# Comparative estimations of the energy content of *Enteromorpha* spp. using different methods*

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*Enteromorpha* spp.
Comparative determination
Energy content
Jurata

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**Abstract**

The energy content of *Enteromorpha* spp. from Jurata (situated in the western part of the Gulf of Gdańsk) was determined. The values were measured directly by combustion in a microbomb calorimeter and indirectly by calculation based on the biochemical composition, and on carbon and nitrogen contents.

On the basis of the results obtained, very little difference – on average 2.98 ± 5.69% (0.54 ± 0.67 J $mg^{-1}$ of DW) – was found between the energy values calculated from the carbon content and those calculated from carbon and nitrogen contents. Moreover, the values calculated from both these sources were higher those that calculated from the biochemical composition by about 14.95 ± 12.24% (2.09 ± 1.72 J $mg^{-1}$ of DW) and 17.28 ± 14.41% (2.63 ± 2.07 J $mg^{-1}$ of DW) for the two methods respectively. At the same time, large differences were found between the values calculated using microbomb calorimetry and other methods (biochemical composition, carbon content, and carbon and nitrogen contents): they were 20.40 ± 15.09%, 33.76 ± 5.86% and 35.74 ± 6.71% for the three methods respectively.

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## 1. Introduction

A study of the energy flux is an important step in the analysis of the dynamics of an ecosystem. To this end, much effort has been expended in measuring the energy content of the tissues of organisms.

Although energy content is used extensively in ecological investigations (Cummins and Wuycheck, 1971), most workers have only determined it by one method. Some have cross-checked their results by a second method but few have used more than two methods on the same material. For example, Geng (1925) found that energy contents determined with a Berthelot bomb calorimeter were higher than the calculated values. Using 1 g of material in each test, Ivlev (1934) obtained very similar results using wet (dichromate) oxidation (corrected for incomplete oxidation of protein) and an oxygen bomb calorimeter. Nilsson (1974) applied a large correction factor (1.411 for *Gamarus pulex*) to energy values determined by wet oxidation as they were less than the values obtained using a Phillipson (1964) microbomb calorimeter. However, Elliott (1976) found no significant difference between energy measurements made by wet oxidation and those obtained from the chemical composition of the material. Furthermore, Szaniawska and Wołowicz (1986) found no significant differences between the values obtained directly by combustion in a microbomb calorimeter and indirectly by calculation based on the biochemical composition. Only one publication concerning the variation in energy value and lipid content of *Enteromorpha* spp. from the Gulf of Gdańsk has been found in the literature (Haroon and Szaniawska, 1995).

This paper compares the results of the different methods (microbomb calorimetry, biochemical composition, carbon and nitrogen contents) used to determine the energy content of dried *Enteromorpha* spp. from Jurata. Much attention was given to selecting the best method of determining the energy content. The advantages and disadvantages of all the methods used in this comparison were assessed.

## 2. Materials and methods

The materials used in the present investigations were collected from Jurata (Fig. 1) during the period from November 1992 to October 1993. Samples collected from the shallow littoral were stored in seawater before removal to the laboratory, where they were cleaned and rinsed in $H_2O$, dried at 60°C to constant weight and homogenised.

The measurements of the energy contents were carried out using the methods described below.

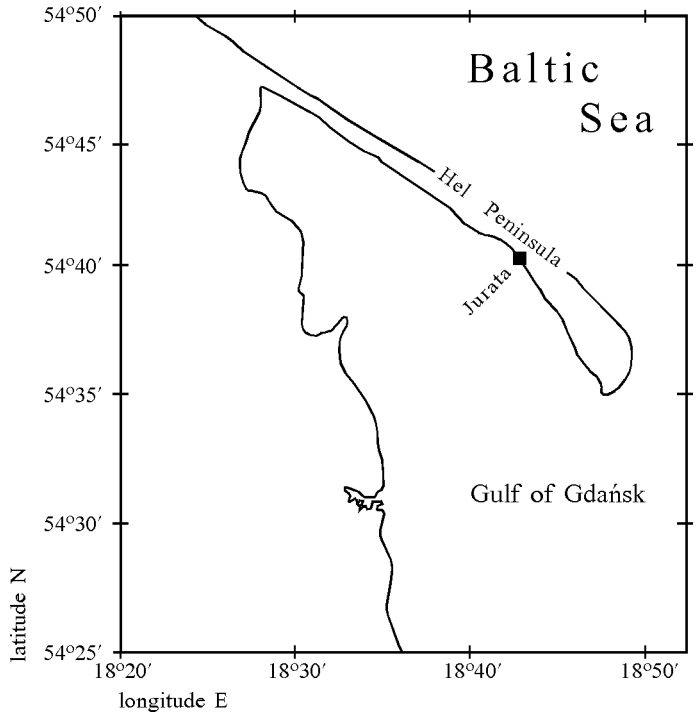


Fig. 1. Location of station

### 2.1. Direct method

Energy contents were determined directly using a Phillipson microbomb calorimeter (Phillipson, 1964), the operating principles of which were described by Klekowski and Bączkowski (1973). Three combustions were carried out for each sample, followed by the calculation of the average values. The total energy contents were expressed in J $mg^{-1}$ DW.

### 2.2. Indirect methods

#### 2.2.1. Determination of the energy content on the basis of the biochemical composition

This method involves determining the energy content on the basis of the biochemical composition (lipid, protein and carbohydrate contents) using the standard conversion factors (Wenne and Styczyńska-Jurewicz, 1985): for lipids, this factor is 39.57 J $mg^{-1}$; for proteins it is 23.65 J $mg^{-1}$ and for carbohydrates it is 17.16 J $mg^{-1}$.

A mixture of chloroform, methanol and water was used to separate lipids, utilising the method of Blight and Dyer (1959). For determining lipids, the method of Marsch and Weinstein (1966) was used: this involves measuring

the extinction and reading off the lipid level from a model curve plotted on the basis of glyceral tripalmitin. Extinctions were read off using a SPEKOL II Carl Zeiss Spectrophotometer at a wavelength of 360 nm. For each sample three results were calculated and the average found; the results are expressed in % of DW.

The protein fraction (% of DW) was calculated from the elemental N determinations using the nitrogen-protein conversion factor of 5.78 proposed by Gnaiger and Bitterlich (1984) for aquatic organisms.

The carbohydrate contents were assayed by the phenol-sulphuric acid method (Dubois *et al.*, 1956), in which a weighed sample (10 mg DW) of material was homogenised with 3 ml TCA 15% (trichloroacetic acid) and left at 4°C for one hour. This was followed by centrifugation for 10 minutes at 2800–3000 revs/minute. 0.5 ml of the supernatant was then transferred to another test-tube, mixed with 1.0 ml 5% phenol solution and allowed to stand for 40 minutes at room temperature. 5 ml concentrated $H_2SO_4$ was then added. The contents of the tubes were now homogenised in an electric shaker and again left to stand for 10 minutes at room temperature. Finally, the optical thickness was measured at 490 nm using a Carl Zeiss Jena Spekol II Spectrophotometer with glucose as standard.

### 2.2.2. Determination of the energy content on the basis of carbon and nitrogen contents

The carbon and nitrogen contents of *Enteromorpha* spp. were determined using a CHNS/O (EA 1108) Elemental Analyzer. Triplicate samples of 1–4 mg DW were sealed in a tin boat and weighed on a Cahn 25 electro-microbalance. Samples were either stored in a desiccator or immediately transferred to the automatic sampler of the Elemental Analyzer. The combustion temperature was 1025°C and sulphanilamide served as reference. Depending on the values obtained for carbon and nitrogen the energy contents were calculated using the following equations (Platt and Irwin, 1973):

$$\text{Total energy content (J mg}^{-1}\text{ DW)} = [0.632 + 0.086(\%\text{C})] \times 4.18;$$

the standard error for an estimate from this equation is 0.757 J $mg^{-1}$ DW.

$$\text{Total energy content (J mg}^{-1}\text{ DW)} = [-0.555 + 0.113(\%\text{C}) + 0.054(\text{C:N})] \times 4.18;$$

the standard error for an estimate from this equation is 0.644 J $mg^{-1}$ DW.
Where
%C – the percentage of carbon in the total dry weight,
C:N – the carbon-to-nitrogen ratio in the total dry weight.

## 3. Results

During the period from November 1992 to October 1993, the energy contents of *Enteromorpha* spp. collected from Jurata were determined using different methods.

The results of all the analyses are set out in Tab. 1. The energy contents obtained from the carbon content ranged on average from 10.39 ± 0.02 J $mg^{-1}$ DW in November 1992 to 15.17 ± 0.04 J $mg^{-1}$ DW in May 1993. For the remaining months the variations in values are about 1.00 J $mg^{-1}$ DW. The carbon and nitrogen content method yielded values ranging from 9.48 ± 0.01 J $mg^{-1}$ DW in November 1992 to 16.15 ± 0.01 J $mg^{-1}$ DW in August 1993. At the same time these values were higher than those obtained by combustion in a microbomb calorimeter and from the biochemical composition.

**Table 1.** Comparative determination of total energy contents (J $mg^{-1}$ of DW) of *Enteromorpha* spp. from Jurata

| | Direct method | Indirect methods | | |
|---|---|---|---|---|
| | calculated from microbomb | calculated from biochemical composition | calculated from carbon content | calculated from carbon and nitrogen contents |
| Months | Average ± SD | | | |
| November 1992 | 6.99 ± 1.90 | 9.81 ± 0.40 | 10.39 ± 0.02 | 9.48 ± 0.01 |
| May 1993 | 10.81 ± 0.44 | 13.10 ± 0.65 | 15.17 ± 0.04 | 15.82 ± 0.07 |
| June 1993 | 8.25 ± 0.91 | 14.86 ± 0.03 | 14.64 ± 0.01 | 15.32 ± 0.03 |
| July 1993 | 8.34 ± 0.53 | 9.36 ± 0.59 | 13.54 ± 0.03 | 14.24 ± 0.01 |
| August 1993 | 10.10 ± 0.72 | 14.15 ± 0.63 | 15.12 ± 0.00 | 16.15 ± 0.01 |
| September 1993 | 9.53 ± 2.47 | 11.21 ± 0.42 | 14.35 ± 0.08 | 14.88 ± 0.11 |
| October 1993 | 10.49 ± 0.71 | 10.24 ± 0.22 | 14.18 ± 0.20 | 15.26 ± 0.26 |
| Average ± SD | 9.22 ± 1.40 | 11.82 ± 2.21 | 13.91 ± 1.65 | 14.45 ± 2.28 |

On the basis of calculations (Tab. 2 and 3) it was found that there was very little difference – on average 2.98 ± 5.69% (0.54 ± 0.67 J $mg^{-1}$ DW) – between the energy contents calculated from the carbon content and those calculated from the carbon and nitrogen contents. However, the respective values calculated using these two methods were higher than those obtained from the biochemical composition by about 14.95 ± 12.24% (2.09 ± 1.72 J $mg^{-1}$ DW) and 17.28 ± 14.41% (2.63 ± 2.07 J $mg^{-1}$ DW).

**Table 2.** The difference (expressed as %) between the energy contents of *Enteromorpha* spp. from Jurata calculated using different methods

| Months | B + M | B + C | B + (C + N) | M + (C + N) | M + C | C + (C + N) |
|---|---|---|---|---|---|---|
| November 1992 | 28.75 | 5.58 | –3.48 | 26.27 | 32.72 | –9.60 |
| May 1993 | 17.48 | 13.65 | 17.19 | 31.67 | 28.74 | 4.11 |
| June 1993 | 44.48 | –1.50 | 3.00 | 46.15 | 43.65 | 4.44 |
| July 1993 | 10.90 | 30.87 | 34.27 | 41.43 | 38.40 | 4.92 |
| August 1993 | 28.62 | 6.42 | 12.38 | 37.46 | 33.20 | 6.38 |
| September 1993 | 14.99 | 21.88 | 24.66 | 35.95 | 33.59 | 3.56 |
| October 1993 | –2.44 | 27.79 | 32.90 | 31.26 | 26.02 | 7.08 |
| Average ± SD | 20.40 ± 15.09 | 14.95 ± 12.24 | 17.28 ± 14.41 | 35.74 ± 6.71 | 33.76 ± 5.86 | 2.98 ± 5.69 |

Legend:

B – total energy content calculated from biochemical composition,
M – total energy content calculated from microbomb calorimetry,
C – total energy content calculated from carbon contents,
C + N – total energy content calculated from carbon and nitrogen contents,
SD – standard deviation.

**Table 3.** The difference (expressed as J $mg^{-1}$ of DW) between the energy contents of *Enteromorpha* spp. from Jurata calculated using different methods

| Months | B – M | C – B | (C + N) – B | C – M | (C + N) – M | (C + N) – C |
|---|---|---|---|---|---|---|
| November 1992 | 2.82 | 0.58 | –0.33 | 3.4 | 2.49 | –0.91 |
| May 1993 | 2.29 | 2.07 | 2.72 | 4.36 | 5.01 | 0.65 |
| June 1993 | 6.61 | –0.22 | 0.46 | 6.39 | 7.07 | 0.68 |
| July 1993 | 1.02 | 4.18 | 4.88 | 5.2 | 5.9 | 0.7 |
| August 1993 | 4.05 | 0.97 | 2 | 5.02 | 6.05 | 1.03 |
| September 1993 | 1.68 | 3.14 | 3.67 | 4.82 | 5.35 | 0.53 |
| October 1993 | –0.25 | 3.94 | 5.02 | 3.69 | 4.77 | 1.08 |
| Average ± SD | 2.60 ± 2.23 | 2.09 ± 1.72 | 2.63 ± 2.07 | 4.70 ± 1.00 | 5.23 ± 1.43 | 0.54 ± 0.67 |

Symbols as in Tab. 2.

At the same times large differences were found between the values calculated by microbomb calorimetry and the other methods (biochemical composition, carbon content, and carbon and nitrogen contents): they were 20.40 ± 15.09%, 33.76 ± 5.86% and 35.74 ± 6.71% for the three methods respectively.

Furthermore, comparison of standard deviations and the smallest differences between the energy values obtained by microbomb calorimetry and those calculated from the carbon content, carbon and nitrogen contents, and the biochemical composition of the experimental material has shown that the results obtained using the first three methods are more accurate than the ones calculated from the biochemical composition.

## 4. Discussion and conclusions

The four methods used for determining the energy content of *Enteromorpha* spp. from Jurata (Gulf of Gdańsk) yielded different results.

As stated by Craig (1977), the differences between the values obtained by bomb calorimetry and conversion from lipids (using a factor of 9.45 cal $mg^{-1}$) and proteins amounted on average to 7.2%. Using the same conversion factors (9.45 cal $mg^{-1}$ for lipids, 5.65 cal $mg^{-1}$ for proteins) and correcting for non-protein nitrogen, there was a difference of 4–3% between the two methods. Craig *et al.* (1978) also ascribe this difference to an overestimate of lipid content owing to the presence of impurities.

As reported by Bieńkowski (1990), the energy value determined by the Gnaiger equation is relatively closest to the true value, although it is markedly lower (by *ca* 20%) than that determined by microbomb calorimetry. Additionally, in Berthelot's modification of Dulong's method or Aliev's (1973) method it is lower by 30 and 50% respectively.

At Jurata the energy values computed from carbon and nitrogen contents were higher than those obtained by combustion in a microbomb calorimeter or calculated from the biochemical composition.

The results we have obtained, together with the following reasons, lead us to conclude that the best method of determining energy content is based on the carbon and nitrogen content for the following reasons:

- It can be used with a very small weight of sample – 1.00–4.00 mg DW. For microbomb calorimetry from 5.00–10.00 mg and for the biochemical composition at least 30 mg DW are required, and at most times a sufficient quantity of dry tissue is not readily available.
- The CHNS/O Elemental Analyzer used for C and N determination is very rapid and analysis can be completed in as little as 12 minutes. The possibility of analysing 4 elements simultaneously in one sample,

and to run up to almost 200 analyses in a complete automatic mode reduces the cost. Furthermore, the gases analysed can be used for further quantitative analyses, as they have not been diluted, split or otherwise modified during the analysis.

- When using the carbon and nitrogen content method, there is no possibility of personal error, whereas in the microbomb and the biochemical composition methods, this can always occur. Such an error may result from the loss of dry matter during the preparation of the microbomb, or of some lipid, protein or carbohydrate during extraction or lipid separation. Moreover, the effects of incomplete extraction, coextraction of impurities or calorimetric interferences are unpredictable and must be considered significant (Gnaiger and Bitterlich, 1984). It would be excessively time-consuming to analyse an organism or organic deposit into its major biochemical constituents slowly for the purpose of estimating energy content, unless the protein, carbohydrate and lipid fractions were required for some other purpose.
- Carbon and nitrogen contents can be used for determining both energy contents and lipid, protein and carbohydrate contents.

At the same time, however, if we wish to discover only the energy content of an organism, a microbomb calorimeter is sufficient. In addition, if we wish to learn something more about the metabolism of an organism and its principal source of energy, we must measure lipid, protein and carbohydrate contents. In this case, calculating the energy content from the biochemical composition is a better method.

## References

Aliev A. S., 1973, *Bioenergetics of organic matter*, Izd. Elm, Baku, 66 pp., (in Russian).

Bieńkowski P., 1990, *Evaluation of methods of calculation of energetic value of organic matter from its elementary composition*, Pol. Ecol. Stud., 16, 245–258.

Blight E. G., Dyer W. J., 1959, *A rapid method of total lipid extraction and purification*, Can. J. Biochem. Physiol., 37, 911–917.

Craig J. F., 1977, *The body composition of adult perch, Perca fluviatilis L., in Windermere, with reference to seasonal changes and reproduction*, J. Anim. Ecol., 46, 617–632.

Craig J. F., Kenley M. J., Talling J. F., 1978, *Comparative estimations of the energy content of fish tissue from bomb calorimetry, wet oxidation and proximate analysis*, Fresh Wat. Biol., 8, 585–590.

Cummins K. C., Wuycheck J. C., 1971, *Caloric equivalents for investigations in ecological energetics*, Mitt. Int. Ver. Theor. Angew. Limnol., 18, 1–158.

Dubois M., Gilles K. A., Hamilton J. K., Rebers P. A., Smith F., 1956, *Calorimetric methods for determination of sugars and related substances*, Analyt. Chem., 28, 350–356.

Elliott J. M., 1976, *Body composition of brown trout (Salmo trutta L.) in relation to temperature and ration size*, J. Anim. Ecol., 45, 273–289.

Geng H., 1925, *Der Futterwert der natürlichen Fischnahrung*, Z. Fisch., 23, 137–165.

Gnaiger E., Bitterlich G., 1984, *Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept*, Oecologia, 62, 289–298.

Haroon A. M., Szaniawska A., 1995, *Variations in energy values and lipid content in Enteromorpha spp. from the Gulf of Gdańsk*, Oceanologia, 37 (2), 171–180.

Ivlev V., 1934, *Eine Mikromethode zur Bestimmung des Kaloriengehalts von Nährstoffen*, Biochem. Z., 275, 49–55.

Klekowski R. Z., Bączkowski J., 1973, *A new modification of microbomb calorimeter*, Ecol. Pol., 21, 229–236.

Marsch J. B., Weinstein D. B., 1966. *Simple charring method for determination of lipids*, J. Lipid Res., 7, 574–576.

Nilsson L. M., 1974, *Energy budget of a laboratory population of Gammarus pulex (Amphipoda)*, Oikos, 25, 35–42.

Phillipson J., 1964, *A miniature bomb calorimeter for small biological samples*, Oikos, 15, 130–139.

Platt T., Irwin B., 1973, *Caloric content of phytoplankton*, Limnol. Oceanogr., 18, 306–310.

Szaniawska A., Wołowicz M., 1986, *Energetic values of the body of Macoma balthica L. from the Gulf of Gdańsk*, Oceanologia, 24, 55–62.

Wenne R., Styczyńska-Jurewicz E., 1985, *Microgeographic differentiation in condition and biochemical of Macoma balthica (L.) from the Gdańsk Bay*, Pol. Arch. Hydrobiol., 32, 175–194.