A PHOTOGRAMMETRIC METHODOLOGY FOR SIZE MEASUREMENTS: APPLICATION TO THE STUDY OF WEIGHT–SHELL DIAMETER RELATIONSHIP IN JUVENILE *CANTAREUS ASPERSUS* SNAILS

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ABSTRACT

A photogrammetric methodology is proposed to measure the diameter of snail shells on digital pictures. Digital photographs were taken of juvenile *Cantereus aspersus* snails. AutoCAD image analysis software was used for the measurements and the results were contrasted with data obtained using a digital caliper in the same snails to compare the accuracy of both methods. The snails were individually weighed and the shell diameter was measured once a week, for a total of 7 weeks. After the third week, there were no significant differences between both methods, whereas in the first 2 weeks the measurements obtained with a digital caliper scored larger diameters than the photogrammetric measurements. This is probably due to the difficulty to define the end of the shell with the caliper, whereas the photogrammetric analysis does not involve any risk for the snail. To test the reproducibility and repeatability of both methodologies seven snails were measured five times by three different examiners. Using the variance components analysis, the repeatability was 4.8% of the total variation for the photogrammetric methodology and 6.9% for the conventional methodology, while the reproducibility was 0.0% and 2.7% for the photogrammetric and conventional methodology, respectively. These findings indicate that the photogrammetric methodology can be used with confidence to measure the diameter of snail shells. The great advantage of this method is the ability to magnify the images to make more precise measurements from snails of any size, which is especially useful in the early stages of development. The growth data were used to construct a model for live weight (LW) estimation based on shell diameter. The snails showed high growth rates both in terms of shell diameter and LW. The shell diameters showed a low individual variation (16.2% CV) and were normally distributed, whereas the LWs showed greater variability (36.8% CV) and were not normally distributed. The best model for estimating LW from shell diameter in juvenile snails is the multiplicative model, with a high determination coefficient (96%).

INTRODUCTION

Morphometric traits used to describe snails growth usually include the live weight (LW) and the shell dimensions (Roberson & Moorhead, 1999). Snail body weight is the most relevant trait for commercial or experimental purposes (e.g. estimations of feed conversion ratio, productivity, etc). Its measurement is easier and faster than the shell dimensions and can be easily automated (Dupont-Nivet *et al*., 1997). However, there is a great variability of individual weights which may be due to genetic factors, the momentary physiological state, environmental conditions and water content (Daguzan, 1982; Dupont-Nivet *et al*., 1997; Roberson & Moorhead, 1999). Consequently, the fitting of weight–age growth models is complex and lacks precision (Lucarz, 1982; Sanz Sampelayo *et al*., 1990).

In contrast, other authors have used shell dimensions rather than LW to describe snail growth (Albuquerque de Matos & Serra, 1988; Madec, 1989), owing to lower variability of shell dimension as compared to LW and the large repeatability (Albuquerque de Matos, 1989).

The measurement of the snail shell diameter is usually scored with callipers, which requires both delicate handling and a skilled operator in order to avoid damage to shells and to minimize measurement errors, especially in juvenile snails. In addition, shell measuring is of little value in farms, where it is necessary to measure large numbers of snails in short periods.

To solve these problems, new methodologies based on the measurement of digital photographs have been applied in the field of animal experimentation (Andersen, 2005). These photogrammetric techniques show many advantages and have applications to biometry and related fields (Becker & Svensson, 1998). Furthermore, it is an excellent method to assess both linear and angular measures on images where the planification of structures does not alter the real 3D image dimensions (Siu *et al*., 1996; Aigner *et al*., 2004).

The determination of the shell diameter through measurements of digital pictures constitute a suitable method to study the growth of terrestrial snails, because it simplifies the sampling process, reduces the time of measurement and avoids the manipulation of the snail.

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Therefore, the aim of this study was to establish and validate a method to measure shell diameter on digital pictures and to analyse the shell diameter–weight relationship of juvenile *Cantareus aspersus* when cultured under laboratorial conditions. This study may provide a basis for the establishment of a practical conversion protocol to simplify management practices in commercial cultures and laboratorial experiments.

MATERIAL AND METHODS

Biological materials and rearing conditions

The snails (*Cantareus aspersus*) were originally collected from the Andalusian Agrarian Research and Training Centre (Cordoba, Spain). At the end of incubation, a tray containing around 3,000 (5 ± 1 days old) neonates was available. A sample of 300 individuals with an LW of 16.5 ± 0.5 mg was selected and distributed into five groups of 60 snails. The animals were measured during their juvenile stage until they were 6 weeks old (Daguzan, 1982).

The study took place in a laboratory under semi-controlled conditions as described by García *et al*. (2006). Each of the five groups was kept in a translucent plastic box (20.5 × 20.5 × 7.5 cm) maintained under natural light conditions, avoiding direct sunlight, and at room temperature. The boxes were cleaned daily to avoid the negative effects of excreta, mucus and high population density (Mayoral *et al*., 2004). The experiment was conducted between May and June 2006, during increasing daylight hours (14:10 light/dark cycle). The relative humidity in the rearing boxes was regulated by spraying with water twice daily. During the day, the average relative humidity and temperature were 73% and 25°C, respectively. At night, the mean relative humidity rose to 89% and the temperature decreased to 18°C. Under these conditions, snails rested during the day and actively moved and foraged at night. The animals were fed *ad libitum* with flour made up of commercial layer mash to which 20% calcium carbonate was added and its main constituents were dry matter (92.6%), crude protein (14.4% of dry matter), lipids (3.1% of DM), carbohydrates (51.8% of DM), neutral detergent fibre (11.8% of DM) and ash (30.7% of DM, including 10.3% calcium).

Measurements

The snails were individually weighed and shell diameter was measured weekly, for a total of seven observations for each of the 300 snails. The deaths of some snails reduced the resulting set of data to 1996 observations. The shell diameter was measured using a digital caliper (SDC, shell diameter by conventional methodology) with a precision of 0.1 mm and using the digital photogrammetric methodology (SDD). The LW was measured using a digital analytical balance with accuracy of 0.1 mg. The measurements were carried out when the snails were resting, during about 5 h (from 9.00 to 14.00).

In a second stage, to test reproducibility and repeatability of both methodologies, seven snails ranged from 16 to 540 mg were measured five times by three different examiners.

Photogrammetric methodology

Each group of snails was photographed inside their own box. The snails on the walls of the box were moved to the ground with the help of a lamp brush. A ruler with 1 mm divisions was placed at the bottom of the box. All boxes were placed horizontally in the same standard position. Photographs (1,024 × 768 pixels) were taken at a distance of 30 cm using a Canon A540 6.0 MP digital camera with a 35–140 mm lens.

The measures of the shell diameters were performed by Autocad® image analysis software (Autodesk Inc., San Rafael, CA, USA). The software converts photographs into line images, and calibrates the scale by using the ruler in each photograph as a reference. The shell of each photographed snail is fitted to an elliptic shape and its larger axis is measured to determine the diameter of the shell.

Statistical study

The statistical analysis was performed using Statgraphics Plus v 5.0. To confirm if the values obtained with the digital photogrammetric methodology (SDD) were significantly different from the conventional methodology (SDC), an ANOVA-test was applied at each time point.

The repeatability and reproducibility of both digital (SDD) and conventional methodologies (SDC) was calculated by an ANOVA-test using a variance components method similar to that described by Duncan (1986).

The normality of the distribution of LWs and shell diameters was verified using a χ^2 test. The variability of the individual weights and shell diameters is expressed as the coefficient of variation. The relationship between the shell diameter (SDD) and the LW was modelled using simple, linear and nonlinear regression analysis.

RESULTS

Figure 1 shows weekly development of snail shell diameter from conventional and photogrammetric digital measurements. Every week, the mean value of the conventional measurement was larger than the mean value of the digital measurement. However, this difference was only significant for the first 2 weeks (Table 1, $P < 0.05$). At the start of the experiment, the mean value obtained with the caliper was 25% greater than that obtained from the digital picture; this difference was reduced with age, being 0.08% at the sixth week. Variation coefficients were low for both procedures but, with snail ageing, they increased from 10.9% to 17.0% for SDD and from 9.4% to 23.4% for SDC.

Table 1 shows weekly development of snail LW from birth until 6 weeks old. At the start of the experiment, LW showed lower variability, which gradually increased with snail age. The final weight (sixth week) showed a variation of 44.5%, which was higher than that obtained with the caliper shell measurement or the digital photogrammetric measurement.

The variance components analysis results for SDC and SDD repeated measurements, involving three examiners each measuring seven snails three times, are shown in Table 2. The estimated standard deviation of the conventional measurement

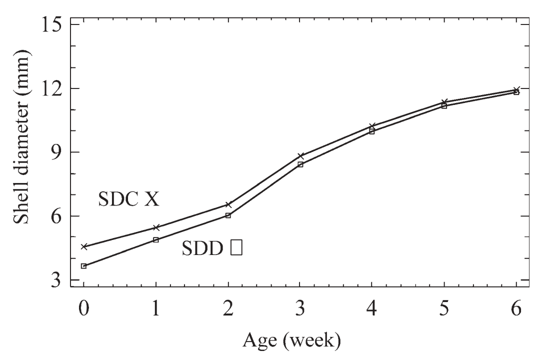

Figure 1. Mean shell diameter (mm) from 0 to 6 week. Abbreviations: SDC, conventional measurement; SDD, photogrammetric measurement.

Table 1. Snail live weight (LW, mg), conventional measure (SDC, mm) and photometric measure of shell diameter (SDD, mm) from 0 to 6 weeks.

Trait	Weeks						
	0	1	2	3	4	5	6
SDD	3.6 ± 0.02[a] (10.9)	4.8 ± 0.04[a] (14.9)	6.0 ± 0.04[a] (16.4)	8.4 ± 0.08 (16.2)	10.0 ± 0.10 (18.9)	11.2 ± 0.18 (19.1)	11.8 ± 0.24 (17.0)
SDC	4.5 ± 0.06[b] (9.4)	5.4 ± 0.06[b] (10.1)	6.5 ± 0.07[b] (16.1)	8.8 ± 0.15 (17.9)	10.2 ± 0.22 (20.3)	11.3 ± 0.32 (22.2)	11.9 ± 0.30 (23.4)
LW	16.4 ± 0.94 (9.1)	34.0 ± 1.07 (32.1)	69.0 ± 2.96 (36.7)	131.8 ± 5.68 (36.4)	247.7 ± 12.10 (48.9)	428.8 ± 21.54 (49.9)	532.9 ± 24.51 (44.51)

Mean ± SE (CV); a, b, superscript letters indicate significant differences between diameters ($P < 0.05$).

Table 2. Variance components analysis by ANOVA for repeated conventional measurement (SDC, mm) and photogrammetric measurement of shell diameter (SDD, mm).

	SDC		SDD	
	Repeatability	Reproducibility	Repeatability	Reproducibility
Variance (σ^2)	0.041	0.006	0.019	0.000
Standard deviation (σ)	0.20 mm	0.08 mm	0.14 mm	0.00 mm
95% Confidence variation ($\pm 2\sigma$)	±0.40	±0.16	±0.28	±0.00
% Total variation	6.9 %	2.7 %	4.8%	0.00%
% Overall mean [($\pm 2\sigma$/mean) × 100]	±0.47 %	±0.07 %	±0.23%	±0.00%

process was 0.20 mm. The variation due to repeated measurements (repeatability) was 6.9%, while the variation due to multiple examiners (reproducibility) was 2.7% of the total variation. The digital measurement process obtained an estimated standard deviation of 0.14 mm, which was lower than that for the conventional measurement process. The repeatability of the digital measurement process was 4.8% and the reproducibility was 0.0% of the total variation.

A χ^2 test was performed to confirm the assumption that individual LW and shell diameter (SDD) displayed a normal distribution. While individual shell diameter showed a normal distribution ($P > 0.38$), the individual LWs were not normally distributed ($P < 0.05$). Figures 2 and 3 show the histogram of individual shell diameter (SDD) and LWs during the sixth week. The distribution of individual shell diameter shows the mean value close to the median, whereas the distribution of individual LW shows an average value around 6% higher than the median value.

Once the normal distribution had been verified, the relationship between shell diameter (SDD) and LW was studied. The shell diameter–weight relationship showed a good fit to multiplicative, square-root and exponential models, with a determination coefficient of over 96% (Table 3, Fig. 4).

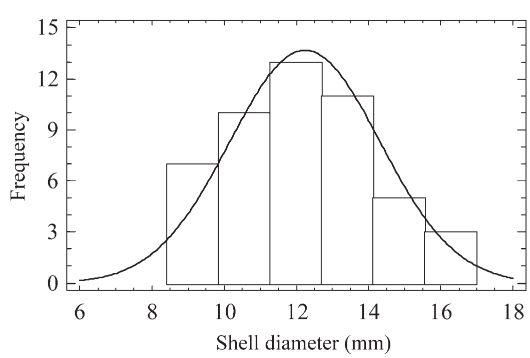

Figure 2. Snail shell diameter (SDD) distribution at 6 weeks. Mean diameter 11.8 mm; median diameter 11.9 mm.

DISCUSSION

Until now, shell diameter measurements have been performed directly on snails using calipers or compasses. Although the methodology for conventional measurements is well established and has been frequently used in laboratory studies (Madec,

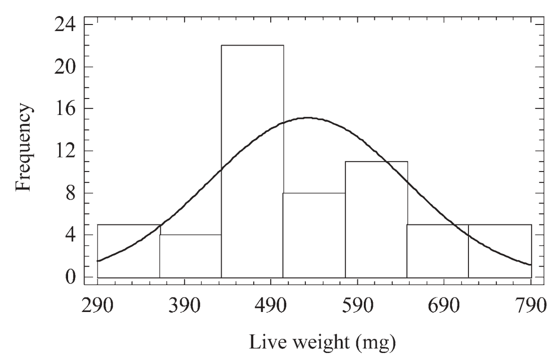

Figure 3. Snail live weight distribution at 6 weeks. Mean weight 532.9 mg; median weight 503.0 mg.

Table 3. Relationship between snail weight (Y, in mg) and shell diameter (SDD) (X, in mm).

Model	Parameters				
	r	*a*	*B*	R^2 (%)	SE
Multiplicative $Y = aX^b$	0.98	0.54578	2.70603	96.5	0.17
Square-root y $Y = (a + bX)^2$	0.98	−4.34562	2.21837	96.3	1.1
Exponential $Y = e^{(a+bX)}$	0.97	2.12085	0.342785	94.5	0.22
S-curve $Y = e^{(a+b/X)}$	−0.96	7.47876	−18.4491	92.3	0.26

r is the regression coefficient, R^2 is the determination coefficient; SE is the standard error, *a* and *b* are the parameters of the equations.

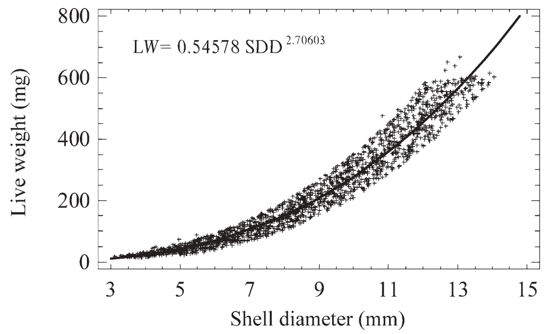

Figure 4. Distribution of the observed values of live weight (LW, mg) and shell diameter (SDD, mm) of juvenile *Cantereus aspersus* cultured under laboratorial conditions. The regression curve shown represents the estimations of LW from the best fitting regression between LW and SDD (multiplicative model: $Y = aX^b$; $R^2 = 96.5$).

1989; Roberson & Moorhead, 1999) as well as in field studies (Albuquerque de Matos, 1989), it has certain disadvantages that limit its use. In juvenile snails, it is particularly difficult to determine the edges of the shell without damaging them and without incurring large measurement errors. In addition, the excessive manipulation required for the process of direct measurements may alter growth rates, particularly in the early stages of development (Jess & Marks, 1995; Perea, 2003).

The results of this study show that during the early stages of *Cantareus aspersus*, measurements with calipers recorded larger diameters than photogrammetric measurements; these differences diminished as the age of the animals increased. This is probably related to the difficulty of defining shell margins with the caliper. In contrast to this, the use of photogrammetric analysis is simpler and does not involve any risk to the snail. Furthermore, enlargement of pictures allow for better resolution of shell edges (Shaner *et al.*, 1998).

The successful use of the photogrammetric digital methodology to measure the diameter of snail shells depends on the possibility to distinguish small differences in snail sizes. The photogrammetric digital methodology described here has been shown to be both repeatable and reproducible, which indicates that this method can be reliably employed to measure the shell diameter of *C. aspersus*.

The determination of the shell diameter through measurements on digital pictures has other advantages that support its use in studies to assess growth: (1) It allows to obtain accurate measurements from snails of any size, adult or juvenile, and it is especially useful in the early stages of development when determining the edges of the shell accurately is difficult. (2) It minimizes the direct manipulation of the snails, which explains why the measurement process does not alter the growth of the animal as shown by Jess & Marks (1995) and Perea (2003), who found a direct relationship between snail handling and low growth rate. (3) It does not involve any risk of damage to the shells since manipulation is not necessary. (4) In laboratory studies, all snails of each experimental unit can be measured on a single photograph, reducing the measuring time. (5) Digital photographs may be taken not only in laboratories but also in commercial farms and some natural environments. Therefore, the study can be completed *ex-situ*, simplifying the sampling and measuring process. (6) The digital photographs can be checked immediately to determine proper position and lighting as reported by Guyot *et al.* (2003). Furthermore, according to Becker & Svensson (1998) and Moreira *et al.* (2006) the pictures and the studied protocol of each experimental unit (individual snail, rearing box, leaves, etc) can be stored in digital media and retrieved when necessary for re-evaluation.

However, it is necessary to consider some aspects to ensure the exactness of the measurements obtained by means of digital photogrammetry. Diliberti & Olson (1991) suggested that the main sources of error are related to focal angle, distance between the focus and the snails, unreliable edges and measurement errors on photographs. Alignment is the principal problem for three-dimensional objects such as snail shells (Andersen, 2005). Alignment problems involving landmarks located on different planes. Photogrammetric measurements of shell diameter are reliable only if the points being measured are located on the same plane.

On the other hand, although the snails showed high growth rates both in terms of shell diameter and LW, the shell diameter showed a low individual variation, following a normal distribution, whereas the final weight showed greater variability and was not normally distributed. These results are consistent with other studies on *C. aspersus* which also report high variability and asymmetrical distribution of individual LW (Pollard *et al.*, 1977; Gomot *et al.*, 1986; Sanz Sampelayo *et al.*, 1990). However, these results contrast with those of García *et al.* (2006) who, using the same experimental conditions as the present study, found similar growth rates, with a normal distribution of weight values and a low variation.

The individual variation of LW can be due to genetic or environmental factors, such as current physiological state, environmental conditions, water content, etc (Daguzan, 1982; Dupont-Nivet *et al.*, 1997; Roberson & Moorhead, 1999; García *et al.*, 2006). In the present study, the snails originated from a homogeneous sample with a low individual variation in initial LW. The experimental conditions were favourable and similar for all the animals. The individual variation of the LW reported here is possibly due to physiological differences at the moment of measurement and to the handling routine during the measurement process. Garcia *et al.* (2006) weighed randomly 60 snails per week, whereas in this study all the snails (300) were weighed weekly and measured. Consequently, the animals undergo unfavourable handling that can produce alterations of their physiological state during the measurements (Perea *et al.*, 2003). In addition, during the 5 h that weighing of the animals lasted, small variations of laboratory temperature or relative humidity could modify the physiological state or the water content of the snails (Dupont-Nivet *et al.*, 1997; Takeda & Ozaki, 1986). The diameter of the shell is not affected either by the physiological state of the snail or by the environmental conditions at the moment of the measurement, which allows the use of shell diameter as a better growth estimate as compared to LW. Furthermore, it can be measured accurately in laboratory studies or in commercial farms, while the LW can only be used as a growth estimate when it is possible to standardize the measurement conditions to reduce the error generated by environmental factors or physiological state of the snail (Dupont-Nivet *et al.*, 1997).

The application of morphometric relationships could be a simple alternative to estimate LW of snails. A shell diameter–weight relationship can be used for a number of purposes. It has been used in the estimation of weight from diameter for snails, such as *Cepaea nemoralis* (Williamson, 1976). It can also be used in the conversion of shell diameter growth equations into weight growth to predict weight at a certain age and its subsequent use in assessment models (Pollard, 1973; Illano *et al.*, 2004; Leontarakis & Richardson, 2005). The diameter–weight relationship also allows for life history and morphology comparisons between species or between populations of species from different habitats or locations (Wolda, 1970; Staikou, 1998). Morphometric relationships of shell diameter and weight determined in adults (Dupont-Nivet *et al.*, 1997) or immature *C. aspersus* (Lazaridou-Dimitriadou *et al.*, 1998; Roberson & Moorhead, 1999) may have resulted in erroneous extrapolation for juvenile individuals, since morphometric relationships may vary among the different life stages (Primavera *et al.*, 1998). The results of the present study show a consistent positive relationship between LW and shell diameter under laboratory conditions and a high determination coefficient ($R^2 > 95\%$). Despite the advantages of the method proposed in this study, caution is necessary when applying the model to estimate snail weight due to the observed high variation in individual LWs which were used in the construction of the model. Moreover, the morphometric relationships may also be largely affected by environmental factors such as experimental conditions, habitats, seasons or geographical location (Bagenal & Tesch, 1978; Tzeng *et al.*, 2001).

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