# Evaluation of three protein methodologies for marine plankton

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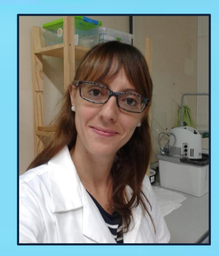


## INTRODUCTION

**Protein is an important biomass parameter and critical in the enzyme analysis of plankton. When plankton biomass is abundant, obtaining protein samples is not difficult. However, when biomass is a scarce quantity and it needs to be used for many other measurements, obtaining sufficient material for a protein sample is a challenge. There are several methods for determining total protein content. Among these, some are based on nitrogen content estimation, others on colorimetric measurement after dye binding, and still others on the biuret and Lowry methods. One of the biggest problems, limiting the application of all these methods, is the interference with the buffer compounds used in sample homogenization.**

**In this experiment, we try to determine the optimal method for measuring protein content in plankton samples prepared for enzyme analysis, testing three commonly used protein determination methods.**

**ORCID ID**

## MATERIAL AND METHODS

**To test the three methods of protein (one of these also modified), we applied each of one to the same sample, from each standar curve and each experiment sample. The standard curve and all samples were done per triplicate.**

**STANDARD CURVE**

Standard protein solutions containing 0-500µg·ml$^{-1}$ from a stock solution of Bovine serum albumina (500µg·ml$^{-1}$) dissolved in phosphate buffer containing Triton X-100

**SAMPLES**

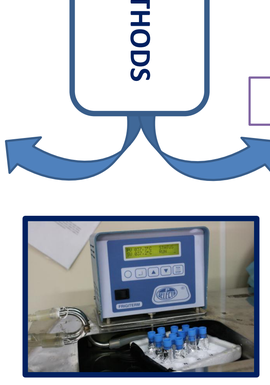
**KNOWN CONCENTRATIONS**

From the stock solution of Bovine serum albumina (500µg·ml$^{-1}$), we obtained eight samples with different protein concentrations.

**UNKNOWN CONCENTRATIONS**

- Mesozooplankton samples (200-2000µm, WP2 net-100µm mesh)
- Microplankton samples (0.7-50µm, 50µm mesh and GF-F filters – 0.7µm)

**PROTEIN METHODS**



### Lowry modified by Rutter (Rutter, 1967) – 'RUTTER'

✓ **REAGENTS:**

*R.A.*: Sodium carbonate, Sodium hydroxide, Sodium potassium tartrate

*R.B.*: Cupric sulfate pentahydrate

*R.C.*: 50 parts R.A. + 1 part R.B.

Folin: Folin diluted 1:1

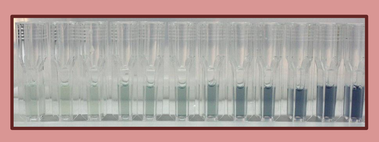
✓ **ASSAY:**

*Step 1.* Mix 0.1ml sample with 0.5ml R.C.

*Step 2.* Incubate during 10'

*Step 3.* Add 0.05ml Folin, mix well

*Step 4.*Incubate during 40' in darkness and read the absorbance at 750nm



### Bradford method (Bradford, 1976) – 'BRADFORD'

✓ **REAGENTS:**

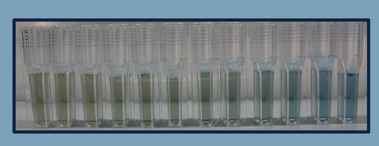
*R.A.*: Coomassie Brilliant Blue G-250, ethanol

*R.B.*: Phosphoric acid

*R.C.*: R.A + R.B. diluted to a final volume of 1L

✓ **ASSAY:**

Mix 0.02ml of sample with 1ml of R.C., and read the absorbance at 595nm



### Rutter slightly modified (Markwell et al., 1981) – 'RUTTER-SDS'

✓ **REAGENTS:**

*R.A.*: Sodium carbonate, Sodium hydroxide, Sodium potassium tartrate, Sodium dodecyl sulfate

*R.B.*: Cupric sulfate pentahydrate

*R.C.*: 50 parts R.A. + 1 part R.B.

*Folin*: Folin diluted 1:1

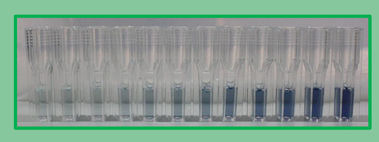
✓ **ASSAY:**

*Step 1.* Mix 0.1ml sample with 0.5ml R.C.

Step 2.Incubate during 10'

Step 3. Add 0.05ml Folin, mix well

Step 4.Incubate during 40' in darkness and read the absorbance at 750nm



### Bicinchoninic acid method (Smith, 1985) – 'SMITH'

✓ **REAGENTS:** (Pierce BCA Protein Assay Kit)

*R.A.*: Sodium carbonate, Sodium bicarbonate, Bicinchoninic acid, Sodium tartatre, Sodium hydroxide

*R.B.*: Cupric sulfate

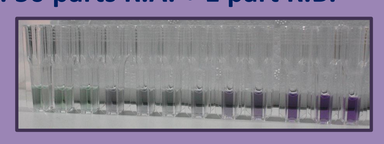
*R.C.*: 50 parts R.A. + 1 part R.B.

✓ **ASSAY:**

Step 1. Mix 0.025ml of sample with 0.5 ml of R.C.

Step 2. Incubate during 30' at 37ºC

Step 3. Cool samples to room temperature and read the absorbance at 562nm



## RESULTS

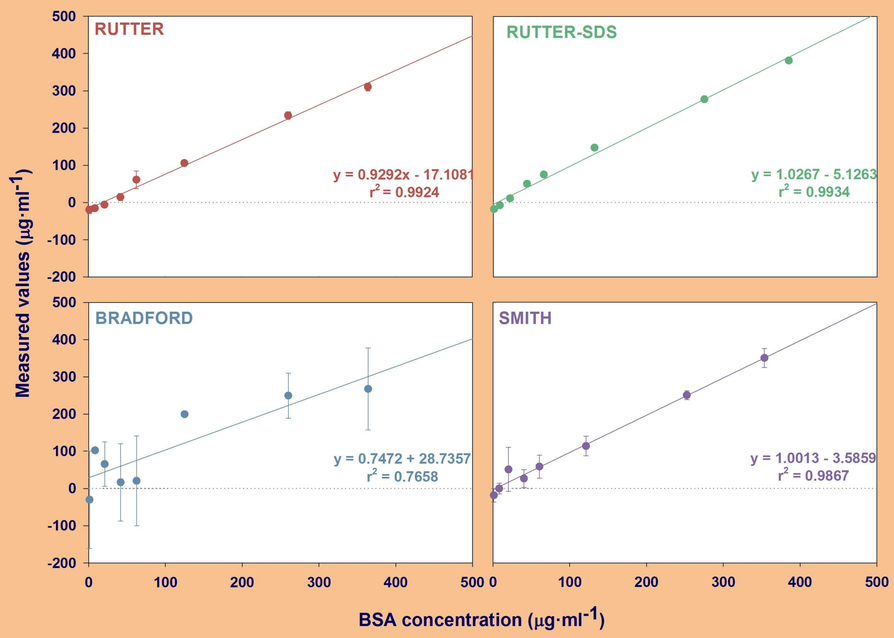



**Fig1. Known concentration samples. This figure shows the accuracy of the methods to determine the real concentration of the samples.**

**The most accurate are the SMITH and RUTTER-SDS methods, being RUTTER-SDS which has the less variability between replicates.**

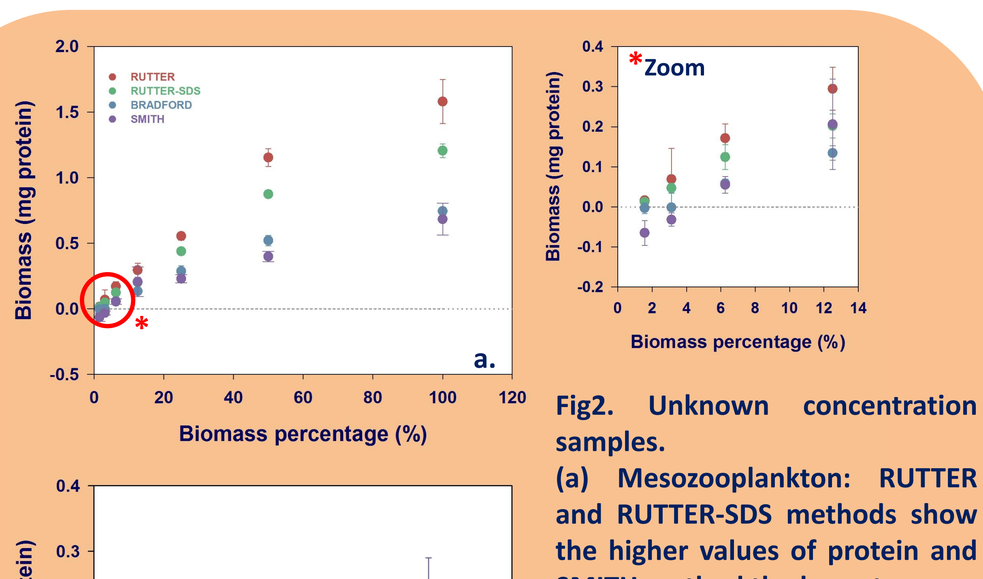



**Fig2. Unknown concentration samples.**

**(a) Mesozooplankton: RUTTER and RUTTER-SDS methods show the higher values of protein and SMITH method the lowest.**

**(b) Microplankton: In this case, the higher values were obtained with SMITH method and the lowest with BRADFORD method.**

**The variability of all methods increase if concentrations are low.**

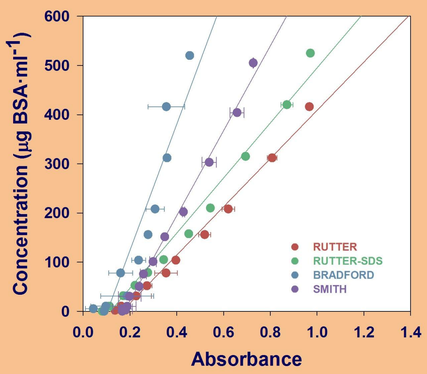
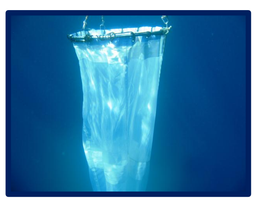
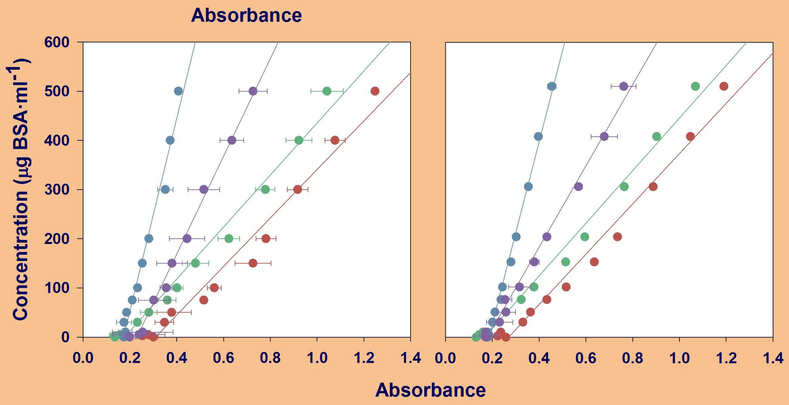



**Fig3. In most of the cases, the greatest standard curves was obtained with BRADFORD and SMITH methods, with $r^2$ of 0.99, but don't reach the maximum absorbance (ABS=1).**

**RUTTER and RUTTER-SDS methods shows the lower slopes.**






## CONCLUSIONS

- **In general, 'RUTTER-SDS' is the method with the lower variability**
- **'RUTTER-SDS' and 'SMITH' seems to be the best methods to determine the 'real' protein concentration in samples**
- **For mesozooplankton and microplankton samples, the best methods to determine the protein biomass are RUTTER and RUTTER-SDS. In microplankton samples, SMITH and BRADFORD values probably failed because the samples was a very low biomass (they present the same value independently of filtered volume)**
- **'SMITH' and 'BRADFORD' probably works better in higher protein concentrations, so the second step should be to test the micro-assays of these methods**

## REFERENCES


- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry, 72(1-2), 248-254.
- Markwell, M. A. K., Haas, S. M., Tolbert, N. E., Bieber, L. L. (1981). [16] Protein determination in membrane and lipoprotein samples: Manual and automated procedures. Methods in enzymology, 72, 296-303.
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- Smith, P., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M., Fujimoto E.K., Goeke N.M., Olson B.J., Klenk, D. C. (1985). Measurement of protein using bicinchoninic acid. Analytical biochemistry, 150(1), 76-85.