

# Estimation of body volume and body density in California sea lion pups

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Body volume was measured directly by the water displacement method and using three different geometric models, consisting of a series of cones and truncated cones, in California sea lion (*Zalophus californianus*) pups from the Gulf of California. Volume is required for the estimation of density, an indicator of the ratio of fat to lean mass. A model consisting of two cones and four truncated cones predicted body volume with greatest accuracy (volume =  $1.11 + 1.06 \times$  estimated volume,  $r^2 = 0.90$ ,  $P < 0.001$ ,  $SEE = 0.673$ ,  $N = 274$ ). This model, however, was not adequate to estimate absolute pup body density (mass to volume ratio), as it yielded values poorly correlated with observed body density ( $r = 0.14$ ,  $P = 0.02$ ). However, the regression line of mass on volume indicated that density decreased with volume, so the mass to volume ratio is a biased estimate of density and is not appropriate for comparing whole body density among sea lion pups. The direct analysis of the relationship between body mass and body volume (observed or estimated) through analysis of covariance provided a better tool to compare the relative density among pups of different sex, populations, or born in different years. The results from such analyses are consistent with previous evidence of sex and age effects on body composition.

## INTRODUCTION

Differences in the density of lipid-bearing tissues compared to lipid-free tissues have been exploited to determine the relative amount of fat in a variety of animals, including humans (Dunham et al., 1985; Lin et al., 1993; Fogelholm et al., 1997). Lean tissues are denser than fat-bearing ones, so that animals with a low fat to lean mass ratio will be denser than others with a higher ratio. Whereas body mass can usually be measured with great accuracy and without much difficulty, body volume is considerably more difficult to measure. Therefore, body density as a measure of the ratio of fat to lean mass has not been extensively used in field studies. Other methods are generally used to measure body condition in these cases, including isotope dilution (Castellini et al., 1987; Oftedal et al., 1996; Schulte-Hostedde et al., 2001), total body electrical conductivity (Walsberg, 1988; Voltura & Wunder, 1998) or bio-impedance analysis (Bowen et al., 1998), and indices derived from morphometric relationships (Virgl & Messier, 1993; Krebs & Singleton, 1993; Jakob et al., 1996). These methods, however, can be very expensive and time consuming, inaccurate, or suffer from conceptual flaws (Speakman, 2001).

Pinnipeds, especially phocids, regularly undergo considerable changes in relative fat stores (Boyd & Duck, 1991; Le Boeuf & Laws, 1994), which can be large enough to affect their buoyancy (Webb et al., 1998; Beck et al., 2000). Hence, density can potentially be used as an indicator of fatness in these animals. This study, therefore, aims at comparing the accuracy of three geometric models for estimating body volume of California sea lion (*Zalophus californianus*) pups and assessing their usefulness

for the calculation of body density. The density estimates are compared with expected values from pups with differing body composition.

## MATERIALS AND METHODS

Sea lion pups for this study were captured at 11 rookeries in the Gulf of California, during late June and early July 1997. The location of eight of these rookeries, the number of pups captured in each one, and the capture procedure followed are described in Luque & Aurióles (2001). For the purposes of this study, pups were captured in three additional rookeries during 1997; 20 (12 males, 8 females) from Los Islotes (24°35'N 110°23'W) on 26 June, 28 (15 males, 13 females) from Roca Blanca (28°54'N 113°26'W) on 2 July, and 30 (15 males, 15 females) from Rocas Consag (31°7'N 114°29.5'W) on 8 July. Pups were, on average, 17 days old (Luque & Aurióles-Gamboa, 2001).

### *Morphometric measurements*

Each pup was placed inside a nylon mesh bag and weighed to the nearest 0.25 kg with a spring scale. Pups were then placed inside a cylindrical water tank (diameter: 0.45 m, height: 1.30 m) for measuring the amount of water displaced by their body. The volume of water in the tank was read to the nearest 0.25 l on a graduated plastic tube as long as the tank, which was connected to its base on its outer wall. The difference between readings recorded before and after placing a pup was taken as the volume of the pup, referred to as observed volume hereafter. Although a few pups struggled initially when placed inside the tank,

all of them allowed for a stable reading within 3 min and were not kept completely submerged for more than 30 s. Measurement error was defined as the precision (0.25 l) divided by the measured value.

Standard length and girth were measured to the nearest 0.25 cm with the pup in a belly-down position. Girth was measured around five different reference planes: the pinnae, front of shoulders, axillae, umbilicus, and at mid-distance between femoral trochanters and the tip of the tail. These measurements were used to construct three geometric models of pup volume: model A, consisting of two intersecting cones (as in Castellini & Calkins, 1993), model B, consisting of two cones and two truncated cones, and model C, consisting of two cones and four truncated cones. The volume of the cones and truncated cones were calculated as described in Luque & Auriolles (2001). The performance of the models at estimating body volume was assessed by simple linear regression analysis of observed against modelled volumes and comparing  $r^2$ ,  $SEE$ , and the values and SE of slopes and intercepts. The volume calculated from these regressions is, hereafter, referred to as estimated volume.

The relationship between mass and volume (observed and estimated) was analysed directly to determine whether it could be used as a relative measure of density. This was done by comparing the mass of male and female pups, after adjusting for differences in observed body volume, by analysis of covariance (ANCOVA) (Sokal & Rohlf, 1995).

Observed density was defined as  $\rho = M \times V^{-1}$  where  $\rho$  is density,  $M$  is body mass (kg), and  $V$  is observed body volume (l). Estimated density was defined as  $M$  divided by estimated volume (l). The sum of the measurement errors (i.e. precision divided by measured value) in  $M$  and  $V$  is taken as the measurement error in density.

#### *Precision of density estimates*

Density values were compared with expected values, based on available information on body composition for

otariid pups. A two-compartment model was considered for the pups' body, composed of lean body mass (LBM) and total body lipid (TBL). The LBM was assumed to have a constant composition of 74.7% water, 21.4% protein, and 3.9% minerals and bone (same as the ash content), as reported for Antarctic fur seal pups (Arnould et al., 1996). The densities of protein ( $1.340 \text{ g} \times \text{ml}^{-1}$ ), water ( $0.994 \text{ g} \times \text{ml}^{-1}$ ), and minerals and bone (weighted average of  $3.042 \text{ g} \times \text{ml}^{-1}$ ) (Bastarrachea-Sosa et al., 1999) were multiplied by their fraction in the LBM and summed to estimate the density of the LBM ( $1.148 \text{ g} \times \text{ml}^{-1}$ ).

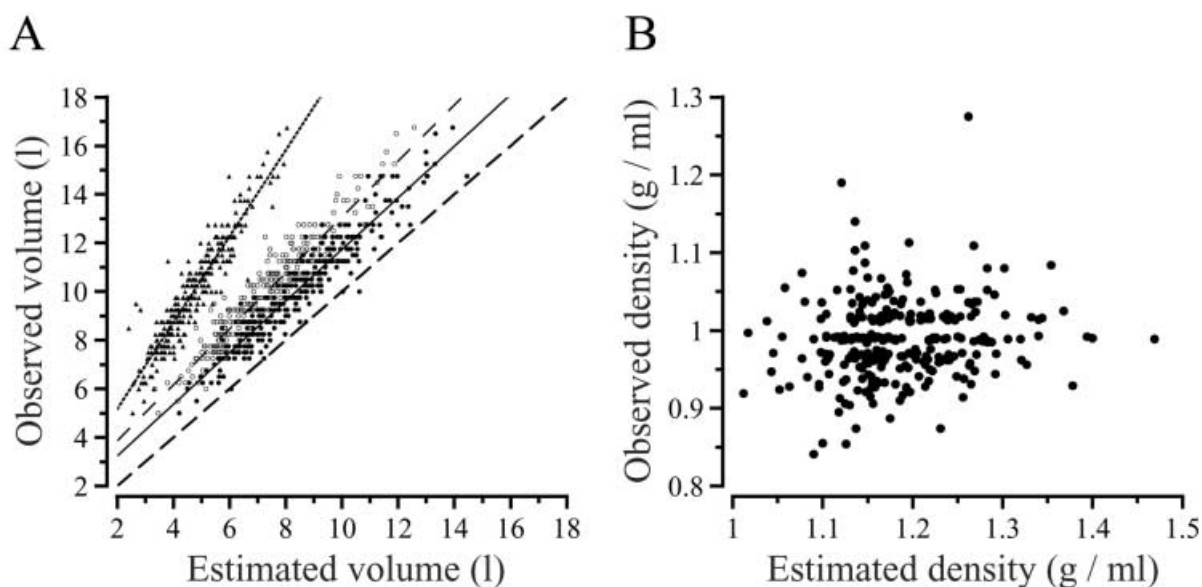
Varying the percentage total body water (TBW%) from 40% to 75%, as found in previous studies (Ofteidal et al., 1987; Arnould et al., 1996; Arnould et al., 2001; Beauplet & Guinet, unpublished data), and estimating the percentage total body lipid (TBL%) from these values (Arnould et al., 1996), resulted in TBL% ranging from 3.2% to 26.0%, which includes observed values in otariids (Ofteidal et al., 1987; Arnould, 1996). Whole body density was thus calculated by multiplying the densities of lipid ( $0.9007 \text{ g} \times \text{ml}^{-1}$ ) (Bastarrachea-Sosa et al., 1999) and LBM by their respective fractions in the body and summing up the products. This procedure was repeated for the whole range of TBL% to estimate a maximum plausible range of total body densities.

## RESULTS

### *Body volume and body density estimation*

All three models of body volume were relatively good predictors of observed volume ( $r^2=0.87-0.90$ ), although they all underestimated it, thereby reducing their usefulness in the calculation of absolute body density. Pup volume could be most accurately estimated by using model C, which had the highest  $r^2$  value, but most importantly, the lowest  $SEE$  (Figure 1A). Equations for predicting volume from each of the models are presented in Table 1.

Males were heavier than females for any given volume, irrespective of whether observed or estimated volume was



**Figure 1.** (A) Relationship between observed and estimated body volume, for three geometric models for calculating volume, and fitted least squares lines: model A (filled triangles, dotted line), model B (unfilled circles, dashed line), and model C (filled circles, solid line). A one to one reference line is also shown (bold dashed line). (B) Relationship between observed and estimated (using model C) densities.

used as covariate in the analysis (ANCOVA, using observed volume:  $F_{1,270}=9.183$ ,  $P=0.003$ ; model C,  $F_{1,270}=10.251$ ,  $P=0.002$ , Figure 2). Adjusted mean male mass was 10.05 kg, while the corresponding value for female pups was 9.85 kg. The sex difference was not evident in the observed density of the pups ( $t_{271}=0.015$ ,  $P>0.1$ ). However, the slope of the relationship between mass and volume (weighted average slope= $0.90\text{ g}\times\text{ml}^{-1}$ ,  $\text{SE}=0.0152$ , from ANCOVA), which did not differ among the sexes ( $F_{1,269}=1.692$ ,  $P>0.1$ ), was significantly different from 1.0 ( $t_{271}=-6.513$ ,  $P<0.001$ ), indicating that density decreases with volume. Furthermore, the intercept of the relationship was significantly different from zero in both males and females ( $P<0.001$ , both cases). Therefore, the mass to volume ratio is a biased measure of density and the direct analysis of mass and volume through ANCOVA provided a more sensitive comparison of relative density among pups of different groups.

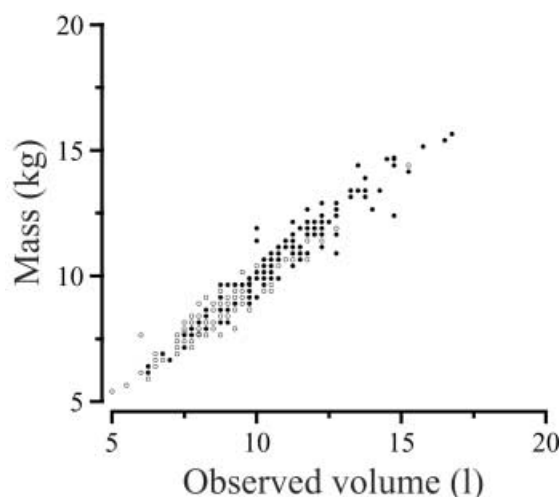
#### Precision of density estimates

Observed and estimated density (using model C) were poorly correlated ( $r=0.14$ ,  $P=0.02$ ), suggesting that the model was not accurate enough to predict density (Figure 1B). Observed body density varied from  $0.841\text{ g}\times\text{ml}^{-1}$  to  $1.275\text{ g}\times\text{ml}^{-1}$  (range= $0.434\text{ g}\times\text{ml}^{-1}$ ) with measurement error ranging from 3.1% to 9.6%, resulting in a maximum possible measurement error of  $0.208\text{ g}\times\text{ml}^{-1}$ .

**Table 1.** Linear regression parameters (Volume= $a+b\times$ estimated volume)( $\pm\text{SE}$ ) relating observed body volume ( $l$ ) with volume estimated from three geometric models ( $l$ ).

Model	$r^2$	$a^*$	$b^*$	SEE
A	0.87	$1.63\pm 0.21$	$1.77\pm 0.042$	0.79
B	0.89	$1.56\pm 0.18$	$1.15\pm 0.024$	0.70
C	0.90	$1.11\pm 0.18$	$1.062\pm 0.021$	0.67

\*Parameter was significantly different from zero for all models ( $P<0.001$ , all cases).  $N=274$ , all cases.



**Figure 2.** Body mass ( $y$ ) as a function of body volume ( $x$ ) in male (filled circles) and female (unfilled circles) sea lion pups. Whereas the slope of this relationship did not differ among the sexes (see ANCOVA results in the text), males had a significantly higher intercept than females (males:  $y=0.99+0.90x$ ,  $r^2=0.93$ ,  $P<0.001$ ,  $N=141$ ; females:  $y=0.80+0.90x$ ,  $r^2=0.93$ ,  $P<0.001$ ,  $N=133$ ).

Expected density, however, should vary from  $1.0668\text{ g}\times\text{ml}^{-1}$  to  $1.140\text{ g}\times\text{ml}^{-1}$ , due to variations in the percentage TBL. Maximum differences in density (i.e. the range of expected density:  $0.0731\text{ g}\times\text{ml}^{-1}$ ), representing extreme differences in TBL%, would thus require density estimates with a maximum measurement error of 6.8% to be detected.

## DISCUSSION

### Volume and its relationship with mass

A geometric model (model C) using five girth measurements and standard length provided a reliable estimate of body volume. This is, to the best of our knowledge, the first attempt at validating volume estimates with direct measurements in pinnipeds.

Despite the errors (discussed below) in the estimation of absolute body density, the direct analysis of the mass–volume relationship offered a useful tool for comparing the density of pups on a relative basis. Furthermore, this analysis demonstrated that the ratio of mass to volume is not appropriate for comparing the density of animals of different volume. The slope of the relationship indicated that density decreased with volume for both male and female pups, consistent with evidence of increasing TBL% as young mammals grow (Adolph & Heggeness, 1971). More importantly, the intercept of the relationship, which was significantly different from zero, meant that the mass to volume ratio would not be independent of volume (Packard & Boardman, 1999). The use of such ratios has been repeatedly criticized (Packard & Boardman, 1988, 1999). Nonetheless, studies validating geometric models of body volume and analysing the mass–volume relationship in different age categories and species are encouraged; they should help us to determine how mass and volume could be used to compare pinnipeds' body conditions.

### Density as an index of body fat

The precision of our morphometric measurements and the associated standard error in estimated volume lead to relatively large errors in the estimation of absolute density. Differences in the percentage TBL of pups would lead to small differences in whole body density, requiring mass and volume to be measured with very high precisions (at least 0.1 kg or 1). However, two crucial assumptions had to be made for predicting a maximum plausible density range: first, that the composition of the LBM was constant, and second, that maximum ranges of TBW% and TBL% were at least as large as those found in other studies (Ofstedal et al., 1987; Arnould et al., 1996; Arnould et al., 2001).

In pinnipeds, the hydration of the LBM appears to be positively related to TBW% (Reilly & Fedak, 1990; Arnould et al., 1996), especially in young animals, but this relationship has not been quantified in otariids. If such a relationship is indeed significant, it would imply negative relationships between the hydration of the LBM and/or its bone and mineral content. Because water is less dense than protein or bone and mineral, this would have the effect of reducing the density of the LBM as TBW% increases, thereby decreasing the difference between the densities of LBM and lipid. Therefore, the maximum density range may be smaller than that calculated in this study.

The range of TBW%, and hence TBL%, considered in this study was slightly larger than that reported for California sea lion and Antarctic fur seal pups (Oftedal et al., 1987; Arnould et al., 1996; Arnould et al., 2001). However, it was the same as the range found in Subantarctic fur seal pups (Beauplet & Guinet, unpublished data). The TBW% and TBL% of pups in this study are therefore likely to be included in the ranges considered. The estimation of absolute body density, as a measure of fat stores, in otariid pups thus requires highly accurate mass and volume measurements.

Compared to the ratio of mass to volume, ANCOVA was a more sensitive tool to compare the density among pups of different sex. The relative differences in mass, thus adjusted for observed volume, between sexes are similar to those previously found using model B (Luque & Auriolles-Gamboa, 2001). These differences were evident irrespective of whether observed or estimated volume (model C, using the equation in Table 1) was used in the analysis. Therefore, the use of ANCOVA, with body mass and an estimate of volume from model C, offers a powerful tool for investigating relative differences in body density, and hence condition, among sea lion pups of different sex, populations, or born in different years.

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