THE RELATIONSHIP

BETWEEN DIET AND OXYGEN UTILIZATION

IN TOMOCERUS FLAVESCENS (COLLEMBOLA)

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by

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ABSTRACT

James B. Dewar, Jr. THE RELATIONSHIP BETWEEN DIET AND OXYGEN UTILIZA-TION IN TOMOCERUS FLAVESCENS (COLLEMBOLA). (Under the direction of Clifford B. Knight) Department of Biology, June 1968.

The purpose of this study is to detect the possible differences in the metabolism of different diets by Tomocerus flavescens by measuring the oxygen uptake following a specific diet. The specimens were collected alive on distilled water and maintained for six days in sterile growth chambers with a specific diet of one of the following: Oedocephalum, Rhizopus nigricans, autoclaved litter, or no food. Following the feeding period the oxygen uptake by each dietary group and recently collected specimens was measured with Warburg manometers at 10°, 20°, and 25°C.

Results above the minimum accuracy of standard manometers were obtained for one group at 10°, none at 20°, and three at 25°C. Since the oxygen consumption per milligram per hour is so low in T. flavescens, the standard manometer tubes are not practical for measuring the oxygen uptake.

Those specimens with no food had the highest oxygen usage over a period of two hours at 10°C while those with a diet of R. nigricans had the lowest. However, at 25°C those fed R. nigricans had the greatest consumption and those with no food had an uptake which was only slightly lower. Specimens fed Oedocephalum or autoclaved litter never showed an uptake greater than the minimum readable value. This may give some indication as to the relative nutritive value of the various diets which have been reported for the collembolans.

ACKNOWLEDGEMENTS

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INTRODUCTION

A wide variety of foods has been reported to make up the diet of Collembola. Poole (1959) listed the main constituents of the diet to be fungi, decaying plant remains and detritus. Evidence that some species fed exclusively on spores, others fed on spores and hyphae, and still others never feed on fungi at all was given by Farahat (1966) who further cited the nutritive factor as being important in the distribution of Collembola. In selective feeding experiments Tomocerus exhibited a decided preference for spores (Knight and Angel, 1967). In this paper, the authors stated that (p. 510), "Since fungi are the most apparent source of food for humus and soil microfauna, it is distinctly possible that some Collembola adapt to a diet of a particular fungal species."

It appears that if there is an adaptation for selection of a fungal species, it is possible that the collembolan obtains nutritive benefits from such a selection. In the present investigation an attempt is made to detect the possible differences in the nutritive value of different diets by measuring the oxygen uptake following a specific diet.

REVIEW OF LITERATURE

The collembolan diet consists of a wide variety of foods. MacNamara (1924) separated the Collembola into two groups according to their feeding habits. According to MacNamara, those with molar plates were adapted to a vegetable diet and those without molar plates were carnivorous. However, even with the capability of grinding food, collembolans with molar plates had particles of wood, uncrushed fragments of fungal hyphae, and spores in the gut. In the gut of specimens collected in the Douglas fir plantation in North Wales, fungal hyphae and spores comprised the major portion of the recognizable material, mineral particles were a common inclusion, and higher plant tissues were a minor component (Poole, 1959). The availability of food in the form of fungal colonies was suggested by Knight (1963) as a limiting factor in the seasonal distribution as well as the microstratification of Tomocerus. Other foods cited by MacNamara included sap of maple trees, algae, pollen, seedlings, and the bodies of several animals including other collembolans. Christiansen (1964) added bacteria, live animals, and perhaps fungal juices to the above list. Singh (1964) reported that Tomocerus longicornis and Neanura muscorus fed on small particles (bacteria and fungal spores) and were necrophagous. Thus he altered Mac-Namara's groupings to solid food feeders and fluid feeders.

Several species of Collembola have been observed feeding on nematodes and their cysts (Brown, 1954 and Murphy and Doncaster, 1957). Folsom (1933) listed forty-three injurious species of Collembola including those which damage crops as well as cultivated mushrooms. Edwards (1962) confirmed that springtails do considerable damage to bean seedlings. Although springrails fed on dead animals, a

significant absence was observed on the carcasses of guinea pigs (Bornemissza, 1957).

In the laboratory Achorutes armatus has been maintained on syrup, starch, butter, cheese, and mycelia of fungi; but yeast was used with best success (Britt, 1951). Folsomia candida was given commercial baker's yeast as food (Marshall and Kevan, 1962); Isotoma notabilis thrived on yeast, but decaying leaves of American elm (Ulmus americana) and sugar maple (Acer saccharum) and fungi were readily accepted (Sharma and Kevan, 1963a). Decaying maple leaves and yeast were used to maintain Folsomia similis with good results, but leaves and yeast alone were not sufficient for the organisms to flourish (Sharma and Kevan, 1963b). Pseudosinella petterseni was fed commercial baker's yeast and P. alba was fed decaying leaves and yeast. Both avoided fungal mycelium (Sharma and Kevan, 1963c). Farahat (1966) reports that members of three families, Entomobryidae, Poduridae, and Isotomidae, were successfully reared in cages with growing fungi and displayed apparently normal activity. On the other hand, members of the family Sminthuridae exhibited no vital activities in the presence of growing fungi. He also found that some species fed exclusively on spores, while others merely preferred spores but would feed on hyphae. In selective feeding experiments, Tomocerus showed a decided preference for fungal spores over hyphae or decaying plant tissues (Knight and Angel, 1967).

Using a Warburg apparatus with micromanometers and 2,2,4-trimethylpentanals in lieu of Brodie's fluid, Schaller and Zinkler (1963) measured the oxygen uptake of Istoma saltans at various temperatures between -2.5° and $9^{\circ}C$. The uptake ranged from 0.922 mm^3 O_2/mg/hr. to 2.510 mm^3 O_2/mg/hr. The oxygen uptake by I. saltans, a snow dwelling species, was 0.922 cm^3 O_2/g/hr. at -2.5° while Orchesella flavescens,

a forest species, showed an uptake of 0.527 cm^3 O_2/g/hr. at 20°C, indicating an adaptation to a cold mode of existence by I. saltans.

METHODS AND MATERIALS

Specimens of Tomocerus flavescens Folsom were collected from litter on the East Carolina University campus in Greenville, North Carolina. Live specimens were separated from the litter with a Tullgren funnel apparatus and collected in jars containing distilled water. Cultures of Tomocerus were maintained in four-and-one-half ounce baby food jars filled one-third full with a plaster of Paris and charcoal solution (9:1) which was allowed to harden and sterilized.

The fungal genera, Oedocephalum sp. (Barnett, 1960) and Rhizopus nigricans, were isolated from forest litter and stocks cultured on a one-half strength potato dextrose agar medium. Fifteen specimens were isolated in each of four sterile growth chambers. Specimens in the first growth chamber were fed Oedocephalum sp.; in the second chamber they were fed R. nigricans. In the third chamber the collembolans were fed sterile crushed litter; in the fourth chamber they had no food. Since collembolans feed on their feces (Poole, 1959), each group was transferred daily with an aspirator to sterile chambers with fresh food supply for a period of six days in order to minimize the ingestion of fecal remains.

On the seventh day the oxygen consumption of each group as well as recently collected specimens was measured. Each of the aforementioned diets was offered (15) fifteen separate groups for a period of one week. At the end of the feeding period the oxygen uptake of five groups with each diet was measured at $10^{\circ}C$, five others at $20^{\circ}C$, and the last five groups at $25^{\circ}C$. The oxygen uptake of three groups of recently collected specimens was measured at $10^{\circ}C$ and that of five groups was measured at 20° and $25^{\circ}C$.

Manometer flasks with a volume of approximately seventeen milliliters contained 0.8 ml of distilled water in the side arm and 0.2 ml of 10% potassium hydroxide in the center well with a 2 x 2 cm fluted strip of filter paper used as a wick. The microliters (μl) of oxygen consumed was determined by using the method of Umbrite, et. al. (1957) and a flask constant of less than 1.42 did not render valid results in the present work. A thermobarometer which was void of animals was used to negate changes due to variations in temperature and atmospheric pressure.

Animals were weighed on an analytical balance and oxygen consumption calculated per milligram of tissue.

Some of the specimens fed on each diet were cleared with 10% lactic acid. The guts were then removed and squashed on a microscope slide for microscopic observation to verify the ingestion of foods offered them.

RESULTS

During the week of feeding and transferring about five specimens of $\underline{T}$. flavescens usually died or were lost. Further, it was practical to use only about ten individuals in the manometer flasks since more than this number increased the possibility of death by their jumping into the center well containing potassium hydroxide. Therefore, beginning with fifteen animals resulted in a workable number for use in the manometer flasks.

The activities of the specimens seemed to be as normal as laboratory conditions allow, with no differences observed in respect to the different diets. Exuviae were seen from specimens with each diet at some time during the feeding period. When the substrate was saturated with water a higher death rate occurred. Some organisms maintained on each diet were seen feeding on dead remains. Gut analysis revealed that many of the specimens had ingested the food offered them. However, some of them had apparently empty guts; others contained collembolan scales and particles of the substrate.

The weight of 462 $\underline{T}$. flavescens was 268.6 mg with an average weight of 0.58 mg per individual.

The oxygen consumed at ten-minute intervals for each of five trials was averaged (See Appendix). This was done for all experiments except for those using recently collected specimens at $10^{\circ}C$ for which only three trials were averaged. The graph points in figures 1-3 were the averages at thirty-minute intervals.

At $10^{\circ}C$ those with no food had a total two-hour oxygen consumption of 1.55

μl and was the only group to show an uptake more than 1.42 μl, the critical point

for accurate measurement (figure 1). Figure 2 indicates that none of the test groups

had an uptake greater than 1.42 μl at 20°C. At 25°C recently collected specimens

consumed 1.61 μl of oxygen and those specimens with no food showed a total uptake

of 2.22 μl of oxygen. Specimens fed R. nigricans consumed 2.46 μl of oxygen at

the same temperature. Those with a diet of Oedocephalum and litter had a total

oxygen uptake of less than the minimum readable (figure 3).

The ten-minute readings showed an accumulative oxygen consumption. How-

ever, some of the average ten-minute readings shown in the appendix actually show

a drop in consumption or negative results. A pressure change of less than one milli-

meter must be estimated for the manometers with the test animals and the thermo-

barometer. Only a slight variation in the angle of sight while reading can result

in erroneous resultant values. If this occurred in the thermobarometer as well as the

test manometers, the error would be magnified. With the very low oxygen uptake

which was apparent for T. flavescens it became necessary to estimate the pressure

change for several readings. Therefore, standard manometer tubes used with the

Warburg apparatus resulted in questionable data, making it necessary to consider

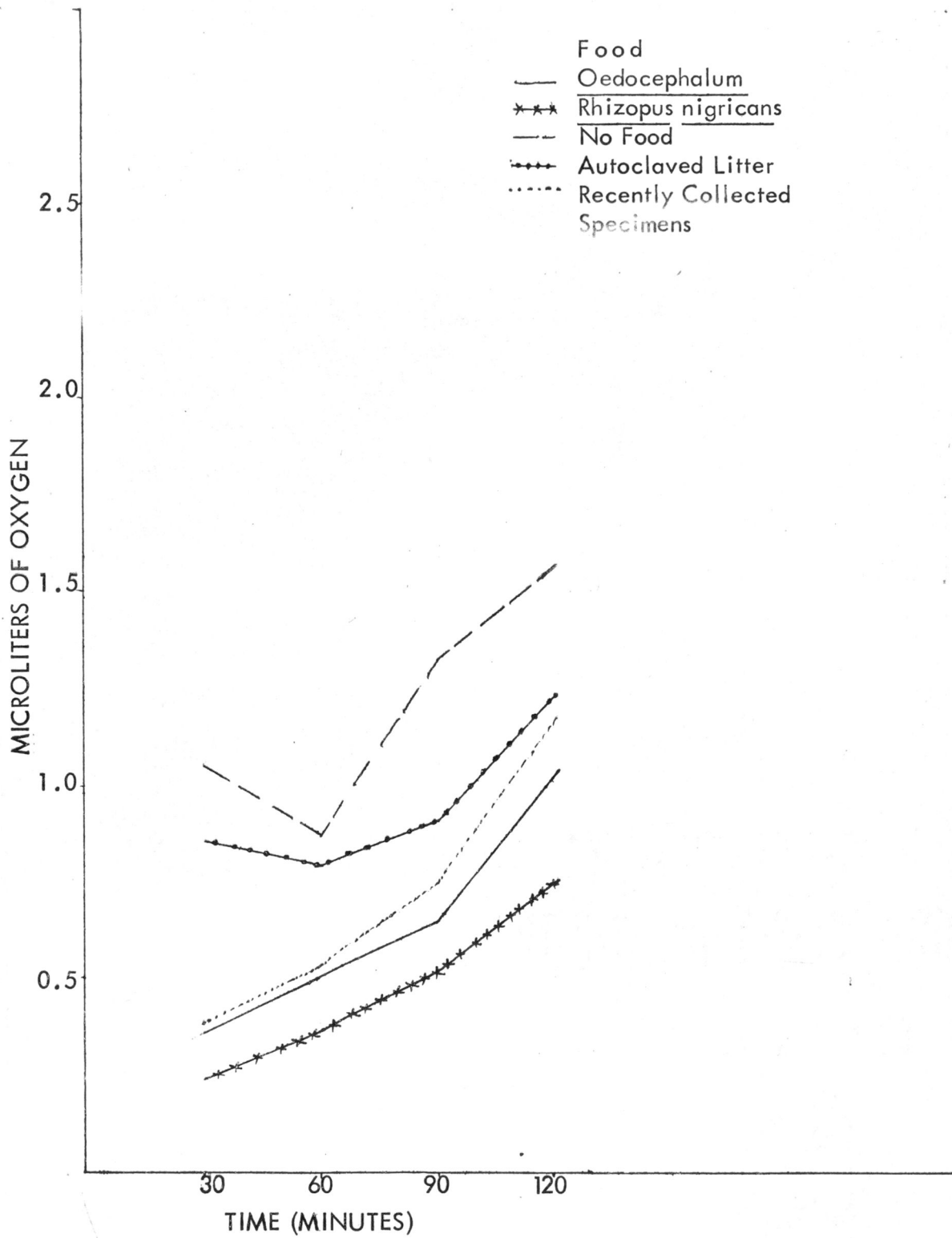the total two-hour oxygen consumption rather than that at shorter time intervals.

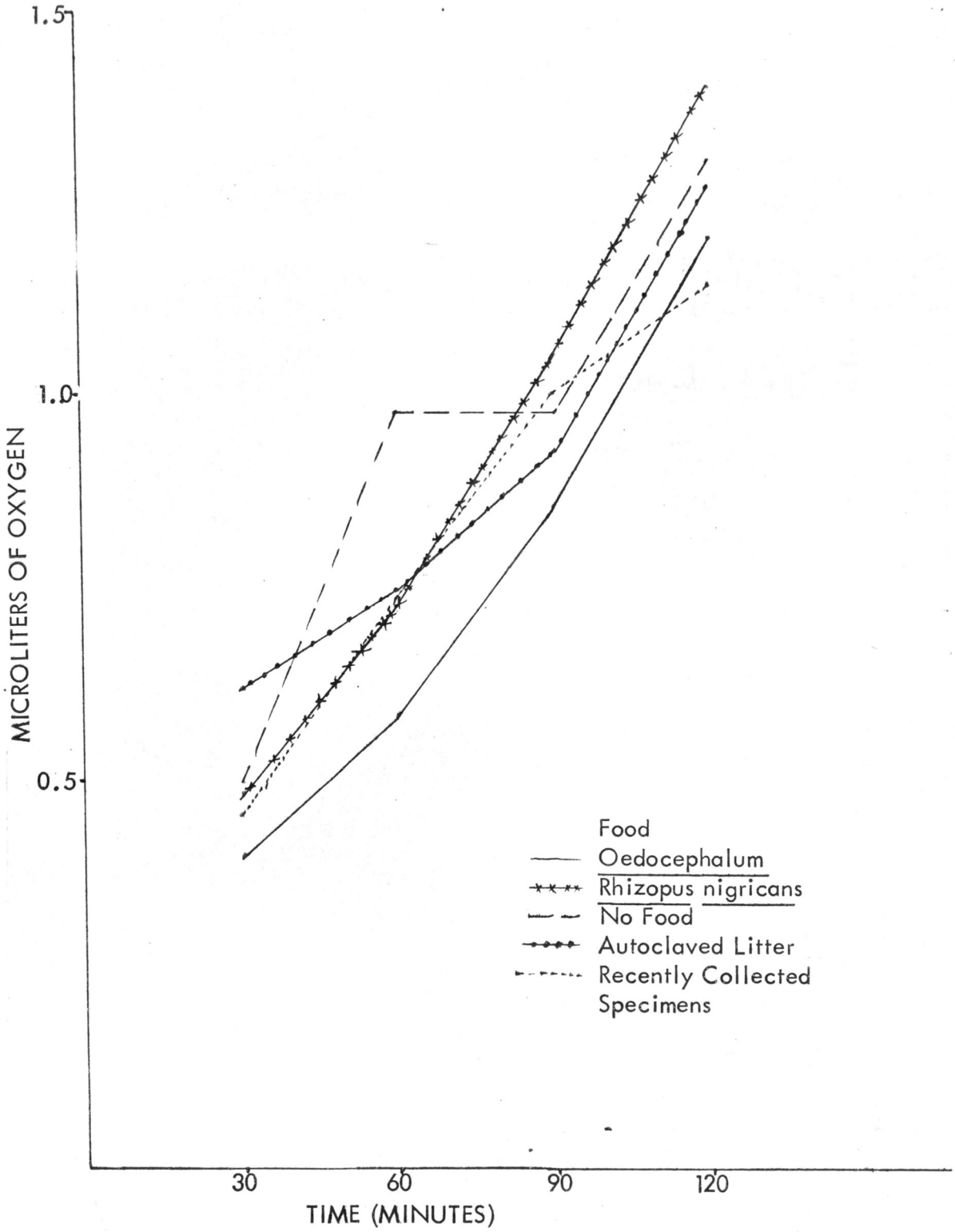Figure 1. Microliters of oxygen consumed per milligram at 1.0°C.

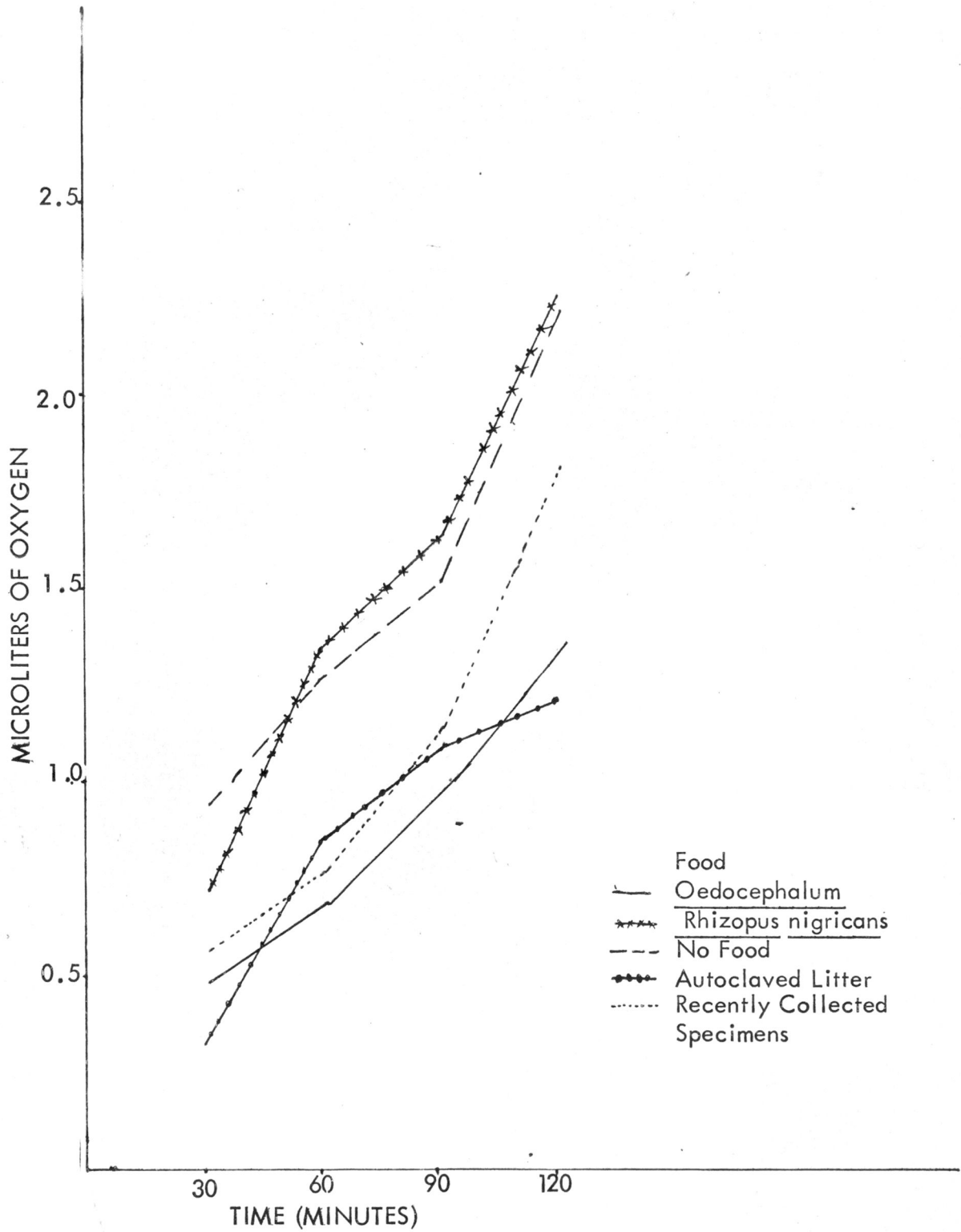Figure 2. Microliters of oxygen consumed per milligram at 20°C.

Figure 3. Microliters of oxygen consumed per milligram at 25°C.

DISCUSSION

The differing metabolic rates of T. flavescens with varying diets was reflected in the oxygen uptake. Since metabolism is dependent upon the enzymatic digestion of foods, an effect on the enzymatic activity will effect a change in the metabolic rate. With a diet of R. nigricans there was a reduction in oxygen consumption with a decrease in temperature which was probably due to a decrease in metabolism. The increase in the metabolic rate which occurred with an increase in temperature was shown in recently collected specimens. This group showed a metabolic rate which was intermediary at all temperatures suggesting a natural diet consisting of several foods rather than a single component.

The higher oxygen consumption of organisms at low temperatures with no food may be interpreted as an adaptive feature wherein during periods of low temperatures, when conditions for fungal growth are less than optimal, the springtails rely on internal food reserves. The metabolic rate at low temperatures was greater than at intermediate temperatures and may serve to maintain a body temperature slightly above that of the environment.

With autoclaved litter as a diet, oxygen utilization was nearly constant at all temperatures. Thus, the ability of T. flavescens to metabolize litter is probably limited, and reports of litter as a food may represent accidental ingestion while feeding on fungi.

Evidence that the selectivity exhibited by collembolans in the choice of food is related to the utilization of that food is indicated herein. Farahat (1966) cited Forsslund (1943) and Weis-Fogh (1948) who pointed to the nutritive value of the diet

as a factor in the distribution of some species of Collembola as well as other soil micro-arthropods. The nutritive potential of the food eaten cannot be realized, however, until it is digested to a form which can be metabolized. Christiansen (1964) stated that several sets of enzymes are probably involved in the gradual digestion of food as it passes through the digestive tracts of many individuals. The fact that fungal spores and hyphae passed through the gut virtually unchanged was attributed to the resistance of fungal walls to digestion (Poole, 1959). It is most likely that the enzymes occurring in the alimentary tract of T. flavescens are relatively specific in their digestion of different species of newly ingested fungi. These enzymes appear to be more effective in their action on R. nigricans than Oedocephalum or autoclaved litter. With this greater effectiveness of enzymes on R. nigricans there is a corresponding increase in the metabolic rate with this diet which does not occur with the latter diets. Although at present it is not possible to say conclusively, it is distinctly conceivable that the selectivity in the collembolan diet is ultimately determined by the metabolic requirements of the organism.

It would appear that the use of standard manometer tubes for the measurement of oxygen uptake in T. flavescens is not practical unless the apparatus has been arranged to render a low flask constant. Even though this would not change the minimum readable change in pressure, it would allow an accurate measurement of smaller volumes of oxygen used. Schaller and Zinkler (1963) reported the successful use of micromanometers to record a minimum oxygen uptake of $0.05 \mu l$ by Isotoma saltans. Giese (1962) reports the Fenn-Winterstein monometer, which consists of two flasks attached to a capillary tube containing a droplet of kerosene, as being more

sensitive than the Warburg manometer but less sensitive than the Cartesian diver in

measuring the oxygen usage. The Fenn-Winterstein manometer offers greater sim-

plicity than the Cartesian diver since the successful operation of the diver requires

considerable skill. However, the diver has a sensitivity of 2.5 to 5.0×10^{-5} μl

which can measure the oxygen uptake in a single egg and could be used to accurately

measure that of a single individual collembolan. With more sensitive apparatus than

the Warburg manometers, detection of differences in metabolic requirements in

collembolans can be accomplished.

SUMMARY

1. Tomocerus flavescens specimens were collected alive on distilled water and maintained for six days in sterile growth chambers with a specific diet of one of the following, Oedocephalum, Rhizopus nigricans, autoclaved litter, or no food.

2. Following the feeding period the oxygen uptake by each group with the above diets and recently collected specimens was measured with Warburg manometers at 10°, 20°, and $25^\circ C$.

3. Results above the minimum accuracy of standard manometers were obtained for one group at $10^\circ C$, none at $20^\circ C$ and three at $25^\circ C$.

4. The oxygen consumption per milligram per hour is so low in T. flavescens that the manometer tubes are not practical for measuring the uptake.

5. At $10^\circ C$ specimens with no food had the highest usage and those with a diet of R. nigricans had the lowest. Those fed R. nigricans showed the greatest consumption over a period of two hours at 25° and those with no food had an uptake which was only slightly lower. This may give some indication as to the relative nutritive value of the various diets which have been reported for the collembolans. It is suspected that the enzymes occurring in the digestive tract of T. flavescens are specific for certain fungi.

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APPENDIX

Readings at 10 minute intervals for five trials were averaged for all groups,
except the recently collected specimens at 10°C for which only three trials were
averaged, to arrive at the following figures.

Time-min.	Oedocephalum	R. nigricans	no food	Autoclaved litter	recently collected

Temperature - 10°C

10	0.12	0.14	0.41	0.43	0.05
20	0.16	0.18	0.53	0.34	0.20
30	0.38	0.24	1.06	0.85	0.39
40	0.71	0.34	0.94	0.93	0.41
50	0.46	0.39	1.04	0.76	0.53
60	0.50	0.37	0.88	0,80	0.53
70	0.53	0.47	0.88	0.76	0.54
80	0.81	0.58	1.39	0.93	0.95
90	0.64	0.52	1.31	0.90	0.76
100	1.18	0.65	1.43	1.26	0.76
110	1.02	0.73	1.46	1.16	1.01
120	1.04	0.75	1.55	1.23	1.19

Temperature - 20°C

10	0.29	0.31	0.37	0.40	0.27
20	0.33	0.30	0.40	0.48	0.34
30	0.40	0.49	0.51	0.62	0.42
40	0.59	0.61	0.83	0.70	0.57
50	0.65	0.72	1.06	0.74	0.71
60	0.58	0.74	0.98	0.75	0.74
70	0.66	0.82	1.03	0.77	0.82
80	0.78	1.02	1.00	0.83	0.89
90	0.86	1.05	0.98	0.93	1.00
100	1.03	1.13	1.08	0.97	1.06
110	1.07	1.22	1.18	1.12	1.17
120	1.20	1.39	1.30	1.27	1.14

Temperature - 25°C

10	0.19	0.42	0.57	0.14	0.11
20	0.09	0.44	0.37	0.07	0.30
30	0.47	0.74	0.96	0.34	0.57
40	0.40	0.82	0.79	0.49	0.63
50	0.45	0.92	1.04	0.54	0.68
60	0.69	1.36	.1.27	0.84	0.78
70	0.71	1.31	1.06	0.71	0.82
80	0.85	1.52	1.41	1.02	0.93
90	0.99	1.62	1.52	1.10	1.13
100	1.07	1.61	1.63	1.08	1.29
110	1.39	1.84	2.13	1.28	1.51
120	1.35	2.25	2.22	1.21	1.81