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Metabolic loss of organic carbon by the polychaete *Capitella capitata* (Fabricius) estimated from initial weight decrease during starvation, oxygen uptake, and release of ^{14}C by uniformly-labeled animals*

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ABSTRACT: Rate of metabolic loss of organic carbon by the polychaete *Capitella capitata* (Fabricius) was estimated by following the initial decrease in weight during starvation. This estimate was compared to estimates derived from measuring oxygen uptake and loss of ^{14}C as $^{14}CO_2$ and $DO^{14}C$ from uniformly-labeled worms. There was general agreement between the methods at all 3 experimental temperatures (10, 20, 30 °C). The weight loss method may be a useful alternative to O_2 uptake for estimating metabolism in some instances.

INTRODUCTION

Probably the most common method of estimating loss of assimilated carbon by marine invertebrates in studies of ecological energetics is to measure the uptake of oxygen (O_2) and then to apply a conversion factor (the respiratory quotient) to estimate the release of carbon dioxide (CO_2) or energy (for example Hargrave 1971; Cammen, 1980). This method, however, does not account for anaerobic generation of CO_2 which may be significant for some invertebrates such as polychaetes (Cammen, in press) and bivalves (Famme et al., 1981 and references therein). In addition, metabolic release of organic material in the form of dissolved organic carbon (DOC) and particulate organic carbon (POC) is not included; these losses may also be important (e. g. Hargrave, 1971; Tenore and Gopalan, 1974; Kofoed, 1975; Famme and Kofoed, 1982).

During a series of studies of the nutrition of the polychaete *Capitella capitata* (Fabricius) and its response to varying levels of food supply (Tenore, 1977, 1981, 1982), the question arose as to whether some relatively simple method of estimating the total metabolic release of organic carbon could be found. Under steady state conditions, carbon absorption by an organism approximates carbon loss with the difference being carbon utilized for somatic growth or gamete production. If it were possible to eliminate the input of carbon to an organism without affecting the loss rate, then the decrease in total carbon content over time would be equal to the total loss rate as CO_2, DOC and POC. In practice, however, metabolism ordinarily begins to decrease with the onset of starvation (e. g. Vernberg, 1959; Wallace, 1973; Lane and Lawrence, 1979; Cuzon et al., 1980; Famme and Kofoed, 1982) and the loss rate of carbon presumably decreases similarly. It should be possible, however, to extrapolate back to the rate present when starvation began if the decrease can be expressed in linear form. Although the decrease in metabolic rate is not regular over a long time-scale, but instead occurs in steps as different pools of carbon reserves are metabolized (Wallace, 1973), there is an initial, short-term decrease that does appear to be regular (see references above).

The purpose of this study was to estimate the total loss rate of assimilated carbon by *Capitella capitata* by following the decrease in weight of starving worms

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over time and extrapolating back to the initial rate of decrease at the onset of starvation. These estimates were compared to estimates derived from measurements of O_2 uptake by similar-sized individuals. In addition, loss of ^{14}C from uniformly-labeled *C. capitata* in the form of $^{14}CO_2$ and $DO^{14}C$ was measured to give an idea of the degree of non-respiratory losses of carbon as well as to give an additional estimate of the loss rate for assimilated carbon.

METHODS

Capitella capitata (Type I) used in these experiments were taken from stock cultures maintained at 20 °C by K. R. Tenore. Experiments were carried out either at room temperature (20 °C) or in 10 and 30 °C controlled-temperature rooms. All seawater was 1µm filtered with a salinity of 26 ‰ unless otherwise noted, and the sand used in these experiments was clean beach sand < 300 µm which had been ashed at 500 °C for several hours.

Weight loss during starvation. Worms were obtained either directly from the stock culture (20°) or from intact portions of the stock culture sediment that had been transferred to controlled temperature rooms (10 and 30°) and left for 12 d. Worms were grouped into small (0.4 to 0.9 mg ww), medium (1.7 to 2.7 mg) and large (2.7 to 4.3 mg) size classes and then 25 to 100 similar-sized individuals from each group were weighed wet and placed in small plastic dishes (41.0 cm^2) along with 50 g ashed sand which made a layer about 1 cm deep. Initially 3 dishes for each size class were submerged in aerated seawater. Worms were sieved from sediment each day for the next 3 d; live worms were weighed and then returned to new dishes with fresh sediment. After final measurement, the data for each dish were fitted to an exponential equation relating days of starvation (T) to body weight (W): $W = ae^{-bT}$. Although the calculated r^2 values were similar for both a linear decrease in body weight with time and an exponential decrease, the latter equation was used as a model since previous studies have indicated the initial decrease in metabolism following the onset of starvation reaches a plateau after a period of days (Vernberg, 1959; Wallace, 1973). In order to make these data comparable to those generated by the ^{14}C and O_2 methods, which used 1 to 3 h incubations, loss in body weight was calculated only for the first 2 h from the equation and then multiplied by 12 to give a daily loss.

O_2 uptake. Worms were obtained as described above for the weight loss experiments; 10 °C worms were allowed to acclimate for 15 to 16 d, 30 °C worms for 11 to 14 d. Ten to 100 worms, depending on size, were given 3 to 4 h to clear their guts and then sealed in small plexiglass respiration chambers (33.2 cm^2) along with 50 g ashed sand; the chambers held 110.8 ml water. A stirring bar was suspended from the lid of each chamber and rotated slowly during the incubations so that the sand surface was not disturbed. O_2 uptake from the water was measured with Radiometer Type E5046 PO_2 electrodes inserted through the lids and connected to a Radiometer PHM71 Mk 2 Acid-Base Analyzer and a recorder. The procedure was to first establish a stable baseline uptake with only 0.2 µm filtered seawater and sand in the sealed chambers (usually about 1 h) and then open the chambers, add the worms, reseal the chambers, and follow the decrease in O_2 for 0.5 to 2 h until a consistent rate of change was established; the difference between the final uptake rate and the baseline uptake, after correction for barometric pressure and water temperature, represented the O_2 uptake of the worms. A respiratory quotient of 0.9 was assumed to convert O_2 uptake to the equivalent release of CO_2. In general, O_2 saturation remained above 80 % during the incubations.

$^{14}CO_2$ and $DO^{14}C$ release. Juvenile *Capitella capitata* were obtained by placing small dishes of clean sand in the culture tray and removing them after larvae had settled (3 to 4 d). These juveniles were then removed from the sand by pipette, cleaned and placed in ashed sand with ^{14}C-labeled ground *Gracilaria folifera* v. *angustissima* (Stock G-20, 32.29 % C, 2.56 % N, specific activity of 1.91×10^8 DPM $[g\ C]^{-1}$ after 3 d of leaching in seawater) as the carbon source. Worms were allowed to grow for 9 to 23 d at either 10, 20 or 30 °C before being removed from the sediment and used in these experiments. Prior to incubations the worms were rinsed well and allowed to clear their guts for 4 to 5 h. Three to 50 worms, depending on size, were then placed in a scintillation vial along with 10 ml 0.2 µm filtered seawater and 2.0 g ashed sand. The vials were sealed and left in the dark at the appropriate temperature for either 2 h (20 and 30 °C) or 3 h (10 °C). At the end of the incubations, two 4 ml aliquots of the water were drawn carefully from each vial. One of the 4 ml aliquots was mixed immediately with 15 ml of a phenethylamine-toluene-Triton X scintillation cocktail. The other aliquot was placed in a vial with 0.1 ml 0.5 N phosphoric acid and bubbled for 15 min to remove the $^{14}CO_2$ (Theodórsson and Bjarnason, 1975) and then scintillation cocktail was added. Worms were removed from the vials, allowed to clear their guts for 3 h, rinsed in 10 % HCl and distilled water, dried at 60 °C, and then burned in a Packard Tri-carb Sample Oxidizer to oxidize all the ^{14}C to $^{14}CO_2$. All samples were counted in a Packard Liquid Scintillation Spectrometer for at least 100 min or 10,000 counts. Using the DPM (g dry weight)$^{-1}$ determined for the worms and a value of 37.1 % carbon of dry weight, specific activity was calculated as DPM (g C)$^{-1}$. All specific

activity determinations for 10 and 30 °C (the 20 °C data were lost due to malfunction of the sample oxidizer) were then fitted to an exponential equation relating specific activity in DPM (g C)$^{-1}$ to body size (W) in mg wet weight:

$$\text{Sp. act.} = 1.92 \times 10^8 (1 - e^{-3.856\,W}) \quad (N = 32, r^2 = 0.95).$$

For each measurement of $DO^{14}C$ (the acidified vial) and $^{14}CO_2$ (the difference between the unacidified and acidified vials), actual carbon release was estimated using the above equation and the appropriate body size to give the specific activity; we assumed that the specific activities at 20 °C were predictable from that equation since no temperature effect was apparent in the 10 and 30 °C data.

Treatment of data and conversion factors. Worm size throughout this paper refers to wet weight (ww) determined by blotting a group of worms dry and weighing; these measurements were reproducible to within 5 %. Dry weight (dw) of *Capitella capitata* is 22.5 % of ww (S. D. 1.8 %, N = 17) at a salinity of 26 ‰ and organic carbon is about 37.1 % of dw (K. R. Tenore, unpubl.). All regressions in this paper are GM regressions as recommended for situations where measurement error exists in both variables (Ricker, 1973).

RESULTS

Despite the diversity of approaches, the 3 methods of estimating the rate of metabolic loss of assimilated carbon gave comparable results at 20 °C (Fig. 1, Tables 1 and 2); however, there was no consistent relation between the methods at either 10 or 30 °C. Estimated weight loss of carbon was not always higher than the estimates derived from measurements of oxygen uptake or ^{14}C release. There was no apparent relation between the percentage of ^{14}C released as $DO^{14}C$ and worm size or temperature; overall, an average of 38 % (S. D. 19 %) of the labeled carbon was lost as $DO^{14}C$.

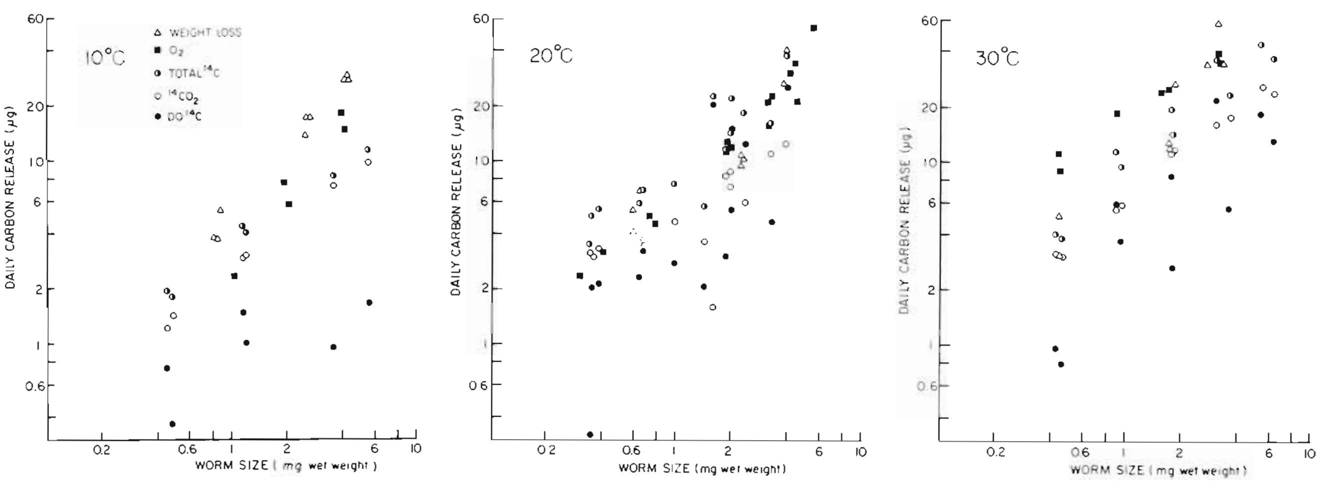

Fig. 1. *Capitella capitata.* Comparison of daily carbon loss at 10, 20 and 30 °C, estimated by weight loss during starvation, release of ^{14}C from uniformly-labeled individuals, and O_2 uptake

Table 1. GM regression lines calculated for data in Fig. 1. Regressions were calculated for the equation $\log C = \log a + b \log W$, where C = μg C released d^{-1} worm^{-1}; W = body size in mg ww. In addition, coefficients have been calculated for the equation $C' = a' W'^{b}$, where C' = μg released d^{-1} (mg worm C)$^{-1}$; W' = body size in mg C

Temperature	Method	N	a	b	r^2	a'	b'
10 °C	Weight loss	9	5.17	1.17	0.98	95.16	0.17
	O_2	5	2.13	1.41	0.96	69.97	0.41
	Total ^{14}C	6	3.43	0.72	0.99	20.50	−0.28
20 °C	Weight loss	8	7.02	0.93	0.76	71.37	−0.07
	O_2	14	6.03	0.99	0.96	70.75	−0.01
	Total ^{14}C	14	8.93	0.85	0.77	73.74	−0.15
30 °C	Weight loss	8	9.62	1.25	0.88	213.09	0.25
	O_2	7	16.25	0.67	0.97	85.33	−0.33
	Total ^{14}C	10	9.54	0.90	0.93	89.13	−0.10

Table 2. Comparison of various methods of estimating daily carbon loss by *Capitella capitata*. Carbon loss (μg C d^{-1} $worm^{-1}$) was estimated from the equations in Table 1 for 3 hypothetical individuals with body weights of 0.4, 1.2 and 4.0 mg ww. Values in parentheses are ± 1 standard deviation

	10°			20°			30°		
Method	0.4	1.2	4.0	0.4	1.2	4.0	0.4	1.2	4.0
Weight loss	1.8	6.4	26.3	3.0	8.3	25.6	3.1	12.1	54.3
	(1.7, 1.9)	(5.6, 7.3)	(15.4, 45.0)	(2.5, 3.5)	(5.3, 13.0)	(5.0, 130.4)	(2.6, 3.6)	(7.8, 18.7)	(11.5, 256.9)
O_2	0.6	2.8	15.0	2.4	7.2	23.9	8.8	18.4	41.0
	(0.5, 0.6)	(2.0, 3.8)	(3.8, 58.8)	(2.3, 2.6)	(6.3, 8.3)	(14.5, 39.2)	(8.3, 9.3)	(15.8, 21.3)	(24.9, 67.6)
Total ^{14}C	1.8	3.9	9.3	4.1	10.0	26.5	4.2	11.2	33.3
	(1.7, 1.9)	(3.5, 4.3)	(6.5, 13.4)	(3.6, 4.7)	(7.3, 13.7)	(9.2, 76.6)	(3.9, 4.6)	(9.1, 13.7)	(15.9, 69.6)
% $\frac{\text{Total } ^{14}C}{\text{Weight loss}}$	100	61	35	137	120	104	135	93	61
% $\frac{O_2}{\text{Weight loss}}$	33	44	57	80	87	93	284	152	76

DISCUSSION

Starving marine invertebrates can lose a significant fraction of their body weight each day. In my experiments, *Capitella capitata* lost 5 to 8 % of their body weight d^{-1} at 10 °C, 8 to 9 % at 20 °C, and 9 to 16 % at 30 °C (Table 2). Fractional weight losses reported for other polychaetes have been lower, namely 0.3 to 2.8 % of initial body weight d^{-1} for *Nereis virens* (Kay and Brafield, 1973; Tenore and Gopalan, 1974), 2.3 to 3.8 % for *N. succinea*, and 1.2 to 4.3 % for *N. diversicolor* (Neuhoff, 1979), but these were larger animals that would be expected to have lower metabolic rates per unit weight. Similar weight losses have been reported for starving zooplankton, including 0.4 to 1.4 % of initial body weight d^{-1} for the copepod *Euchaeta norvegica* (Båmstedt and Holt, 1978), 2 to 3 % for the copepod *Chiridius armatus* (Alvarez and Matthews, 1975), and 10 to 15 % for the cladoceran *Daphnia pulex* (Lemcke and Lampert, 1975 as cited in Båmstedt and Holt, 1978).

The weight loss method should give estimates of carbon loss rate greater than those estimated from the O_2 or ^{14}C methods (as presented in this study) since it includes loss of dissolved and particulate carbon as well. A summary of respiration data for 37 polychaete species at 15 °C (Cammen, in press) yielded a general relation for resting aerobic metabolism of

$$R = 0.42 \cdot W^{0.850} \qquad (1)$$

where R = joules released d^{-1}; W = mg body dw. Assuming an intermediate value of 19 joules (mg $dw)^{-1}$ this expression is equivalent to

$$\%\ \text{daily weight loss} = 2.2\ W^{-0.150} \qquad (2)$$

Since the range of body weights considered was 0.27 mg to 3.17 g dw, the predicted range in fractional body weight loss at 15 °C due solely to aerobic respiration would be 0.7 to 2.7 % d^{-1} with higher rates for smaller worms. If Krogh's normal curve (as given in Grodzinski et al., 1975) is used to adjust these rates to the temperatures used in this study, the predicted fractional weight loss would be 0.4 to 1.5 % at 10 °C, 1.1 to 4.2 % at 20 °C, and 2.5 to 9.4 % at 30 °C, substantially less than the weight losses actually observed in this study.

In fact, though, the discrepancy between the weight loss predicted from the above equation for aerobic respiration and that actually measured in this study may not be as large as it appears. Most of the previous respiration measurements for polychaetes have been carried out on animals isolated from sediment which may tend to reduce activity and thus O_2 consumption. The O_2 consumption rates measured in this study, where the animals were allowed to burrow in sediment, were about twice those predicted from the above equation. A comparison of the loss rates for carbon estimated from the weight loss and O_2 methods in this study (Table 2) shows that in general the weight loss method gave higher estimates at 10 °C and lower estimates at 30 °C; both methods were similar at 20 °C.

Temperature did not seem to affect the weight loss measurements as much as the O_2 measurements. One possible explanation could be a reduction in the fraction of total carbon lost as DOC and POC as the temperature increased. Although the loss rate of $DO^{14}C$ increased from 10° to 20 °C, there was no further increase at 30 °C (Fig. 1). No measurements were made of POC loss; in fact, the only data for polychaetes appear to be that of Tenore and Gopalan (1974) for *Nereis virens* showing that at 20 °C only 4.4 % of the total carbon lost during the first week of starvation was in the form of mucus. A second possibility is that although dead animals were removed daily, a few of those individuals may have decomposed enough in that time to be fed upon by the survivors; mortality generally increased with temperature, and decomposition rate certainly increased. Because only living worms were weighed, the effect would have been to lower the estimate of weight loss. Mortality was greatest for the small size-class at 30 °C (73 % after 4 d) and this is the size-class where weight-loss was lowest relative to the O_2 estimate. Although it might have been preferable to have sampled more frequently and removed the dead worms, that would also have entailed more handling and disturbance.

This study has shown that metabolic rate of *Capitella capitata* can be estimated by following weight loss in starving individuals over time. Weight loss may in fact be a preferred method when all pathways of loss of assimilated carbon are to be measured. However, each of the methods used in this study suffers from a common fault: use of non-feeding individuals to estimate the energetics of a natural population. The calorimetric method used by Pamatmat and Findlay (1983) offers a possibility of estimating metabolism of feeding animals but is difficult to apply. The weight loss method used here, while perhaps less elegant than calorimetry, appears to provide adequate estimates of carbon metabolism and when coupled with the new technique for determining wet weights of animals proposed by Andersen and Famme (1983) may prove to be accurate enough to be applied to a variety of animals.

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