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Measuring carbon content in nematodes

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ABSTRACT: A rapid and accurate method for determining the carbon content in nematodes is described based on the carbon analyzer of Salonen (1979). Individual nematode specimens are introduced on a needle into the furnace-tube and placed there in a drop of 10 µl deionized water and combusted at high temperature. The effect of preserving animals in 4 % formaldehyde at room temperature and in 4 % formaldehyde at −20 °C on the carbon content showed marked differences. Preservation at room temperature rapidly resulted in 8 to 24 % loss of carbon, whereas freezing in formaldehyde yielded values similar to those of living reference animals. Carbon content of nematodes is found to be 12.4 % on a wet weight basis.

INTRODUCTION

Free-living nematodes make an important contribution to energy flow in a variety of aquatic ecosystems (cf. Gerlach, 1971, 1978; Elmgren, 1976; Fenchel, 1978; Heip et al., 1982; Platt & Warwick, 1980; Jensen, 1983, 1984). However, little attention has been paid to the development of accurate methods to determine and quantify nematode biomass values. Currently, the most usual method is to estimate volume by measuring length and width, assuming a cylindrical body shape (Andrassy, 1956), and convert to wet weight using a specific weight of 1.1. Recently, Faubel (1982) presented dry weight values (oven dried at 60 °C) measured on a microbalance and related these values to definite size classes of animals. As noted by Platt & Irwin (1973), the energy equivalent for biomass expressed in terms of organic carbon should theoretically vary less than when calculated from dry weight. Yet, Ernst (1975) described and tested a new combustion method with IR-detection of CO_2 for the microdetermination of organic carbon in marine sediments, and Goerke & Ernst (1975) determined the ATP content of two estuarine nematodes and arrived at the assumption that the carbon content of nematodes is in the range of 10 to 15 % on a wet weight basis.

This paper is concerned with a method, based on the carbon analyzer developed by Salonen (1979), which makes it possible to determine the carbon content of individual nematodes in a rapid and accurate way. The method also allows determination of the effect on biomass of two different preservation methods.

The study is part of a project on the behaviour and ecology of Baltic Sea nematodes carried out at the Tvärminne Zoological Station and Lammi Biological Station, University of Helsinki, Finland.

MATERIAL AND METHODS

Collection of samples

All nematode species were isolated from the sediment or submerged vegetation close to Tvärminne Zoological Station, southern archipelago of Finland (cf. Jensen, 1984).

Chromadorita tenuis (Schneider), *Punctodora ratzeburgensis* (Linstow) and *Hypodontolaimus balticus* (Schneider) were raised in the laboratory at 18 °C in 8.7 cm Petri dishes with 0.8 % agar, 6 ‰ S seawater and inoculated with the pennate diatom *Nitzschia palea* var. *debilis* (Kütz.) Grunow.

Monhystera disjuncta Bastian was cultivated in a similar way, but fed on unidentified bacteria attached to *Fucus vesiculosus*.

Adoncholaimus thalassophygas (De Man) was cultivated in a similar way, but fed with monhysterid and chromadorid nematodes.

Sabatieria pulchra (Schneider) specimens derived from the field.

Preparation of samples

Subsamples of about 50 *Chromadorita tenuis* were used in the comparison of preservation methods. One subsample was analyzed with living animals. Other subsamples were fixed in 4 % unbuffered formaldehyde at room temperature and a final group of subsamples were frozen at −20 °C in 4 % unbuffered formaldehyde; the determination of carbon was then made as soon as the animals melted from the ice.

Carbon determination

The carbon analysis was performed by the high temperature combustion method of Salonen (1979). Individual animals were picked out of the agar with a bent needle and narcotized in 6 ‰ S filtered seawater with crystalline $MgCl_2$. After relaxation, each was washed three times in deionized water, its length and width measured through a camera lucida and then transferred to a 10 µl drop of deionized water on a dense platinum net in the furnace of the carbon analyzer. During a pilot study, living animals were introduced on to a glass-fibre cup on the platinum net in the furnace. The smooth animals adhered firmly to the glass-fibres and the carbon results were reliable. This method is, however, not applicable for 4 % formaldehyde preserved animals which are rigid and do not adhere satisfactorily to the glass-fibre cup but are attached electrostatically to the furnace wall. This latter observation is significant after 10 to 15 successive measurements; i.e. after 10 to 15 min, in which period the electrostatic force in the furnace tube had accumulated to such a level that the individual introduced animals were simply lost before being combusted. This was visualized on the potentiometric recorder attached to the infrared gas analyzer, where the peak height disappeared down to the background level after 10 to 15 measurements and continued at that low level independently of the size of the introduced fixed nematode.

The body volume was derived from measurements of body length and width assuming a cylindrical body shape, and wet weight was calculated using a specific weight of 1.1.

RESULTS AND DISCUSSION

The method described above for measuring the carbon content in nematodes gives an accurate determination in less than 1 min; i.e., a more rapid and advanced method than measuring the wet weight or dry weight. Although the present findings were based on use of the original analyzer, a commercial design is now available ("Unicarb Universal Carbon Analyzer"; Elektro Dynamo Oy, Helsinki, Finland).

The critical point is the introduction of an animal in the right medium. Reliable results with a loss of less than 1 % of living or fixed animals were obtained by exchanging the glass-fibre cup with a drop of 10 µl deionized water on the platinum net. A drop of 10 µl has a surface tension strong enough to adhere a preserved nematode against the electrostatic forces in the tube; the backround noise of 10 µl deionized water is measurable, although very small even compared to a juvenile *Chromadorita tenuis* or *Monhystera disjuncta*. The method is also applicable for living nematodes and is therefore recommended.

Although the method applied by Ernst (1975) has many similarities with the present procedure, we found three main problems with his method for determining carbon content of small samples: (1) it is necessary to open the tube which creates contamination to the sample vessel; (2) contamination of sample vessel from the bottom of the tube is serious and more or less unpredictable for submicrogram samples, however, both problems may be reduced by frequently cleaning (e.g. ignition) of the sample combustion tube; (3) to open and close the tube end is slower than to open and close the side stopper in Salonen his system (Salonen, 1979; Fig. 2).

After preservation for one day in 4 % formaldehyde at room temperature the carbon content of 15-day-old *Chromadorita tenuis* had decreased significantly to 109 ng C per animal compared to the initial value of 119 ng C (Table 1). From the sixth day and

Table 1. Effect of two preservation methods on the carbon content (ng C animal^{-1} ± 95 % confidence limits) of 15 days old male *Chromadorita tenuis*. Number of replicates is given in parentheses

Experimental condition	Initial	1 day	6 days	1 month	2 months	6 months
Live	119 ± 8 (58)	–	–	–	–	–
Preserved in 4 % formaldehyde	–	109 ± 11 (48)	94 ± 10 (56)	91 ± 10 (52)	93 ± 11 (49)	91 ± 10 (57)
Frozen in 4 % formaldehyde	–	–	–	117 ± 8 (52)	116 ± 8 (51)	116 ± 8 (47)

onwards the carbon results did not differ from the final value obtained after 6 months. The final value was 24 % lower than in unpreserved animals and this decrease is also observed in other developmental stages of *C. tenuis*. The subsamples frozen in 4 % formaldehyde yielded carbon values similar to those of living reference animals (Table 1). Both results are in accordance with the findings of Salonen & Sarvala (1980) for the copepod *Megacyclops gigas*.

In general, the organic carbon content of the 6 selected nematode species was 12.4 ± 1.3 % of the wet weight. This is in the range to be excepted from previous findings; the usual carbon value for invertebrates is about 10 % of the wet weight (Steele, 1974), and Ernst (pers. comm.) found an average carbon value of 13.4 % on a wet weight basis.

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