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Notes on Iowa Tardigrades

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Notes on Iowa Tardigrades[1]

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Abstract. Five species of tardigrades, *Macrobiotus areolatus, M. islandicus, M. hufelandii, Milnesium tardigradum,* and *Hypsibius tuberculata* are reported from lichens growing on Iowa trees. Factors influencing tardigrade distribution are discussed, especially with reference to the lichen species represented in the samples, and the tree substrate. *Hypsibius tuberculata* was found to be most abundant about 2 meters above ground level, and to become less abundant below, while the other four species were found to be most abundant near the ground and decline steadily with increasing height. No clear evidence of preference of northern, southern, eastern, or western exposures was found.

The tardigrade fauna of the United States, and especially of the midwestern states, has been largely neglected. This study was undertaken to identify some of the local tardigrades, and to examine some of the factors related to their distribution.

MATERIAL AND METHODS

Samples of lichen-bearing tree bark were flooded with water, covered, and soaked for 24 hours, releasing the tardigrades and inducing asphyxy. After the animals were pipetted off, the sample was treated a second time. Trials showed that over 95% of the tardigrades recoverable from the samples were obtained by two washings.

Washings of bark taken near lichens, but having no lichens attached, were repeatedly negative for tardigrades. Evidently the lichen substrate is required, and the percentage of lichen cover on the sample is a factor in population density. To provide an estimate of the population independent of lichen density, the number of tardigrades found was corrected to the number to be expected if the lichen coverage was 100%. These corrected numbers are used in the tables included in the following pages.

THE TARDIGRADE FAUNA

Five species of tardigrades were encountered, all associated with lichens. None were found associated with mosses, but moss cover has not been studied intensively.

Macrobiotus areolatus Murray, 1907, *M. islandicus* Richters,

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TABLE 1

SUMMARY OF SAMPLES AND ORGANISMS PRESENT IN THEM

Sample Number	Tree Species[1]	Percent Coverage	Conversion Factor	Direction	Height In Meters	Cover Species[2]	Tardigrade Species[3]										Total	
							A		B		C		D		E			
							Raw Number	Converted Number	Raw Number	Converted Number	Raw Number	Converted Number	Raw Number	Converted Number	Raw Number	Converted Number	Raw Number	Converted Number
1	8	75	1.3	S	3	6	6	8	1	1	—	—	—	—	—	—	7	9
2	8	50	2	SE	2	5	—	—	—	—	—	—	15	30	—	—	15	30
3	3	75	1.3	E	2	1	1	1	—	—	—	—	3	4	—	—	4	5
4	3	50	2	W	1	2	—	—	1	2	2	4	4	8	—	—	7	14
5	1	75	1.3	E	1	4	—	—	—	—	—	—	—	—	—	—	—	—
6	1	75	1.3	EE	1	3	3	4	11	15	3	4	—	—	—	—	17	23
7	1	75	1.3	W	1	3	10	13	2	3	—	—	—	—	—	—	12	16
8	1	75	1.3	E	0	8	11	15	2	3	—	—	—	—	—	—	13	18
9	4	75	1.3	S	1	5	13	17	18	24	2	3	—	—	—	—	33	44
10	5	50	2	SE	2	4	5	10	7	14	—	—	—	—	—	—	12	24
11	6	50	2	E	3	2	—	—	—	—	—	—	—	—	—	—	—	—
12	7	50	2	W	2	9	—	—	—	—	—	—	—	—	3	6	3	6
13	5	100	1	N	3	4	—	—	—	—	—	—	—	—	—	—	—	—
14	5	100	1	N	1	1	—	—	1	1	1	1	17	17	—	—	19	19
15	6	75	1.3	S	1	1	—	—	—	—	—	—	53	70	—	—	53	70
16	8	75	1.3	N	1	2	—	—	—	—	—	—	—	—	—	—	—	—
17	8	75	1.3	N	1	moss	—	—	—	—	—	—	—	—	—	—	—	—
18	4	50	2	N	3	1	—	—	—	—	—	—	2	4	—	—	2	4
19	5	50	2	S	1	2	—	—	—	—	—	—	—	—	—	—	—	—
20	8	50	2	N	3	moss	—	—	—	—	—	—	—	—	—	—	—	—
21	2	50	2	S	3	moss	—	—	—	—	—	—	—	—	—	—	—	—
22	8	50	2	E	3	2	—	—	—	—	—	—	—	—	—	—	—	—

TABLE 1 (continued)

Sample Number	Tree Species	Percent Coverage	Conversion Factor	Direction	Height In Meters	Cover Species	Tardigrade Species										Total	
							A		B		C		D		E			
							Raw Number	Converted Number	Raw Number	Converted Number	Raw Number	Converted Number	Raw Number	Converted Number	Raw Number	Converted Number	Raw Number	Converted Number
23	8	50	2	N	2	moss	—	—	—	—	—	—	—	—	—	—	—	—
24	4	75	1.3	NE	2	moss	—	—	—	—	—	—	—	—	—	—	—	—
25	8	25	4	E	0	1	—	—	20	8	6	24	10	40	—	—	36	144
26	1	25	4	E	1	8	—	—	4	16	—	—	—	—	—	—	4	16
27	2	25	4	W	2	6	3	12	7	28	4	16	—	—	—	—	14	56
28	3	50	2	N	0	5	5	10	26	52	—	—	1	2	—	—	32	64
29	1	25	4	E	1	1	2	8	9	36	4	16	1	4	—	—	16	64
30	8	50	2	N	1	6	3	6	1	2	—	—	—	—	—	—	4	8
31	8	75	1.3	W	3	1	—	—	—	—	—	—	1	1	12	16	13	17
32	8	100	1	E	3	1	—	—	—	—	—	—	4	4	—	—	4	4
33	8	100	1	E	1	2	2	2	—	—	—	—	—	—	7	7	9	9
34	4	75	1.3	S	1	1	—	—	—	—	—	—	4	5	1	1	5	6
35	4	75	1.3	N	3	7	—	—	—	—	—	—	—	—	—	—	—	—
36	1	75	1.3	W	1	5	—	—	—	—	—	—	—	—	—	—	—	—
37	1	100	1	E	0	1	1	1	5	5	1	1	3	3	1	1	11	11
38	4	75	1.3	E	2	5	—	—	6	8	—	—	5	7	1	1	12	16
39	4	75	1.3	N	2	4	1	1	6	8	—	—	3	4	—	—	10	13
40	5	75	1.3	S	2	1	—	—	—	—	—	—	—	—	9	12	9	12
					Totals		66	108	127	298	23	69	126	203	34	44	376	722

TABLE 1 (continued)

[1] The code numbers assigned to trees are:

1. *Quercus alba*
2. *Quercus palustris*
3. *Quercus velutina*
4. *Glebitsia triacanthos*
5. *Populus detoides*
6. *Ostrya virginiana*
7. *Acer platanoides*
8. *Ulmus americana*

[2] The code numbers assigned to lichens are:

1. *Candelaria concolor*
2. *Physcia orbicularis*
3. *Physcia elaeina*
4. *Physcia stellaris*
5. *Physcia millegrana*
6. *Physcia ciliata*
7. *Xanthoria fallax*
8. *Parmelia rudecta*
9. *Arthonia caesia*

[3] The code letters assigned to tardigrades are:

A. *Macrobiotus areolatus*
B. *Macrobiotus islandicus*
C. *Macrobiotus hufelandii*
D. *Milnesium tardigradum*
E. *Hypsibius*

1903; *M. hufelandii* Schultze, 1834; *Milnesium tardigradum* Doyere, 1840; and *Hypsibius (Isohypsibius)? tuberculatus* (Plate, 1888) were found in localities in Polk, Madison, Warren, Jasper, and Dallas Counties. Quantitative data on these samples are summarized in Table 1.

Macrobiotus islandicus and *Milnesium tardigradum* have also been taken in qualitative samples from bark of *Juniperus virginiana* and *Quercus albus* in Cedar County, and *Milnesium tardigradum* has been found on unidentified lichens in qualitative samples from *Quercus alba* and *Q. velutina* in the vicinity of Lake Aquabi. These samples are not included in Table 1.

M. areolatus has been reported from North Carolina (Higgins, 1960), the western part of the United States (Schuster and Grigarick, 1965), and Kansas (Beasley, 1968). *M. islandicus* has been reported from Europe, Greenland, and Iceland, as well as from the western United States (Schuster and Grigarick, 1965). *M. hufelandii* has been found in western North America, the District of Columbia, Maryland, and Colorado (Schuster and Grigarick, 1965; Higgins, 1960). *Milnesium tardigradum* has been collected in Colorado, Washington, Illinois, Wisconsin, Maryland, and Kan-

sas as well as western United States. (Higgins, 1960; Beasley, 1968; Schuster and Grigarick, 1965). *Hypsibius tuberculatus* has been found in California and Colorado (Schuster and Grigarick, 1965; Higgins, 1960). To the best of our knowledge, none of these species have been reported from Iowa.

It is evident that a reasonably varied and common fauna of tardigrades is to be found in Iowa, associated with lichens attached to trees. The high percentage of samples that proved to contain specimens indicate that a further analysis of the fauna may be profitable.

Factors Involved in Distribution

A preliminary analysis of some of the factors that may be important in tardigrade distribution was undertaken with the quantitative samples. To permit comparisons despite inequalities in the occurrence of different lichen species in the various samples, a tardigrade frequency index was calculated. The frequency index is the percentage of the total catch of each tardigrade species on a given lichen, if all lichens had occurred with equal frequency, and had they borne a population similar to the ones actually obtained. This rough estimate is, of course, increasingly inaccurate as the number of samples containing the lichen decreases. A high frequency index from a small number of samples is more likely to be meaningful than a low frequency index from a small number of samples, as it indicates a relatively high population density of tardigrades in the few samples examined. To reflect the general suitability of the lichens for supporting tardigrades, the sum of the frequency indices for the various tardigrade species was calculated for each lichen. This sum estimates the total number of tardigrades of all species that might be expected if 500 had been collected from samples containing equal numbers of the various lichens. The results are shown in Table 2.

It is evident that the three species of Macrobiotus inhabited a wider range of lichens than either *Milnesium* or *Hypsibius*. Although the three species appear to have rather similar requirements, the relatively low frequency of *M. areolatum* on *Candelaria concolor* and of *M. hufelandi* on *Physcia millegrana* appear significant, and suggest that requirements are not identical. *Milnesium tardigradum* appears to prefer *Candelaria concolor* and *Physcia millegrana,* of the lichens sampled, and it is evident that *Arthonia caesia* provides a good habitat for *Hypsibius tuberculatus.*

The ability of several species of tardigrades to exist on the same species of lichens raises the question of species interaction. Table I shows the populations obtained from single samples. It is evident that all three species of *Macrobiotus* can and do coexist; in some

Table 2

Frequency Indices of Tardigrades Associated with Iowa Lichens

Lichen Species	Number of Samples	*Macrobiotus areolatus*	*Macrobiotus islandicus*	*Macrobiotus hufelandii*	*Milnesium tardigradum*	*Hypsibius tuberculata*	Sum of Frequency indices
Candelaria concolor	11	2.6	22.6	18.5	57.4	26.8	117.9
Physcia orbicularis	6	0.9	6.6	3.2	5.5	11.5	27.7
Physcia elaeina	2	24.9	6.1	9.8	0	0	40.8
Physcia stellaris	4	8.7	10.3	0	4.2	0	23.2
Physcia millegrana	5	15.8	34.6	2.9	32.7	1.9	87.9
Physcia ciliata	3	25.4	21.3	26.1	0	0	72.8
Xanthoria fallax	1	0	0	0	0	0	0
Parmelia rudecta	2	22.0	3.8	39.2	0	0	65.0
Arthonia caesia	1	0	0	0	0	59.6	59.6

cases all occur together in reasonable abundance, as in sample 27. *Milnesium* occurs with all of the other species, but as a rule, a large number of *Milnesium* is found only when it is alone. The exception is sample 25, where two species of *Macrobiotus* and *Milnesium* occurred together in large numbers. *Hypsibius* has been found with all other species, but as a rule, unless it occurs alone or nearly so, population levels are low. The scanty data suggest that several types of species interactions may occur among tardigrade species. They may prove to be particularly useful for a study of this aspect of population dynamics.

In examining the relationship of the tree substrate to tardigrade distribution, a frequency index was calculated in the same manner as for the lichens. To facilitate comparisons of the importance of trees and lichens, a mean frequency index for the lichens found in the samples taken from the various trees was determined. These results are shown in Table 3.

Although only 35 samples were taken, the results show some interesting points. Each species of tardigrade has a relatively high frequency index on one species of tree. As it happens, each of these "optimal" tree habitats is represented by only one or two samples, presumably a tree inhabited by an unusually high tardigrade population. Evidently more data is required before definite conclusions can be drawn, but there are indications that certain combinations of tree and lichen are especially satisfactory, while other combinations are less so. Certainly, although the tardigrades occur on the lichens rather than the tree bark, the tree substrate is a factor in distribution.

Samples were taken at different heights, and the frequency index for each species of tardigrade for each height was calculated.

It is clear that the population density of tardigrades changes with the height of the lichens of the trees. Four of the species are most abundant in samples taken near the ground. While they are still relatively abundant in samples about 1 and 2 meters from ground level, they have become quite scarce in samples taken about 3 meters from ground level. The exception is *Hypsibius tuberculatus,* which proves to be relatively scarce near ground level, and to reach a maximal density about 2 meters from ground level. It remains abundant at about 3 meters from ground level. The occurrence of two patterns of vertical distribution is quite interesting. No information is available to explain environmental factors that operate to create vertical distribution patterns, but the existence of diverse patterns may help to set up observational conditions under which answers can be sought.

Samples were taken from the north, south, east, or west sides of the trees. Frequency indices were calculated to determine whether

Table 3

Frequency Indices of Tardigrades on Iowa Trees, and Mean Frequency Indices of Lichens Present on the Bark

Tree Species	Number of Samples	Tardigrade Species									
		Macrobiotus areolatus		*Macrobiotus islandicus*		*Macrobiotus hufelandii*		*Milnesium tardigradum*		*Hypsibius tuberculata*	
		Tree F.I.	Lichen F.I.	Tree F.I.	Lichen F.I.	Tree F.I.	Lichen F.I.	Tree F.I.	Lichen F.I.	Tree F.I.	Lichen F.I.
Quercus alba	8	18.6	14.8	13.7	15.5	11.2	14.1	1.5	18.8	1.1	5.7
Quercus palustris	1	43.7	25.4	37.4	21.3	68.6	26.1	0	0	0	0
Quercus velutina	3	13.2	6.4	24.0	21.3	5.7	8.2	8.3	31.9	0	13.4
Glebitsia triacanthos	6	10.8	6.8	8.9	16.8	2.1	5.3	5.9	23.5	2.8	7.0
Populus deltoides	5	7.2	4.0	4.0	13.1	0.8	7.3	5.9	22.4	21.0	12.7
Ostrya virginiana	2	0	1.7	0	24.6	0	10.8	62.9	31.4	0	19.1
Acer platanoides	1	0	0	0	0	0	0	0	0	52.5	59.6
Ulmus americana	9	11.1	11.2	12.3	21.3	11.4	12.7	14.9	23.9	22.3	10.0

population densities differed in these positions. *M. areolatus* proved to be very nearly equally distributed around the tree. *M. islandicus* was most common on the eastern side, while nearly all *M. hufelandii* were found on the east or west sides of the trees. *Hypsibius tuberculatum* was most abundant on the west side, and *Milnesium tardigradum* was most abundant on the south. In view of the lack of consistent findings and the relatively small number of samples, it does not seem safe to draw conclusions.

Discussion

The presence of a relatively rich and abundant tardigrade fauna on Iowa tree lichens is indicated from the scattered sampling that has been undertaken. Although tardigrades are adapted to their semi-aquatic habitat by various physiological devices, they must live under considerable stress, at the best. Differences in population density related to such factors as height, species interactions, and lichen or tree substrate undoubtedly reflect differences in the stresses under which the population exists. It seems safe to assume that these tiny creatures may provide excellent experimental material for the study of populations under varied conditions of stress.

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