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Comparison of three body-size measurements for echinoids

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ABSTRACT: Several measurements can be employed to quantify the body size of echinoids. We evaluate here the accuracy of three measurements on the sea urchin *Paracentrotus lividus* (test diameter, fresh body weight and immersed weight - the weight of the sea urchin when immersed in seawater -) and discuss their respective potentials. The immersed weight appears to be by far the most accurate, providing it is standardized, but also the most time-costly measurement. Allometric relationships and formula for calculating a standard immersed weight for *P. lividus* are also provided.

1 INTRODUCTION

In vivo determination of the body size is a basic approach used in many fields in biology, including individual growth (Ebert 1967, Grosjean et al. 1996, Régis 1969), population dynamics (Allain 1972, Régis & Arfi 1978), morphometry or biometry (Ebert 1968, 1981, Lawrence et al. 1995, Moss & Meehan 1968), physiology (through calculation of gonadal or repletion indices, for instance Agatsuma & Sugawara 1988, Giese 1966, Nedelec 1983, Spirlet et al. 1998). Various kinds of measurements are available, from lengths to volumes or weights. For echinoids, this task is facilitated by the presence of a rigid endoskeleton that restrains both their external dimensions and their total volume / weight. Hence, several measurements are accessible and used to quantify the body size.

Few studies have compared and discussed the accuracy and suitability of these various measurements, except for the classical relationship between the test diameter and the body weight (Agatsuma & Sugawara 1988, Allain 1972, Kaneko et al. 1981). Consequently, authors use different body size measurements that are not always optimal for their studies.

In the present study, the accuracy, reproducibility and suitability of some measurements that can be performed *in vivo* on sea urchins were assessed. The 3 selected measurements are test diameter, fresh body weight and immersed weight.

2 MATERIAL AND METHODS

A stratified sample of 224 *Paracentrotus lividus* sea urchins of various sizes (20 to 25 individuals in each 5 mm size-class ranging from 10 to 60 mm in test diameter) was measured. Half of them where directly collected in the field in Morgat, Brittany (France). The others where cultivated specimens from the Marine Station of Luc-sur-Mer, Normandy (France) (see Grosjean et al. 1998 for the protocol), but which field parents originated also from Morgat. In both field and cultivated individuals, half population was measured in September, and half was measured in March in order to assess a possible seasonal variation (Spirlet et al. 1998).

- The diameter is measured to the nearest 0.1 mm with a sliding caliper to the widest part of the sea urchin (the ambitus), and without considering the spines. In case of a possible oval shape, it is the average of two perpendicular diameters taken to the ambitus that is considered.
- The total fresh weight is measured to the nearest 0.001 g after leaving the sea urchins for 5 minutes (stabilization of weight) on absorbent paper, which prevents possible fluctuations due to residual water on the integument surface.
- The immersed weight is a much less customary measurement and its use is further discussed. In the present case, it corresponds to the apparent weight of a sea urchin in sea water. It is assessed with a scale (precision of 0.1%) provided with a plate or basket immerged in a tank of sea water and containing the individuals to be weighed (see Fig. 1).

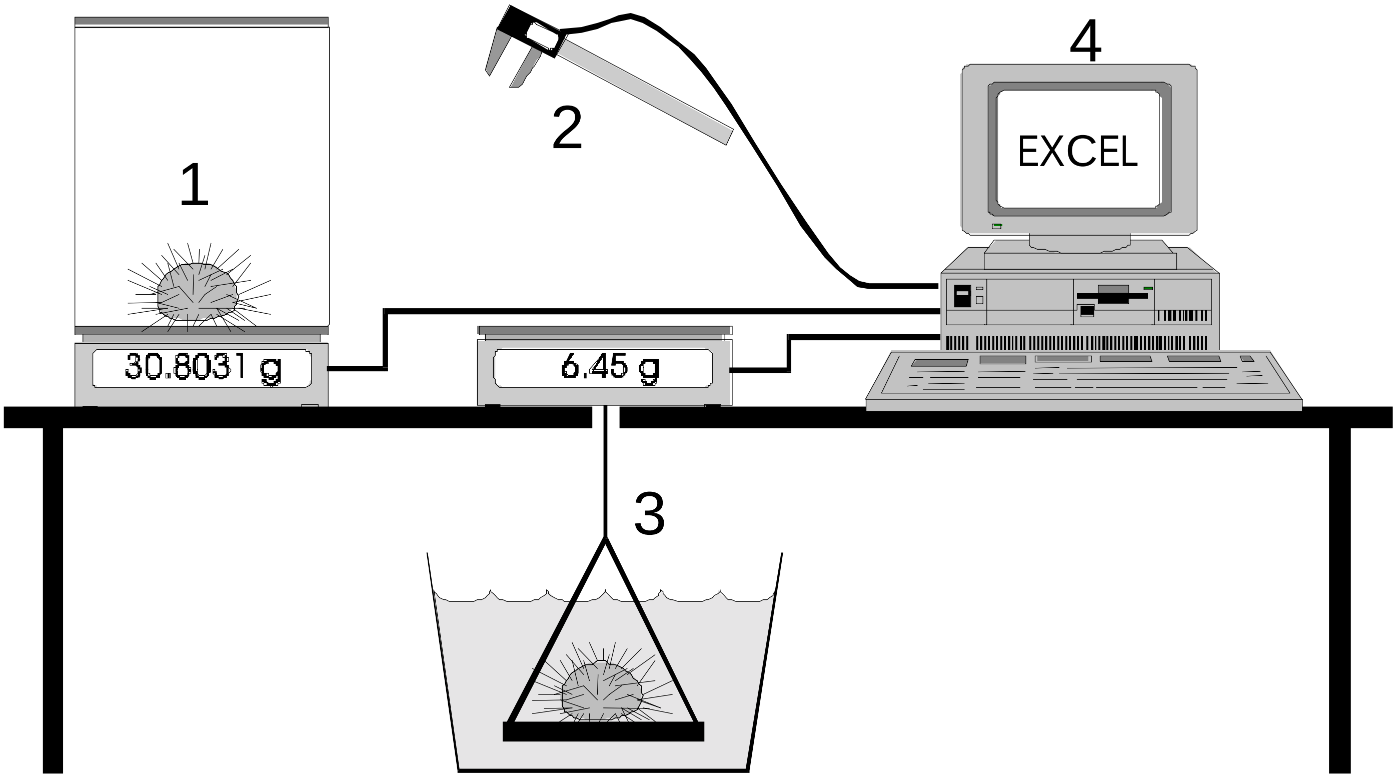

Figure 1. Data acquisition system. A scale (1), an electronic sliding caliper (2) and a second scale equipped with a plate immersed in a tank filled with sea water (3) are connected to a computer (4) for fast data treatment (a software to acquire data directly from scales and calipers into a PC and to calculate the SIW is freely available from the authors).

Each specimen was measured only once. However, to assess the reproducibility of the 3 types of measurement and the possible variation due to the experimenter, an additional 15 echinoids were measured 3 times at a 4 h interval by 7 different people. The individuals were provided in random order to the experimenters. Data were collected by means of a data acquisition system composed of an electronic sliding caliper and electronic scales connected to a computer (Fig. 1).

3 RESULTS AND DISCUSSION

The ambital test diameter and the total fresh weight measurements are exploitable directly. The values of the immersed weight can be compared only if the density of the sea water (depending upon salinity and temperature) is constant between measurements, otherwise a correction must be introduced. The immersed weight is the resultant of 2 opposite forces, the weight and the buoyancy, which compensate each other for organs of the same density as sea water: gonads, digestive tract, and coelomic fluid (Stickle & Ahokas 1974). Their apparent weight in sea water is thus close to zero. Conversely, the calcareous skeleton has a significant positive apparent weight which means that the immerged weight is primarily a measure of the apparent weight of the skeleton in sea water. This is also evidenced in Table 2, showing the immersed weight is directly proportionate to the dry weight of the skeleton (allometric coefficient = 1.00 = perfect isometry).

We define the standard immersed weight (SIW) as the immersed weight that would have been measured in a liquid which density is strictly equal to 1.00 x 10^3 g/l; we calculate it as follows:

$$\mathrm{SIW} = \mathrm{IW} \cdot \frac{2.80 - 1.00}{2.80 - \mathrm{Md}/1000} \qquad (1)$$

where:
- SIW is the standard immersed weight in g
- IW is the measured immersed weight in g
- Md is the mass density of sea water where echinoids are measured in g/l
- 2.80 is the apparent mean density of the sea urchin skeleton in 10^3 g/l (δ_s in eq. 2)

The mass density of the sea water can be determined either directly (with a densitometer), or by calculation (Cox et al. 1970, UNESCO 1981). In the second case, both the salinity and the temperature of the water are needed.

The apparent mean density of the sea urchin skeleton δ_s is calculated from the isometric relationship between the immersed weight measured at a constant sea water density (1.023×10^3 g/l) and the dry weight of the skeleton DW_s (n = 63, $R^2 = 0.999$):

$$DW_s = 1.576 \cdot IW = \frac{\delta_s}{\delta_s - 1.023} \cdot IW \Leftrightarrow \delta_s \approx 2.80 \quad (2)$$

The SIW is usually 2 to 3% higher than the immersed weight actually measured.

3.1 *General comparison*

The diameter is the fastest and easiest in the field measurement (see Table 1). It is the most convenient parameter for separating the sea urchins in size categories or for measuring individually large amounts of echinoids. The others are suited more for batch evaluation (total biomass for instance). Fresh weight and SIW take more time. Since several individuals can be drought simultaneously for the fresh weight, time spent for each measurement drops to around 40 s, instead of the overall 5 min. Hence, the SIW is the longest measurement because the scale takes a while to stabilize. However, it is both the most accurate and the less stressful measurement, which can be of importance when working on sexually mature individuals that can spawn when handled.

3.2 *Reproducibility of measurements*

The diameter of the test to the ambitus is reproducible when it is done by the same person. A two-way ANOVA (measurement order *versus* experimenter) indicates that there is no significant difference between measures from a single experimenter and no interaction between measurements and experimenters ($P > 0.05$ in both cases). However, the experimenters have a great influence ($P < 0.01$) on the values recorded.

The same analysis done on fresh weight and SIW reveals there is only one significant effect ($P < 0.01$): the order of measurements. The difference between 2 successive measurements is steady for all animals, in all cases. The SIW decreases by an average of –0.63% then –0.49% between measurements which can be due to the accidental breaking and loss of spines during handling. Conversely, there is an average increase in the fresh weight of successively +1.70% and +0.64% between 2 series. Such high variation in 4 h intervals can be explained only by slight variations in the volume of the water confined in the echinoid (perivisceral fluid and/or intradigestive fluid; protrusion more or less important of the Aristotle's lantern). This is a drawback that would lower both the accuracy and reproducibility of fresh weight measure in echinoids.

This analysis reveals that the SIW is by far the most reproducible and thus reliable measurement. Its reliability is probably even higher than shown in Table 1 where the loss due to broken spines between measurements was not deduced from the overall recorded variation.

3.3 *Allometry and measurements relationship*

An ANCOVA on log-transformed data ($p > 0.05$) indicates there is no effect of the origin (field or cultivated), or the seasons on the allometric relationship between the three measurements. Hence data re pooled. Table 2 presents model I allometric relations between the 3 measurements considered. All regressions are highly significant ($R^2 \geq 98\%$) for this species in the size range explored. In all cases, the double log data transformation leads to linear regressions with homoscedasticity of variance and random distribution of the residuals. However, caution is the rule as model I is not verified (the independent variable should be measured without error which is not the case here). A non-biased model II would be more adequate (Ebert 1981, 1994, Laws & Archie 1981, Tessier 1948) but only biased model II are available for such data sets (Sokal & Rohlf 1981, p. 549). Since the explained variance is higher than 98%, the bias remains negligible, whatever the model chosen. Thus, in this case, a model I is to be preferred for prediction purposes with independent regressions for reciprocal relationships (Sokal & Rohlf 1981).

Table 1. Comparison of the three selected parameters.

Parameter	Diameter	Fresh weight	SIW
Timing	< 30s	40s (5min)	ca. 2min
Accuracy*	1.33-2.52%**	> 1.31%	< 0.62%
Possible bias**	yes	no	no
Stress	medium	medium	low
Batch measure	no	yes	yes
Field measure***	yes	no	no

* Standard deviation expressed in percent of the mean
** Depending on the experimenter
*** Easily usable in the field and underwater

Table 2. Allometric relations (model I linear regressions on double log transformed data) between parameters for *Paracentrotus lividus* from Morgat, n = 224. Verifications are needed when applying on other strains, or out of the announced validity range.

Measured (x)	Estimated (y)	Allometry	R^2	Std err log(y)	Validity
Diameter (mm)	Fresh weight (g)	$y = 5.50\ 10^{-4}\ x^{2.94}$	0.997	0.037	10 < x < 60
Diameter (mm)	SIW (g)	$y = 2.40\ 10^{-4}\ x^{2.70}$	0.986	0.053	10 < x < 60
Fresh weight (g)	Diameter (mm)	$y = 12.70\ x^{0.35}$	0.995	0.011	0.5 < x < 90
Fresh weight (g)	SIW (g)	$y = 0.22\ x^{0.95}$	0.994	0.034	0.5 < x < 90
SIW (g)	Diameter (mm)	$y = 22.1\ x^{0.37}$	0.984	0.019	0.1 < x < 15
SIW (g)	Fresh weight (g)	$y = 4.95\ x^{1.05}$	0.994	0.036	0.1 < x < 15
SIW (g)	Skeleton (dry w. g)*	$y = 1.56\ x$	0.998	0.021	0.1 < x < 15
SIW (g)	Soma (dry w. g)	$y = 1.74\ x^{0.98}$	0.999	0.010	0.1 < x < 15

* Measured after digestion of the organic matter with sodium hypochloride 10 % under gentle agitation and drying for 48h at 70°C.

The SIW being a direct *in vivo* measurement of the skeleton weight of the sea urchin, the latter can be calculated by the formula in Table 2. As the soma is composed of ca. 90% of skeleton (in dry weight, Grosjean, unpubl.), it is also a reasonably good *in vivo* estimation of the somatic dry weight, after applying possibly a correction calculated after the SIW-soma allometric relationship (Table 2). As such, it allows to follow most accurately the somatic growth of the sea urchins. In some experiments (Grosjean et al., in prep.), we were able to quantify somatic growth of sea urchins within a 7-days period using the SIW, while it would require at least a 1 or 2 months period to get the same accuracy with test diameter or fresh weight! Caution must be taken, of course, when applying conversions on echinoids in particular physiological state that lead to variations in allometric relationships, such with starved individuals (Ebert 1968, Kaneko et al. 1981).

4 CONCLUSIONS

The SIW should be used whenever possible both as a reference measurement for indices (gonadal index, repletion index, …) and for studies involving somatic growth. Test diameter remains the fastest measure, and thus privileged for measuring large number of individuals when accuracy is not of prime importance, or for measures in the field. Fresh weight use should be restricted to the determination of the biomass.

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