and the statistical methodology employed. First, smaller crabs probably using previously abandoned burrows from larger animals, induce a size overestimation and dispersion increase; furthermore, the burrow entrances of *C. guanhumi* individuals that were sampled in Carenero-Venezuela can vary greatly in their shape and form (round, elongated, amorphous), as well as in their consistency (very hard to very soft mud at the entrance of the burrows), causing measuring errors (e.g. tips of the Vernier and the spring caliper penetrating in the very soft mud) that could have produced the lower correlation in the samples taken with the Vernier in the present work. But these measuring problems were not encountered with *U. cordatus*, where burrows were easier to measure. There was less dispersion in data from *U. cordatus* than in *C. guanhumi* and the regression analyzes were much more accurate. On the other hand, from the statistically perspective, the difference could be produced by the statistical methodology employed in the analyzes. Most studies in marine biology typically use Model I regression, but this is inappropriate (Laws & Archie 1981). It is strongly recommended that Model II regression analyzes should be the methodology to relate two set of variables when both are subjected to sampling error (Sokal & Rohlf 1995, Legendre 2001, Quinn & Keough 2002). In this study, two methods of Model II regressions analyzes were applied following the recommendations of Legendre (2001). The above cited references do not make neither mention about the diversity of burrows nor on the Model of regression analyzes used.

Considering the significance of the measuring technique that was analyzed in the present work, at the moment of deciding which one should be utilized, it will depend on how large is the area to be surveyed and the amount of persons that will accomplish the task. If the area is not very large and more than one person is taking the measurements, we strongly suggest using the spring caliper technique in *C. guanhumi* and the Vernier method in *U. cordatus*, for fine estimation when an accurate body size population structure is required; but if the area is substantially large and few persons are measuring, then the photo technique should be regarded as the most efficient method (although less accurate r^2= 0.55 for *C. guanhumi* and 0.53 for *U. cordatus*). The latter is less time consuming in the field, and saves sampling time. This should be taken in mind, especially in areas where they occupy large geographical extensions. In Venezuela, for instance, there are mainly three regions were *C. guanhumi* is reported to inhabit wide land extensions: from San Juan de Los Cayos to Boca de Yaracuy (8 340 ha, Taissoun 1974), from Higuerote to Boca de Unare (more than 8 340 ha, Taissoun 1974), and the region of the Orinoco Delta (98 802 to 455 298 ha, Conde & Alarcón 1993). Also in the latter geographical area, *U. cordatus* is also reported occupying vast mangrove regions (Novoa 2000 and personal observations). But likewise in Brazil, where mangrove extensions are considerably large (1 012 376 ha; Lacerda et al. 1993), both land crabs are being commercial exploited due to their substantial presence in these forests. Finally, due to the ecological and economical importance to estimate body size structure in both land crabs, it is justified the application of the measuring methods that were analyzed in the present work, even when the bias in estimations is a subject to be taken into account in the interpretation of data.

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RESUMEN

Para la estimación de la estructura de tamaños en cangrejos terrestres comerciales y la obtención de información relevante para su manejo, es necesario utilizar métodos rápidos, confiables y no destructivos. *Cardisoma guanhumi* y *Ucides cordatus* son dos cangrejos terrestres que son explotados comercialmente en el Caribe y en Brasil. El propósito de este trabajo es suministrar métodos indirectos para la estimación del tamaño del caparazón de los cangrejos y por consiguiente, de la estructura de tallas. Los muestreos se llevaron a cabo en Carenero (*C. guanhumi*) y en Cumaná (*U. cordatus*) (Venezuela). Se utilizaron tres métodos para estimar el diámetro de sus madrigueras: Vernier, compás y fotografía. Estos se correlacionaron con el tamaño real del cangrejo. Se aplicó el análisis de regresión Ordinary Least Squares Model II y la capacidad de predicción se probó utilizando el modelo II Mayor Axis para las regresiones. *Cardisoma guanhumi* mostró una fuerte dispersión de sus datos en los métodos de Vernier y fotografía. Menos dispersión se obtuvo con el método del compás y fue el más preciso (r^2= 0.61). Para *U. cordatus* las medidas con Vernier fueron la más adecuadas (r^2= 0.71). Sin embargo los tres métodos fueron confiables. Los diferentes métodos mostraron ventajas y desventajas y dependerá del que aplique los métodos, decidir cuál será el más adecuado para sus propósitos.

Palabras clave: Métodos indirectos para medir tamaño corporal, *Cardisoma guanhumi*, *Ucides cordatus*, medidas con Vernier, medidas con compás, medidas con fotografía.

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