LOSS OF SOLUBLE AND PARTICULATE MATERIAL FROM HYDRA POPULATIONS

by

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CHAPTER I

INTRODUCTION

Since the early formulations of energy transfer in ecosystems by Lindeman (1942), the concepts of ecoenergetics has occupied the imaginations of ecologists to an increasing degree. A stimulating body of ecological theory has been built around energy transfer (Odum, 1968; Margalef, 1968; Morowitz, 1968; Watt, 1968, Ch 3; Patten, 1959; Slobodkin, 1962) and at present defines an important direction of inquiry into ecosystem dynamics. Quantitative determinations designed to support this theoretical development are limited and sketchy due to the large costs in time and effort required to obtain meaningful data on energy utilization by either whole or parts of ecosystems. The accumulation of data to date is primarily concerned with the most important and obvious pathways of energy flow which also are the most easily measured.

There are two important reasons for direct measurement and documentation of products released by hydra. First, estimation of excretion products usually has been indirectly accounted for by difference in hydra studies (Slobodkin, 1964; Schroeder, 1969) as well as in Daphnia studies (Richman, 1958; Armstrong, 1959). This calculated rate of release of products should be confirmed or denied by direct estimation.

Second, the amount of dissolved organic matter in aquatic environments is often greater than particulate matter (Birge and Juday, 1926; Ruttner, 1963). Considerable amounts of dissolved organic matter are released into aquatic and marine environments particularly by algae and phytoplankton (Fogg, 1965; Fogg, Nalewajko and Watt, 1965; Hellebust, 1965; Whitton, 1965; Wilson, 1963), but also by animals (Johannes and Webb, 1965; Pomeroy, 1963). Algae may lose up to 50% or more of their photosynthate as dissolved organic matter, probably as glycolate (Wright and Hobbie, 1965) and other substances (Fogg, 1965). Marine zooplankton, including coelenterates, excrete dissolved amino acids at rates that are positively correlated with temperature (Johannes and Webb, 1965). Also in marine environments some particulate matter is thought to be formed by aggregates of dissolved material (Wangersky, 1965; Marshall, 1968).

These substances once released into the aquatic environment become a potential energy source within the community. Some aquatic organisms may benefit by direct uptake of dissolved organic nutrients (Krogh, 1931; Wright and Hobbie, 1965; Conover, 1966). Such nutrients may also act as specific chemical regulators on other organisms (Lucas, 1961, 1959; Saunders, 1957; Ryther, 1954, Lenhoff, 1967).

Little is known or understood about these pathways of energy transfer and they may very well play a significant role in community energetics. In addition to accounting for excretion products in the energy budget of individual organisms or populations, it is of interest to know to what extent organisms at higher trophic levels contribute to the pool of dissolved organic matter in aquatic environments.

Energy Relationships in Hydra

The release of organic matter from hydra has been demonstrated by Muscatine and Lenhoff (1965) and Lenhoff (1961, 1967). Muscatine and Lenhoff (1965) found that Chlorohydra viridissima lost assimilated radioactive sulfur, from S^{35} labelled mouse liver fed to individual animals, over a 5 day period. When deprived of its symbiotic algae C. viridissima lost 23% to 75% of the total S^{35} present at the beginning of experiments. Maximum loss for green hydra with its symbiotic algae present was 37.9 ± 3.1%. However, in a similar study by Lenhoff (1961) the rate of release of radioactive sulfur from H. littoralis was nearly constant and amounted to a total loss of 25% of assimilated label over 5 days' starvation. The organic matter released from H. pseudoligactis should be documented for comparison with H. littoralis since both are species of brown hydra with no algal symbiont.

An energy budget for an individual organism may be described by the simple equation (Schroeder, 1969):

$$I = G + R + E \qquad (1)$$

where

I = rate of ingestion

G = rate of growth

R = rate of respiration

E = rate of excretion, egestion, leaching and sloughing of cells and mucus

Since a population is a collection of individuals of the same species delimited by space and time then the energy budget for the population, if it is immortal, is similar to equation (1):

$$I^P = G^P + R^P + E^P \qquad (2)$$

where

I^P = rate of ingestion of population P

G^P = rate of growth of population P

R^P = rate of respiration of population P

E^P = rate of egestion, excretion, leaching and sloughing of cells and mucus of population P

Since hydra are presumed to be immortal there is no death to consider, although there are physiological changes with age (Burnett, 1961; Forrest, 1963; Stiven, 1962).

The present study is concerned with the direct estimation of the E^P term. Schroeder (1969) determined the energy budgets of populations of H. pseudoligactis at 20°C ± 1°C and two rates of ingestion. At an ingestion rate of 0.5 cal of Artemia per cal of hydra per day (cal/cal/day) 37% went into growth, 44% was expended in respiration [based on 34 μl per hr per mg protein (Lenhoff and Loomis, 1957) and 30% dry weight as protein] and 19% egested. At an ingestion rate of 0.7 cal/cal/day 37% went into growth, 30% was expended in respiration and 33% egested. The fraction of calories egested (calculated by difference) implies total calories lost by egestion, excretion, leaching, and sloughing of cells and cell residues. It is not known what portion of these total amounts, 33% and 19%, constitutes each of the above mentioned pathways of release of energy. The radioactive sulfur studies indicate assimilation to be 95%. Schroeder found assimilation to be 67% and 81% by difference. This discrepancy may be due to lumping all losses, leaching and sloughing of cells, into the egestion term.

Gross growth efficiency, GE, is the ratio of the rate of growth to the rate of ingestion 100(G/I), and net population growth efficiency, GE_n, is the ratio of growth to assimilation 100(G/I-E) (Schroeder, 1969). Egestion, E, is the total non-assimilated energy. Ecological efficiency is the ratio of the rate of yield to predator populations to the rate of ingestion by the prey population 100(Y/I) (Slobodkin, 1960). Since there is no death to consider in the case of hydra and if it is assumed that physiological changes with age have no appreciable effect on hydra energy budgets, and if all growth of hydra tissue is harvested, then population growth efficiency is equivalent to Slobodkin's ecological efficiency (Schroeder, 1969).

Yield as considered by Slobodkin includes only hydra tissue removed by predation; yield as the E^P term previously mentioned is ignored. The E^P term equation (2) should also be considered as yield energy, Y^P, of population P. Y^P may be categorized as Y^P_1, Y^P_2, . . . Y^P_n representing yield from leaching, excretion, sloughed cells, etc. respectively. Then production efficiency, the ratio of total yield to ingestion of a population, is defined as:

$$E_p = \frac{\sum_{i=1}^{n} Y^P_i}{I^P} \qquad (3)$$

where

Y_i^P = rate of yield from category i of population P

I^P = rate of ingestion of population P

E_p = production efficiency

It is the purpose of this investigation to evaluate by direct measurement quantities of matter lost to the aquatic community as:

Y_1 = rate of excretion of NH_3

Y_2 = rate of leaching of dissolved organic matter

Y_3 = rate of sloughing of cells and mucus

as related to temperature, and to determine the population energy budget and production efficiency of H. pseudoligactis at 16^oC. If the population was preyed upon at an intensity equal to the rate of growth then growth becomes Y_4 and production efficiency with respect to all yield may be determined.

Five to six hours after feeding hydra egest the nonassimilated portion of their meal as a cohesive bolus. Yield as egested matter is better applied to the yield from the Artemia trophic level and is not calculated into production efficiency. However, it must be included in the population energy budget.

Hydra are common freshwater coelenterates of the sessile polyp form. It is an ideal experimental animal that is easily reared in the laboratory using Artemia nauplii as a food source. In these circumstances hydra reproduce asexually by budding which facilitates the rearing of pure genetic strains if so desired. They have been the subject of extensive physiological (Lenhoff, 1961; Lenhoff and Bovaird, 1957, 1960; Lenhoff and Loomis, 1961; Lentz, 1961, 1965), cytological (Lentz, 1966), and ecological (Forrest, 1963; Schroeder, 1969; Slobodkin, 1964; Stiven, 1962) investigations.

CHAPTER II

METHODS AND MATERIALS

Populations of Hydra pseudoligactis used for these experiments were taken from stock cultures maintained in this laboratory for several years using methods outlined by Loomis (1953) and Lenhoff and Brown (1970). Experimental populations were cultured in finger bowls containing about 200 ml medium[1] and in square gridded plastic petri dishes containing about 60 ml medium. One group of populations was maintained at 12°C ± 1°C, one group at 20°C ± 1°C, and a third group at 16°C ± 1°C. Each group of populations consisted of 6 cultures having initial densities of 5 to 7 GA[2] per ml of medium. Controls consisted of dishes of medium without hydra populations. For hydra reared at 16°C ingestion rates were determined by taking dry weights of 30 to 40 GA prior to feeding and immediately after ingestion. The release of particulate and dissolved material was measured from samples collected over a 72 hr period beginning 2 hr after feeding, including the egestion period, and thereafter at 24 hr intervals. The bottom and sides of the dish were scraped to dislodge sloughed cells and egested boli prior to decanting. Samples were collected by pouring the culture medium into beakers through a nylon net to catch loose hydranths. Hydra were rinsed and replaced in fresh culture solution

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[1] 10^{-3}N $CaCl_2$, 10^{-4}N KCl, 10^{-4}N $NaHCO_3$ in distilled, deionized H_2O

[2] GA as used here refers to all growth axis detected with the unaided eye except for those hydra reared at 16°C which were examined under a binocular scope at 15X.

until the next sampling time. The rinsings were added to the samples.

Egested material, sloughed cells and mucus were separated from dissolved organic matter prior to quantitative oxidation with potassium dichromate (Maciolek, 1962). Glass fiber filters (Reeve Angel, 2.1 cm, grade 93AH), washed several times in distilled water to remove any organic residues, were used to remove particulate material by filtration in a Millipore vacuum apparatus. The material on the filters was preserved by freezing for later wet ashing. A 5 to 10 ml sample was taken from the filtrate for NH_3-N analysis which was determined by the standard Nessler method (Golterman and Clymo, Eds., 1959). The remaining filtrate was treated with Ag_2SO_4 to remove Cl^- which interferes with dichromate oxidation, filtered and dried at 85^oC to 90^oC.

Initial and final population counts were made. Upon termination of each experiment the entire population, or 50 to 60 GA, was dried at 45^oC for 48 hr over $CaCl_2$ and weighed on a Cahn Electrobalance to a precision of ± 1 μg.

Bacterial growth in the cultures may have been a source of error. However, since the sampling was relatively frequent (at or less than 24 hr intervals) it was assumed that bacterial development over a period of 24 hr had no appreciable effects. Bacteria counts, however, were made by the membrane filter technique (Standard Methods, 1971) on 24 hr old samples, to obtain a measure of the presence of bacteria in the particulate component. Particulate and dissolved components were quantified in terms of mg of oxygen consumed during dichromate oxidation per dry weight of hydra per hr and then converted to calories of product per calorie of hydra per hr. The energy content of NH_3 was calculated from bond dissociation energies.

CHAPTER III

RESULTS AND DISCUSSION

The results of experiments may be divided into three sections. First, the temperature effects on rates of energy loss through soluble and particulate matter is determined. Second, the products released during starvation are examined; and third, an energy budget for hydra reared at 16°C is determined.

Mean values of particulate and soluble products are based on 6 replicates each at two temperatures over a 72 hr period. Caloric equivalents were calculated by multiplying mg of oxygen consumed during dichromate oxidation by 3.4 (Appendix A). This coefficient is suggested by Maciolek (1962) as the best estimate and compares directly with bomb calorimeter determinations of biological material and quantitatively important limnological compounds. Caloric content of Artemia nauplii and hydra are based on oven dry bomb calorimeter measurements made by Slobodkin and Richman (1961), 5600 cal/g and 6140 cal/g respectively.

Bacterial growth in the culture dishes was measured at the end of 48 hr of the 72 hr experimental period. Approximate values are 115,000 and 142,000 bacterial cells per ml at 12°C and 20°C respectively. These bacteria are assumed to have come either from the hydra or from the Artemia, or both. The contribution of bacteria to the particulate material collected on the glass fiber filter was insignificant. A rough estimate, based on E. coli as being 2 μ by 1 to 1.2 μ in size (Smith, 1969) and having a live density of 1 with 10% dry weight, shows a contribution of 0.01 to 0.02 μg per ml of medium containing 5 to 7 GA releasing a total of 6 to 7 μg of particulate matter in a 24 hr period. This constitutes about 1.6% to 3% at $12^{o}C$ and $20^{o}C$ respectively.

Discharge of soluble material at $12^{o}C$ was 0.018 cal/cal (cal of loss per cal of hydra) and at $20^{o}C$ was 0.016 cal/cal (Table 1,2). These amounts which are the total of the first three samples up to and including egested substances may be attributed mostly to lysis of egested nauplii tissues releasing dissolved organic substances into the hydra medium. Soluble material released during starvation is produced by hydra tissues. Total cumulative discharge of soluble material, excluding egested matter, was 0.113 cal/cal at $12^{o}C$ and 0.157 cal/cal at $20^{o}C$. Analysis of variance (Appendix B) showed no significant difference ($p > .01$) due to temperature effect in the release of dissolved organic matter over the total sampling period, nor over the last 64 hr when regurgitated boli is no longer part of the sample (Figure 1).

Regurgitated particulate material was 0.034 cal/cal at $12^{o}C$ and 0.059 cal/cal at $20^{o}C$ (Table 1,2). Total cumulative discharge of particulate material at $12^{o}C$ and $20^{o}C$ was 0.025 cal/cal and 0.048 cal/cal respectively. Analysis of variance (Appendix B) showed a highly significant difference ($p < .01$) between the two temperatures with respect to loss of particulate matter and is in accord with the Q_{10} rule. An 8 degree centigrade increase in temperature increases the rate of particulate discharge 1.7 times (Figure 2).

Loss of particulate matter is greater than soluble losses at both temperatures (Figures 3,4). At increasing temperatures a

TABLE 1

LOSSES OF ORGANIC MATTER FROM HYDRA POPULATIONS REARED AT 12°C OVER A 72 HR PERIOD; MEAN VALUES OF 6 REPLICATES ± STANDARD DEVIATION

Sample	Hr after feeding	Hr between samples	Soluble losses: Rate of loss 10^3 cal/cal/hr	Soluble losses: Cumulative loss 10^3 cal/cal	Particulate losses: Rate of loss 10^3 cal/cal/hr	Particulate losses: Cumulative loss 10^3 cal/cal
1	1	1	3.9 ± 9.9	3.9 ± 9.9	8.3 ± 5.9	8.3 ± 5.9
2	6	5	2.6 ± 1.9	16.6 ± 9.5	2.5 ± 1	20.7 ± 4.9
3	8	2	5.1 ± 1.4	17.7 ± 2.9	6.4 ± 1.2	33.5 ± 2.4
Total soluble egestion				17.7 ± 2.9	Total particulate egestion	33.5 ± 2.4
4	24	17	0.1 ± 0.1	1.3 ± 1.8	0.2 ± 0.2	3.7 ± 3.9
5	48	24	0.2 ± 0.1	4.9 ± 2.4	0.5 ± 0.3	15.5 ± 7.7
6	72	24	0.3 ± 0.2	11.3 ± 5.0	0.4 ± 0.3	24.8 ± 7.9
Total leaching				11.3 ± 5.0	Total sloughed cells & mucus	24.8 ± 7.9
Total leaching and soluble egestion				28.0 ± 7.9	Total sloughed cells & mucus and particulate egestion	58.3 ± 10.3

TABLE 2

LOSSES OF ORGANIC MATTER FROM HYDRA POPULATIONS REARED AT 20°C OVER A 72 HR PERIOD; MEAN VALUES OF 6 REPLICATES ± STANDARD DEVIATION

Sample	Hr after feeding	Hr between samples	Soluble losses: Rate of loss 10^3 cal/cal/hr	Soluble losses: Cumulative loss 10^3 cal/cal	Particulate losses: Rate of loss 10^3 cal/cal/hr	Particulate losses: Cumulative loss 10^3 cal/cal
1	1	1	5.2 ± 4.9	5.2 ± 4.9	24.0 ± 5.7	24.0 ± 5.7
2	6	5	1.7 ± 0.3	13.6 ± 1.3	4.9 ± 1.4	48.3 ± 6.9
3	8	2	1.3 ± 1.9	16.2 ± 3.9	5.1 ± 4.2	58.6 ± 8.4
Total soluble egestion				16.2 ± 3.9	Total particulate egestion	58.6 ± 8.4
4	24	17	0.3 ± 0.2	5.8 ± 2.8	0.8 ± 0.3	12.9 ± 5.7
5	48	24	0.2 ± 0.1	9.9 ± 2.9	0.7 ± 0.1	28.5 ± 3.4
6	72	24	0.2 ± 0.2	15.7 ± 5.1	0.9 ± 0.2	47.8 ± 5.3
Total leaching				15.7 ± 5.1	Total sloughed cells & mucus	47.8 ± 5.3
Total leaching and soluble egestion				31.9 ± 9.0	Total sloughed cells & mucus and particulate egestion	106.4 ± 13.7

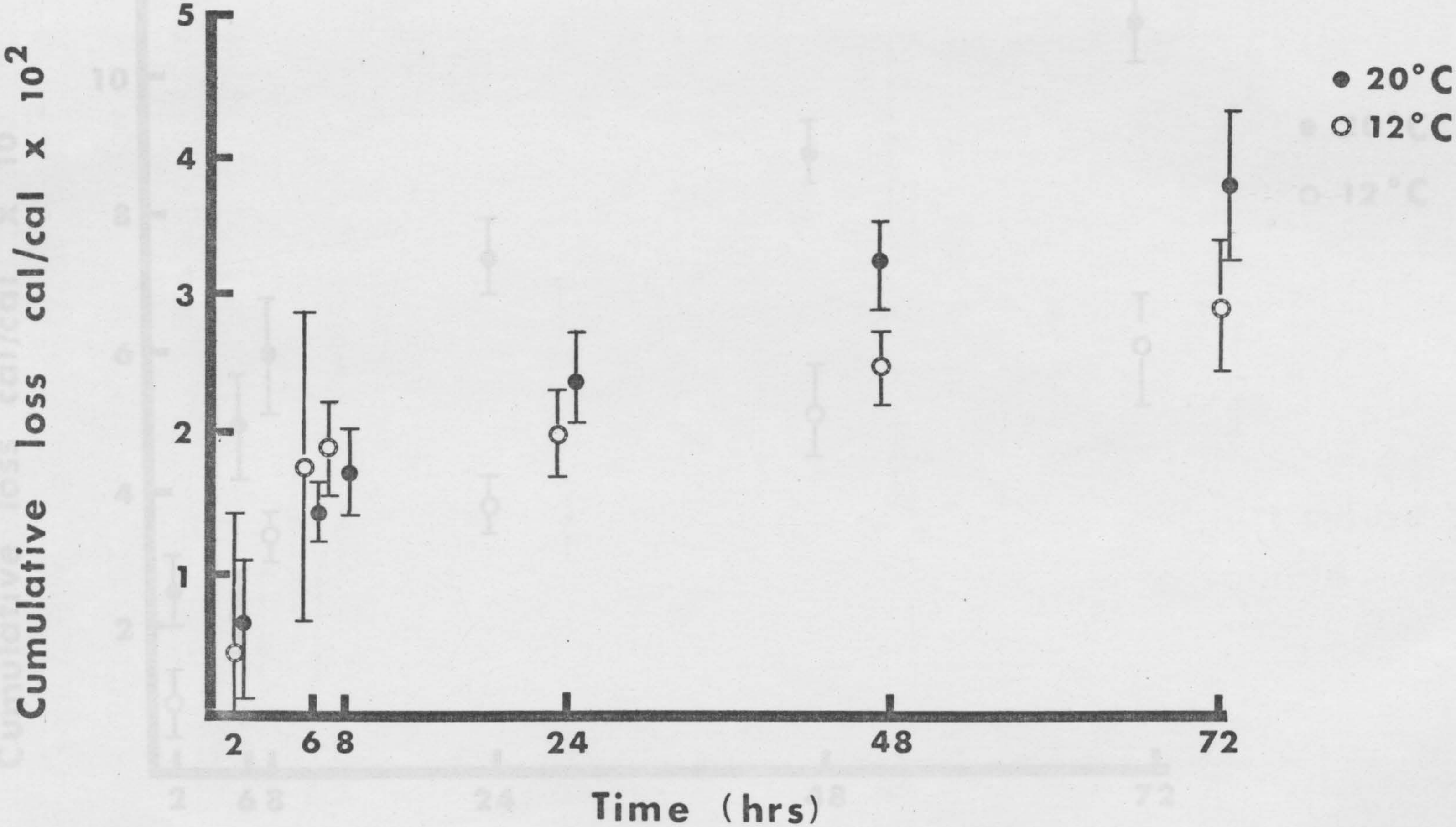

FIG. 1 Soluble losses from hydra populations.

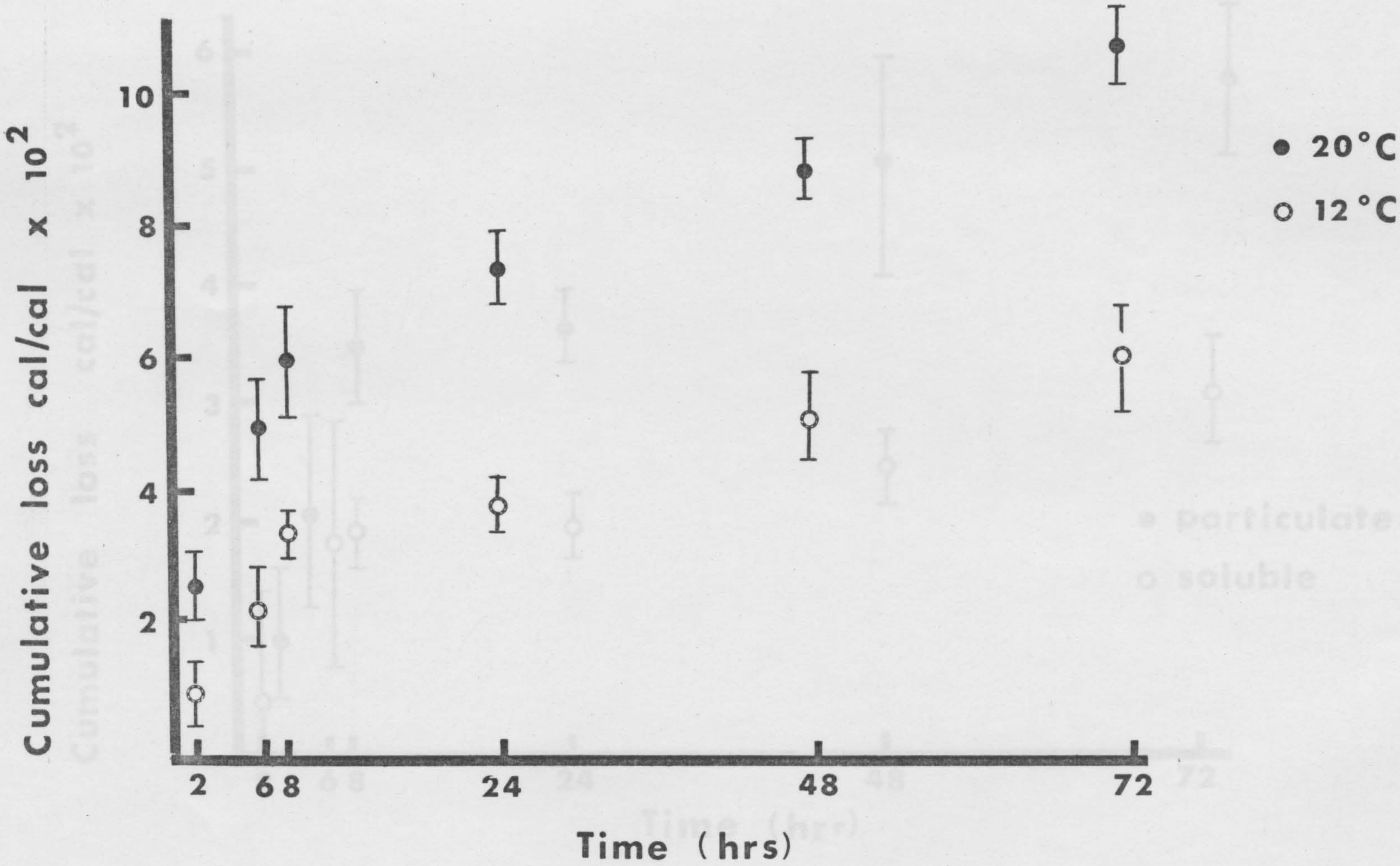

FIG. 2 Particulate losses from hydra populations.

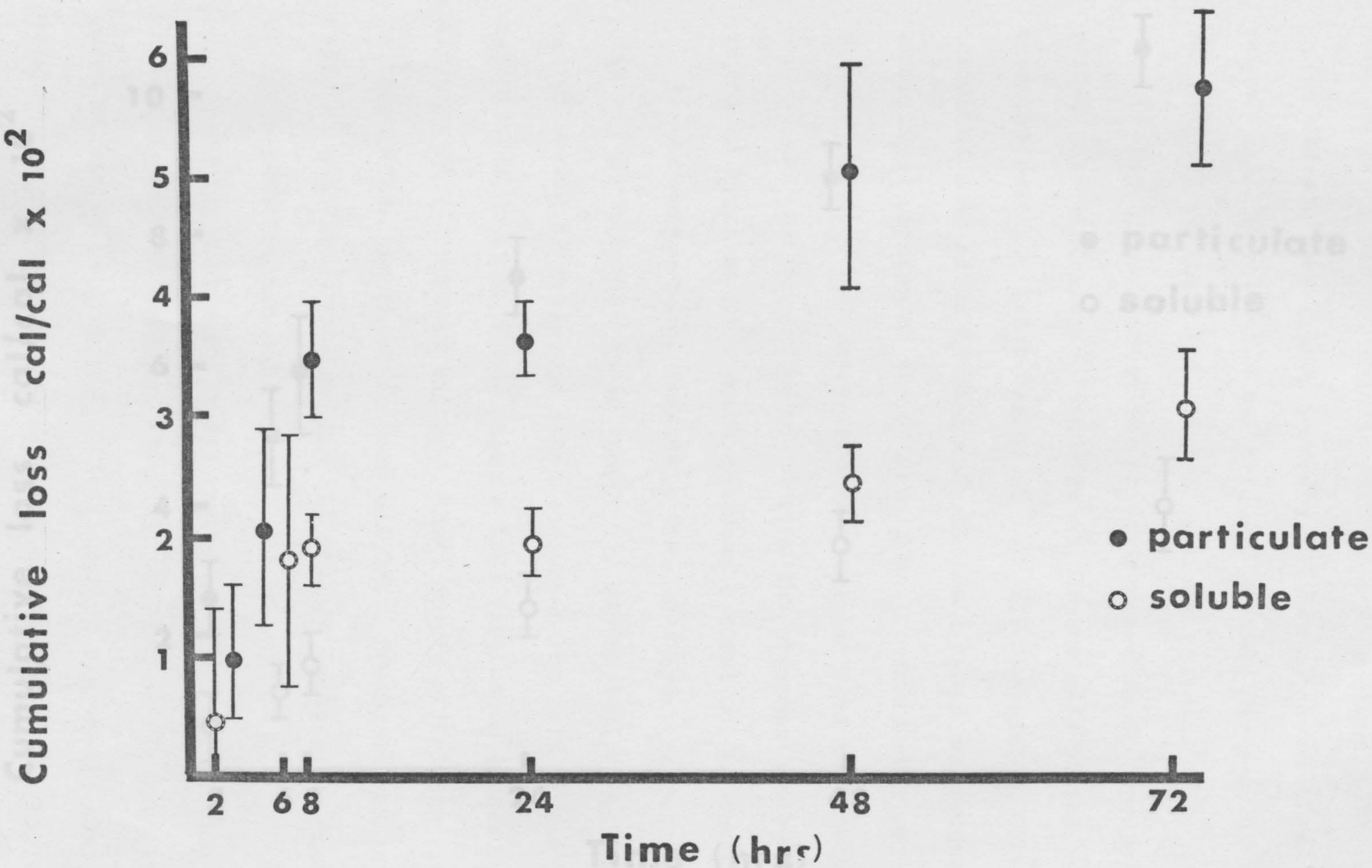

FIG. 3 Soluble and particulate losses from hydra populations at 12°C.

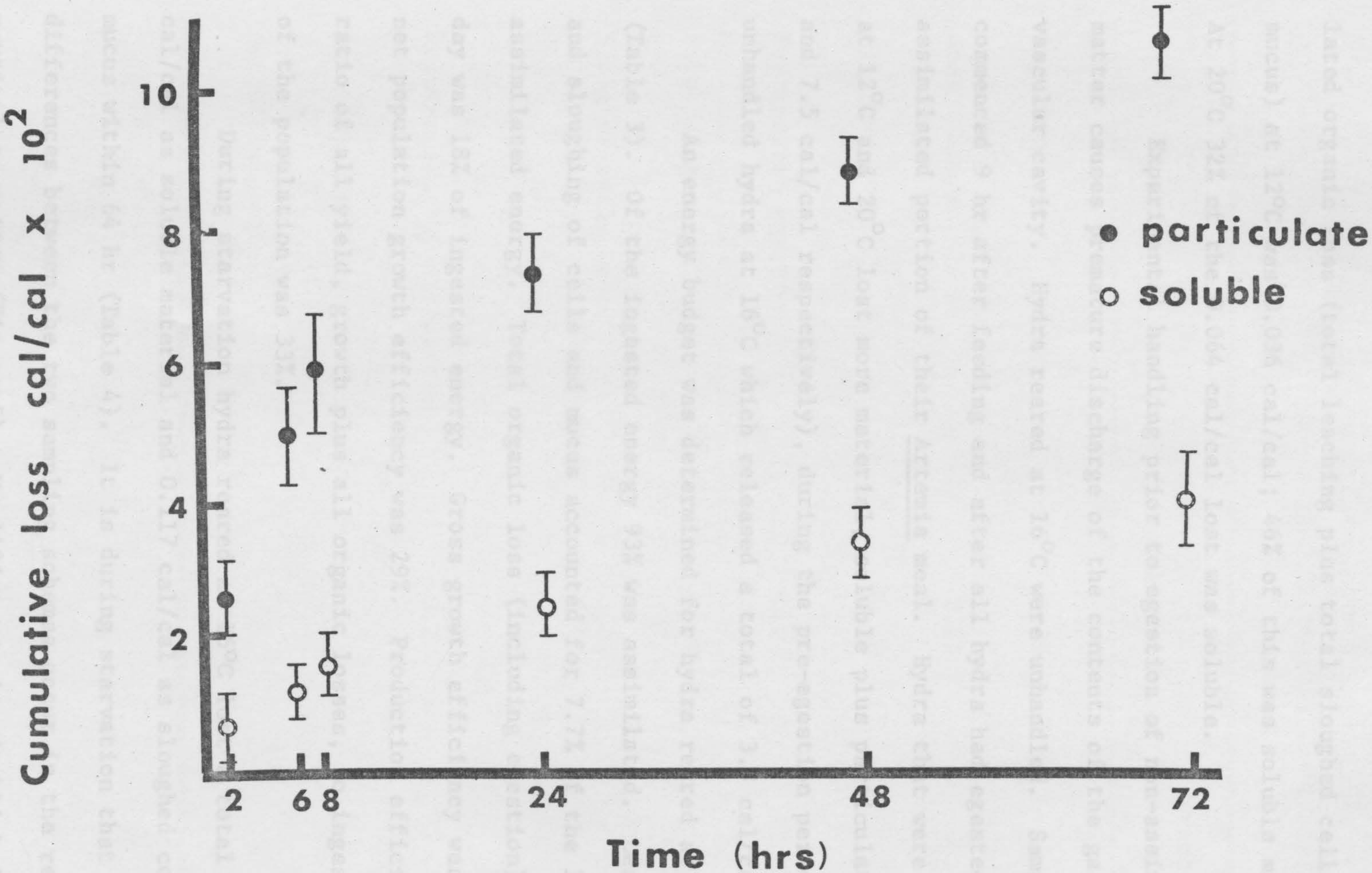

FIG. 4 Soluble and particulate losses from hydra populations at 20°C.

smaller proportion of organic discharge is soluble. The total assimilated organic loss (total leaching plus total sloughed cells and mucus) at 12°C was 0.036 cal/cal; 46% of this was soluble material. At 20°C 32% of the 0.064 cal/cal lost was soluble.

Experimental handling prior to egestion of non-assimilated matter causes premature discharge of the contents of the gastrovascular cavity. Hydra reared at 16°C were unhandled. Sampling commenced 9 hr after feeding and after all hydra had egested the non-assimilated portion of their Artemia meal. Hydra that were handled at 12°C and 20°C lost more material, soluble plus particulate (5.1 and 7.5 cal/cal respectively), during the pre-egestion period than unhandled hydra at 16°C which released a total of 3.8 cal/cal.

An energy budget was determined for hydra reared at 16°C (Table 3). Of the ingested energy 93% was assimilated. Leaching and sloughing of cells and mucus accounted for 7.7% of the loss of assimilated energy. Total organic loss (including egestion) for one day was 18% of ingested energy. Gross growth efficiency was 26% and net population growth efficiency was 29%. Production efficiency, the ratio of all yield, growth plus all organic losses, to ingestion rate of the population was 33%.

During starvation hydra reared at 16°C lost a total of 0.006 cal/cal as soluble material and 0.117 cal/cal as sloughed cells and mucus within 64 hr (Table 4). It is during starvation that large differences between the two sampling schemes occur in the release of particulate matter (Figure 5). No difference is established with respect to soluble material (Figure 6).

TABLE 3

ESTIMATED ENERGY BUDGET FOR H. PSEUDOLIGACTIS
REARED AT 16°C WITH PERIOD OF INGESTION-EGESTION UNDISTURBED

	cal/cal/day	fraction of ingested calories
Ingestion	0.39	
Assimilation	0.35	.93
Growth	0.10	.26
Particulate egestion	0.03	.07
Soluble egestion	0.009	.02
Sloughed cells, etc.	0.03	.07
Leaching	0.002	.005
Respiration (by difference)	0.22	.56
Production efficiency		.33
Net efficiency		.29

TABLE 4

LOSSES OF ORGANIC MATTER FROM HYDRA POPULATIONS REARED AT 16°C OVER A 72 HR PERIOD; MEAN VALUES OF 6 REPLICATES ± STANDARD DEVIATION

			Soluble losses		Particulate losses	
Sample	Hr after feeding	Hr between samples	Rate of loss 10^3 cal/cal/hr	Cumulative loss 10^3 cal/cal	Rate of loss 10^3 cal/cal/hr	Cumulative loss 10^3 cal/cal
1	9	9	1.0 ± 0.4	8.9 ± 3.4	3.2 ± 0.7	28.9 ± 6.5
Total soluble egestion				8.9 ± 3.4	Total particulate egestion	28.9 ± 6.5
2	24	18	0.1 ± 0.1	2.4 ± 1	1.6 ± 0.5	28.4 ± 9
3	48	24	0.1 ± 0.1	4.3 ± 2.2	1.7 ± 0.3	68.9 ± 15.8
4	72	24	0.1 ± 0.03	6.3 ± 3	2 ± 0.3	116.6 ± 23
Total leaching				6.3 ± 3	Total sloughed cells & mucus	116.6 ± 23
Total leaching and soluble egestion				15.2 ± 6.4	Total sloughed cells & mucus and particulate egestion	145.5 ± 29.7

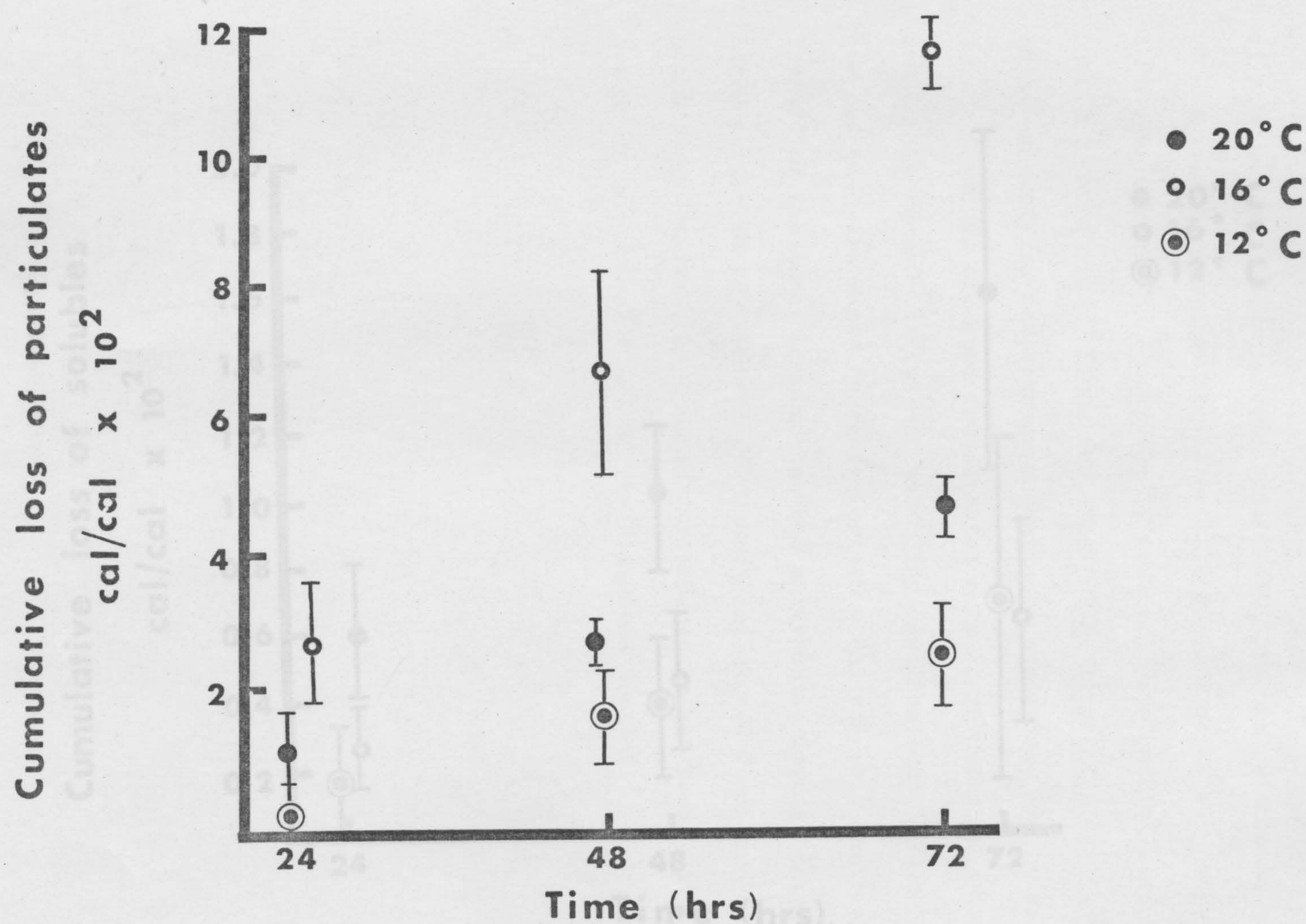

FIG. 5 Particulate losses from hydra populations following egestion at three temperatures.

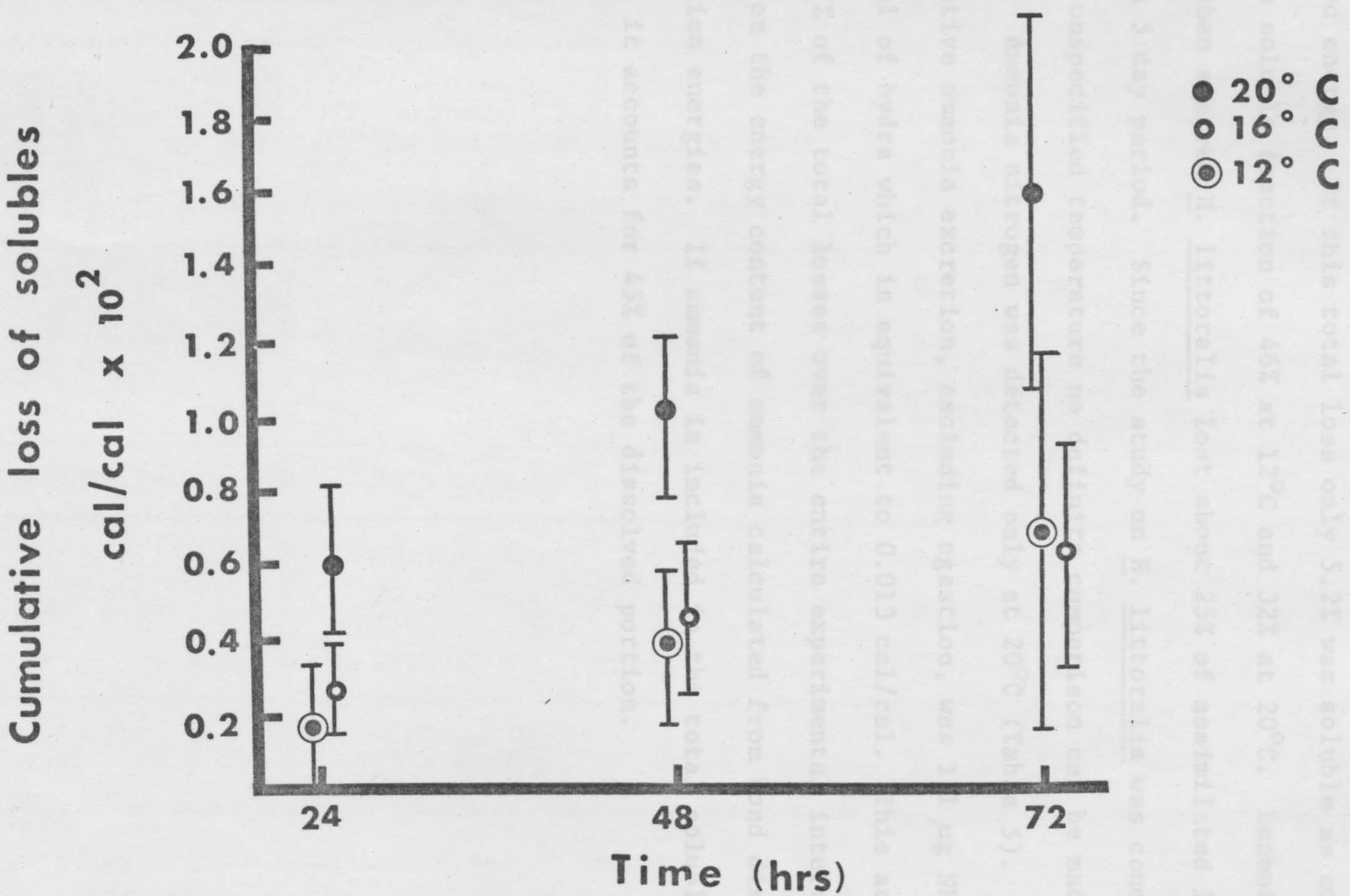

FIG. 6 Soluble losses from hydra populations following egestion at three temperatures.

Total organic discharge at $16^{o}C$ within 64 hr was 35% of assimilated energy. Of this total loss only 5.2% was soluble as compared with a soluble fraction of 46% at $12^{o}C$ and 32% at $20^{o}C$. Lenhoff found that when starved H. littoralis lost about 25% of assimilated label over a 5 day period. Since the study on H. littoralis was conducted at an unspecified temperature no definite comparison can be made.

Ammonia nitrogen was detected only at $20^{o}C$ (Table 5). Total cumulative ammonia excretion, excluding egestion, was 1.1 μg NH_3-N per cal of hydra which is equivalent to 0.013 cal/cal. This accounts for 17% of the total losses over the entire experimental interval, as based on the energy content of ammonia calculated from bond dissociation energies. If ammonia is included in the total soluble losses it accounts for 45% of the dissolved portion.

TABLE 5

EXCRETION OF AMMONIA BY HYDRA POPULATIONS REARED AT 20°C OVER A 72 HR PERIOD; MEAN VALUES OF 3 REPLICATES ± STANDARD DEVIATION

Sample	Hr after feeding	Hr between samples	$\mu g\ NH_3$-N per GA x 10^2	$\mu g\ NH_3$ per cal hydra x 10^2	per cent assimilated[a] loss	cal NH_3 per cal x 10^2	per cent assimilated[b] loss
1	1	1	6.3 ± 0.2	31.8 ± 1		0.3 ± 0.04	
2	6	5	8.3 ± 0.7	43.7 ± 5.3		0.4 ± 0.05	
3	8	6	---	---		---	
Total egestion			14.6 ± 0.9	75.5 ± 6.3		0.7 ± 0.09	
4	24	17	5.7 ± 0.8	28.5 ± 4.3	.44	0.3 ± 0.04	.85
5	48	24	11.2 ± 1.7	56.4 ± 8.1		0.5 ± 0.08	
6	72	24	9.6 ± 0.4	48.4 ± 2.1		0.5 ± 0.02	
Total loss (assimilated)			26.5 ± 2.9	133.3 ± 14.5	2.1	1.3 ± 0.14	3.7

[a]63.62 μg Artemia assimilated/cal of hydra.

[b]0.35 cal Artemia assimilated /cal of hydra (heats of combustion of NH_3 calculated from bond dissociation energy).

CHAPTER IV

CONCLUSIONS

1. Hydra release a significant portion of ingested energy in the form of dissolved and particulate organic matter.

2. Loss of assimilated energy as sloughed cells, mucus, etc. is temperature dependent within a 24 hr feeding interval and over a 64 hr starvation interval.

3. Loss of soluble substances appears to be temperature independent.

4. Sampling scheme affects the energy loss of hydra. Disturbance of cultures prior to egestion results in a lower rate of particulate discharge reflecting lower assimilation rates.

5. At $16^{\circ}C$ and under a sampling scheme designed to reduce disturbance, 33% assimilated energy is lost, most of which is particulate matter.

6. Energy losses by excretion of ammonia is a significant portion of the dissolved fraction at $20^{\circ}C$.

APPENDIX A

Conversion to Energy Values

CONVERSION OF MG O.C. TO CAL/CAL/HR

1. Corrected mg O.C. = O.C. of sample - O.C. of control.

2. Caloric value of population = total wt in mg x 6.140 (based on 6140 cal/g hydra tissue, Slobodkin and Richman, 1961).

3. Mg O.C. per cal hydra per hr = (1)/(2)/number of hr elapsed since last sampling time.

4. Cal loss per cal hydra per hr = (3) x 3.4.[1]

[1] 3.4 is the conversion factor suggested by Maciolek (1962) gcal = O.C. in mg x 3.4 (p. 39, Maciolek, 1962).

MG OXYGEN CONSUMED VALUES
(CORRECTED FOR VOLUME REMOVED FOR NH_3 ANALYSIS)

	Culture	12°C		20°C	
		Diss	Part	Diss	Part
	A1	.6268	1.1068	1.4463	3.1768
	B1	.8467	1.4872	1.2783	2.7188
	C1	.9594	.9212	1.3703	2.8480*
t_1	X1	.7599	.8552	.6760	.7612
	A2	1.793	.7258	.3322	.7933
	B2	.7137	.9072	.5145	.8469
	C2	2.668	.8826	.3822	.7909
	X2	1.229	.5354	.3754	.2653
	A1	.9360	1.0192	1.2937	3.2912
	B1	1.0734	1.5892	1.1021	2.5292
	C1	.9822	.8152	1.1979*	2.7422
t_2	X1	.5718	.6728	.6195	.6188
	A2	1.8145	1.2491	.5179	.8147
	B2	2.3768	1.3171	.4954	.8522
	C2	1.9383	1.6388	.4436	.8040
	X2	.6619	.6552	.2315	.2868
	A1	1.0375	.7652	.8433	.9552
	B1	1.4071	.7832	1.1162	.9992
	C1	.8759	1.2568	.9892	1.2092
t_3	X1	.9030	.5532	.6345	.8448
	A2	.8753	1.3628	.2648	.6539
	B2	.7080	1.4138	.1134	.7558
	C2	.7511	1.4022	.3691	.7290
	X2	.8079	.4077	.2325	.2171
	A1	.8556	.7432	1.0915	1.0952
	B1	.6670	.8393	.9963*	1.324
	C1	.8242	1.0748	.9010	1.1597
t_4	X1	.6024	.8188	.6477	.5432
	A2	1.0035	1.4115	.5502	.6642
	B2	.8128	1.2488	.3944	.8464
	C2	.6551	1.0058	.3174	.6167
	X2	.7785	.7828	.2212	.2251

	Culture	12°C		20°C	
		Diss	Part	Diss	Part
	A1	.8267	.9043	1.1405	1.354
	B1	.8554	1.0541	1.1049	1.742
	C1	.6610	.9139	1.0216	1.782
t_5	X1	.5334	.4968	.5958	.5188
	A2	1.0120	2.2062	.3336	.6299
	B2	1.0302	1.9579	.3486	.6853
	C2	.6712	1.8785	.2622	.8173
	X2	.6070	.7716	.2652	.2486
	A1	.7562	.7834	.7925*	1.520
	B1	.5471	.8256	.8081	2.442
	C1	.7906	.9696	.7767	2.170
t_6	X1	.5446	.6448	.5433	.5568
	A2	1.4649	1.8222	.4160	.6299
	B2	1.3855	1.6635	.3582	.8358
	C2	1.4079	2.1038	.3097	.8939
	X2	.6619	.7803	.2652	.2486

*Estimated missing plot datum (Steele and Torrie, 1960).

X = control dish, medium without hydra.

Culture series 1 and 2 (A1, A2, . . . C2) analysed at different times.

Culture series 1 and 2 at 12°C and culture series 1 at 20°C contained populations of 1000 GA per culture dish.

Culture series 2 at 20°C contained 400 GA per culture dish.

MG OXYGEN CONSUMED VALUES AT 16°C
(CORRECTED FOR VOLUME REMOVED FOR NH_3-N ANALYSIS)

	Culture	Diss	Part	#GA/dish
	A1	.2813	--	301
	B1	.2666	.5910	302
	C1	.1468	--	296
t_1	X1	.0523	.1238	314
	A2	.2962	1.1008	325
	B2	.4826	1.0624	414
	C2	.6188	1.2147	
	X2	.1251	.1144	
	A1	.1073	1.0458	
	B1	.1258	1.0101	
	C1	.0993	--	
t_2	X1	.0523	.1238	
	A2	--	.6284	
	B2	.1468	.6182	
	C2	.1553	.9101	
	X2	.1069	.1227	
	A1	.2297	1.1202	
	B1	.1797	1.1624	
	C1	.1829	.9417	
t_3	X1	.1430	.0808	
	A2	.1468	1.2454	
	B2	--	1.1456	
	C2	.1679	1.3427	
	X2	.1216	.1256	
	A1	--	1.4277	
	B1	.1697	1.4600	
	C1	.1344	1.1227	
t_4	X1	.1017	.0808	
	A2	.1326	1.2442	
	B2	.1812	1.5142	
	C2	--	1.7370	
	X2	.0993	.1297	

HYDRA WEIGHTS IN $\mu g/GA$

	12°C*		20°C*		16°C	
	Initial	Final	Initial	Final	Initial	Final
A1	60.5	48.4	42.2	21.1	43.5	44.1
B1	43.1	34.5	43.6	21.8	45.2	44.3
C1	54.8	42.2	39.2	19.6	43.1	43.8
A2	36.8	29.4	41.4	20.7	49.2	38.2
B2	35.9	28.7	37.8	18.9	53.2	41.0
C2	37.9	30.3	38.4	19.2	50.1	34.0

*At 16°C hydra lost about 30% of their weight in 3 days. Based on the fact that at increasing temperatures hydra show increasing weight loss (Griffing, 1965) initial weights for hydra at 12°C and 20°C were calculated by assuming a 25% loss at 12°C and a 50% loss at 20°C, and adding this portion on to the directly measured final weights.

APPENDIX B

Analysis of Variance

ANALYSIS OF VARIANCE

Dissolved Material

Levels of Factors

A 6 Time
B 2 Temperature (12°C and 20°C)
C 2 Experiments (Culture Series)
R 3 Replicates (in one culture series)

Grand Mean 0.01945

Source of Variation	Sums of Squares	Degrees of Freedom		Mean Squares		F
A	0.02713	5		0.00543		1.52
B	0.00277	1		0.00277		
AB	0.01512	5		0.00302		
C	0.00068	1		0.00068		
AC	0.01856	5		0.00371		1.04
BC	0.00004	1		0.00004		
ABC	0.03901	5		0.00780		2.18
R (Block)	0.00397	2		0.00198		
AR (error)	0.03282	10	36	0.00328	(.00357)	
BR (error)	0.00101	2		0.00050		
ABR (error)	0.04465	10		0.00447		
CR (error)	0.00951	2		0.00476		
ACR (error)	0.03003	10		0.00300		
BCR (error)	0.01064	2		0.00532		
ABCR	0.03101	10		0.00310		
TOTAL	0.26695	71				

Levels of Factors

A 6 Time
B 2 Temperature
R 6 Replicates

Grand Mean 0.01945

Source of Variation	Sums of Squares	Degrees of Freedom		Mean Squares		F
A	0.02713	5		0.00543		1.43
B	0.00277	1		0.00277		.73
AB	0.01512	5		0.00302		
R (Block)	0.01516	5		0.00283		
AR (error)	0.08142	25	55	0.00326	(0.00378)	
BR (error)	0.01168	5		0.00234		
ABR (error)	0.11467	25		0.00459		
TOTAL	0.26695	71				

Particulate Material

Levels of Factors

A	6	Time
B	2	Temperature
C	2	Experiments
R	3	Replicates

Grand Mean 0.04040

Source of Variation		Sums of Squares	Degrees of Freedom		Mean Squares		F
A		0.18203	5		0.03641		80**
B		0.03068	1		0.03068		66**
AB		0.08209	5		0.01642		36**
C		0.00004	1		0.00004		
AC		0.00183	5		0.00037		
BC		0.00048	1		0.00048		1.04
ABC		0.00233	5		0.00047		1.02
R (Block)		0.00157	2		0.00079		1.71
AR	(error)	0.01011	10	36	0.00101	(0.00046)	
BR	(error)	0.00098	2		0.00049		
ABR	(error)	0.00312	10		0.00031		
CR	(error)	0.00019	2		0.00009		
ACR	(error)	0.00138	10		0.00014		
BCR	(error)	0.00094	2		0.00047		
ABCR		0.00238	10		0.00024		
TOTAL		0.32013	71				

Levels of Factors

A	6	Time
B	2	Temperature
R	6	Replicates

Source of Variation		Sums of Squares	Degrees of Freedom		Mean Squares		F
A		0.18203	5		0.03641		85**
B		0.03068	1		0.03068		71**
AB		0.08209	5		0.01642		38**
R (Block)		0.00180	5		0.00036		
AR	(error)	0.01331	25	55	0.00053	(0.000428)	
BR	(error)	0.00240	5		0.00048		
ABR	(error)	0.00783	25		0.00031		
TOTAL		0.32013	71				

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