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SHORT NOTE

# A Volumetric Method for Estimating Fecundity in Decapoda

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ABSTRACT: A new volumetric method for estimating fecundity in Decapoda is presented. Its precision and amount of time spent in the estimation were determined and compared to other two methods available in the literature (Hopkins, 1962, as modified by Díaz, 1974; and Bogorov and Zenkevich, 1947, as modified by Ros and Menocal, 1978). The new volumetric method was found to be more precise and faster than the other two methods referred.

Methods for estimating the number of eggs in Decapoda have been developed in an attempt to determine fecundity in relation to population dynamics. In most such methods a regression or functional relation has been established between volume or weight of the sample and the number of eggs in the berry. Then, the total number of eggs in the egg mass can be estimated. However, chitinous connections among eggs introduce some error in the estimate. The error originates in estimating the volume occupied or the weight of the connections. A variable amount of materials such as sand grains and the degree of humidity in the subsample magnify the error. In order to prevent such errors, most methods described in the literature include disaggregation of the egg mass; however, this is tedious and time-consuming (e.g. Herrick, 1909; Kensler, 1967; Perkins, 1971; Morgan, 1972; Díaz, 1974; Ros and Menocal, 1978).

The present work describes and evaluates a new method for quick and accurate estimation of the egg number without disaggregating the egg mass. The reliability of the method is compared to that of the Hopkins' (1962) technique as modified by Díaz (1974) and the Bogorov and Zenkevich' (1947) technique, as modified by Ros and Menocal (1978).

Egg masses were obtained from 3 ovigerous females of the mangrove crab *Aratus pisonii,* preserved in 10 % formalin in seawater. Groups of 1000 eggs were manually disaggregated, counted and separated into labelled small vials. One of these groups was processed according to each of the methods described by Díaz (1974), Ros and Menocal (1978) and the presented authors. For our new method it is necessary to estimate the mean value of the egg volume. This was done by measuring the diameter of 30 eggs of each sample. Since the eggs of *A. pisonii* are spherical, their diameter could easily be converted into units of volume. The mean volume of eggs was estimated after immersion in distilled water (immersed condition) and after removing the water by means of a vacuum pump at 0.50 atm (humid condition). Egg loss by suction was prevented by means of a piece of phytoplankton mesh. To prevent egg deformation due to overdrying during water removal, suction time for an egg layer, approximately 1 mm in thickness, was set at 30 s, and concave slides with cover slips were used while measuring under a microscope (16 ×, 10 ×). The total sample was then placed into a 5 ml calibrated flask and distilled water added. The amount of water used was recorded by means of a 0.01 ml precision buret. The difference between the volume of the calibrated flask and the amount of water corresponds to the volume of the total sample. Knowing the mean egg volume, it was possible to estimate the total number of eggs contained in the sample.

Following such estimation by all 3 methods, a new sample was prepared by adding another group of 1000 eggs to the group previously processed and estimation processes were repeated. Egg masses obtained from the 3 ovigerous females were processed independently.

Without removing the chitinous ligaments the reliability of our new volumetric method, was tested using

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17 ovigerous *Aratus pisonii* females, only separating the egg masses from the pleopods. Estimation of the total number of eggs followed. Thereafter, the eggs were disaggregated and counted manually.

In all cases the volume of the eggs (disaggregated) or egg mass (chitinous filaments included) was determinated at least 3 times, and the average obtained was considered the best approximation to the real value.

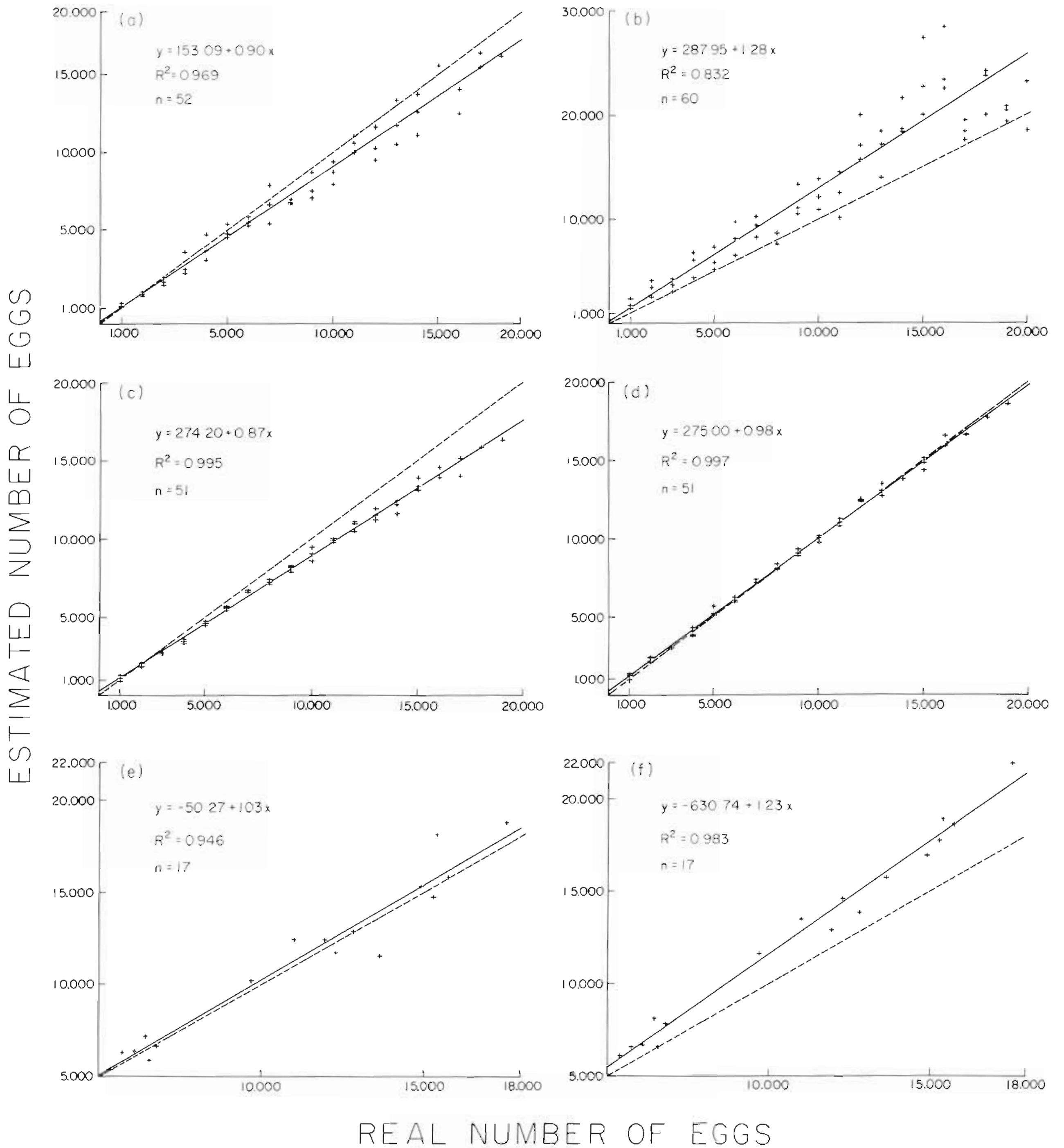


Fig. 1. Regression lines. Estimates of number of eggs contained in disaggregated egg masses by means of: (a) Hopkins' technique modified by Díaz (1974); (b) Bogorov and Zenkevich' method, modified by Ros and Menocal (1978); (c) New volumetric method, mean egg volume determined in immersed condition; (d) New volumetric method, mean egg volume determined in humid condition. Estimates of numer of eggs contained in total egg masses by means of the volumetric method: (e) mean egg volume determined under immersed condition; (f) mean egg volume determined under humid condition. Dashed lines: real values. Equations of the least squared fitted lines, proportion of variation of estimate ($R^2$); number of samples (n) indicated for each case

Table 1. Precision of each method tested (MSE) and time spent (approximately) during estimation process

| Estimation technique | Mean Squared Error (MSE) | Time (min)* Disaggregating** | Counting | Total |
|---|---|---|---|---|
| Hopkins, modified by Díaz (1974) | 1,490,514.25 | 120 | 60*** | 180 |
| Bogorov and Zenkevich, modified by Ros and Menocal (1978) | 23,610,514.70 | 120 | 10*** | 130 |
| Volumetric method | | | | |
| Disaggregated eggs | | | | |
| (a) Humid condition | 86,576.45 | 120 | 5 | 125 |
| (b) Immersed condition | 1,352,511.64 | 120 | 5 | 125 |
| Non-disaggregated eggs | | | | |
| (a) Humid condition | 4,677,506.39 | – | 5 | 5 |
| (b) Immersed condition | 1,068,425.88 | – | 5 | 5 |

* Masses with approximately, 19,000 eggs
**, *** Time consumed approximately proportional to amount of eggs in sample

The precision of each method was determined by calculating the mean squared error (MSE) (Bickel and Doksum, 1977); the degree of association between the real and estimated number of eggs was determined by means of the coefficient of determination ($R^2$) (Sokal and Rohlf, 1969). Results are shown in Table 1 and Fig. 1.

Estimations with disaggregated eggs. Best approximation to the real value was obtained by the new volumetric technique when the egg diameter was measured under humid conditions (Table 1). Despite of the close fit to a straight line found with the volumetric method under immersed conditions, the real values are consistently underestimated (Fig. 1c). This fact can be explained by a significant ($p < 0.05$, t test) increase in egg volume during diameter measurement; on the other hand, egg volume remains rather constant when the diameter is measured under humid conditions.

The data obtained with the modification of the Hopkins (1962) technique (Díaz, 1974) and the modification of the Bogorov and Zenkevich (1947) method (Ros and Menocal, 1978) showed a tendency of increased variation with increases in the number of eggs (Fig. 1a, b). The latter method proved to be the least precise of the 3 techniques compared here (Table 1).

Estimations with non-disaggregated eggs. The regression line of data obtained under immersed conditions is closer to the one corresponding to the real values. The lower MSE value reveals the greater precision obtained. However, the dispersion was higher (Table 1, Fig. 1e). Humid conditions result in an overestimation of the real value (Fig. 1f).

When estimating complete egg masses, the total volume is overestimated due to the presence of chitinous filaments. However, this overestimation apparently compensates for overestimating the mean egg volume, thus resulting in a better approximation of the real number of eggs in the sample (Fig. 1e). This apparent compensation does not occur with the mean egg volume estimated from eggs measured under humid conditions. Egg volume values measured under such conditions would not be expected to increase but rather to decrease due to overdrying. Thus, in this case, the number of eggs obtained from a sample of total egg mass would be expected to result in overestimation (Fig. 1f).

The low variability of the number of eggs estimated under humid conditions is due to the low variability in the mean egg volume under such conditions; water absorption rate might vary from one egg to another, and this variation might influence the estimates.

For estimating fecundity the period of time required to process a sample has to be considered in order to decide which method should be used. Disaggregation of eggs is tedious and time consuming (Table 1). The volumetric method does not necessarily require that step. The small loss in precision of volumetric estimations of non-disaggregated egg masses under immersed conditions with respect to estimations of disaggregated egg masses under humid conditions, is compensated for by substantial savings in time during sample processing (Table 1).

Where the female carries a large number of eggs, or large-sized eggs, it is advisable to use large, calibrated flasks with minimal error in lecture. Special care should be taken to avoid the formation of air bubbles inside the flasks while estimating egg-mass volume. Also, egg shape has to be taken into account for deciding on the method for calculating their mean volume.

The results obtained show clearly that under immersed conditions, the volumetric method provides a precise and time-saving estimate of fecundity in decapods without the need for disaggregation of egg masses. The procedure involves a significant improvement of traditional methods.

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