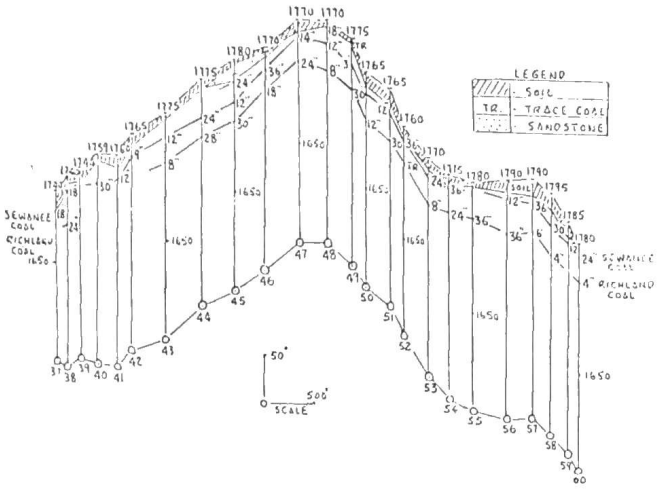

FIG. 6. *Panel diagram based on air rotary borehole data showing the lensing nature of the Sewanee and Richland coal in the Whitwell shale near Kelley Creek, Savage Point Quadrangle, Tennessee.*

ing here would encounter environmental problems. Also, mine drainage would be a serious problem if underground mining were attempted.

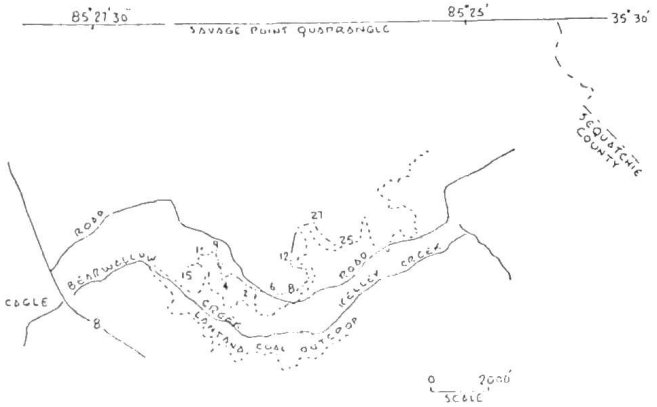

FIG. 7. *Location of boreholes (Fig.) near Bearwallow Creek, Savage Point Quadrangle,* Sequatchie Co., *Tennessee.*

Bearwallow Creek Area, Savage Point Quadrangle— Over 20 air rotary boreholes were drilled in the Bearwallow Creek area (Fig. 7) to test the Vandever Lower Shale for the Lantana coal seam. Holes 1-15 (western portion of diagram, Fig. 8) bottomed in the upper part of the Newton Sandstone. Lantana coal thicknesses range from no coal in holes 5, 6, 10, 13, 14, 11 and 26 to 42 inches in hole 1.

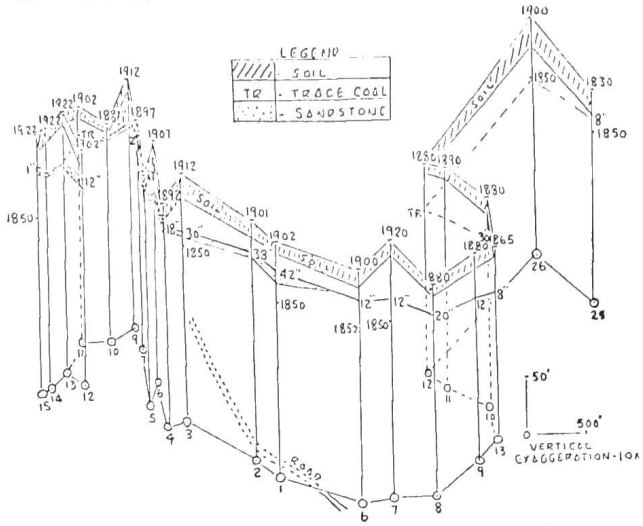

FIG. 8. *Panel diagram based on air rotary borehole data showing the lensing nature of the Lantana coal in the lower Vandever shale near Bearwallow Creek, Savage Point Quadrangle, Tennessee.*

SUMMARY

Coreholes and air rotary boreholes tested portions of the Pennsylvanian Raccoon Mountain Formation, Whitwell Shale and Vandever Lower Shale for coal seam thickness variation. In so doing, part of the subsurface distribution of the lithostratigraphic units in the Gizzard Group and Crab Orchard Mounains Group was determined. This information should be of use to those individuals involved in mapping this area.

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A LABORATORY STUDY OF SUBSTRATE AND TEMPERATURE PREFERENCES OF THREE SPECIES OF FRESHWATER PLANARIANS (TURBELLARIA: TRICLADIDA)

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ABSTRACT

Responses of three species of freshwater, epigean planarians to substrate and a temperature gradient were determined in the laboratory and compared to field observations. One hundred specimens of each species were presented five types of substrate on nine different occasions, and another 100 specimens of each species were arranged in a temperature gradient of 3.5 to 38.5°C on twelve different occasions. *Dugesia dorotocephala*

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showed a slight preference for rocks over leaves, but moss and sand were quite acceptable, and silt was least preferred. Although the most eurythermal species, it chose mostly a range of 27 to 34°C and had a temperature preference of 27.5°C. *Phagocata gracilis* preferred either rocks or moss to the other substrates, selected mainly a range of 4.0 to 22.0°C, and showed temperature preferences of 14.8°C on rocks and 12.6°C on moss. *Phagocata velata* definitely preferred rocks to moss, leaves, sand, and silt. It was stenothermal, mostly in a range of 16.0 to 20.5°C and had a temperature preference of 17.8°C. These results are similar to observations in the field by other workers.

INTRODUCTION

Substrate and temperature long have been recognized as important factors that influence the distribution of stream invertebrates, and their effect has been summarized well by Hynes (1970). Among the stream fauna, planarians have received some attention regarding these factors, but there apparently was no definitive laboratory study published on substrate preferences of the species considered here, although general information from field studies is available. Peaslee (1910) stated that *Phagocata gracilis* was found "mostly under stones and leaves or crawling along the sandy bottom of a brook." Darlington and Chandler (1972, 1979) reported *Dugesia dorotocephala* and *Phagocata velata* on eroding substrates (large rocks and gravel) and *P. gracilis* on a variety of substrates ranging from rocks to sand.

Temperature ranges have been recorded in several field studies and in some laboratory experiments. Hyman (1925) indicated that low temperature was one factor that limited *D. dorotocephala* to spring-fed habitats, and Kenk (1970) referred to the principal occurrence of *P. gracilis* in springs and headwaters of creeks with low temperatures. In a survey of Tennessee and Arkansas triclads, Darlington and Chandler (1972, 1979) noted temperature ranges for all the foregoing species. Mast (1903) subjected *D. dorotocephala* and *P. gracilis* to a temperature gradient, and Eddy and Gleim (1932) did this for *P. gracilis*.

The purpose of the present study was to determine, under laboratory conditions, the substrate and temperature preferences of the above mentioned species and to compare the results with those of previous studies.

MATERIALS AND METHODS

All three species of planarians were collected in the middle Tennessee area (*ca.* 85° to 88° W longitude, 35° to 36° N latitude). *Dugesia dorotocephala* (Woodworth 1897) was collected from a stream partly formed by overflow from a trout farm adjacent to interstate highway 40, Hickman County. At time of collection water temperature was 12°C, and planarians were found under rocks and moving over the sandy bottom. *Phagocata gracilis* (Haldeman 1840) was obtained from a spring-fed stream in an abandoned limestone quarry alongside state highway 141, just below the dam at Center Hill Reservoir, DeKalb County. Planarians were found on a variety of substrates and water temperature was 17°C. *Phagocata velata* (Stringer 1909) was collected from a rivulet flowing from Herring Cave, off the Valley View Road, near Lascassas, Rutherford County. All specimens were tested in the laboratory within two weeks after collection and were kept in porcelain pans containing spring water at room temperature. Planarians were not fed during any experimental period.

A galvanized metal trough (6.3 cm wide, 143.1 cm long, and 10.2 cm deep) was used in both substrate and temperature tests. Five types of substrate were placed in the trough, each covering the same amount of trough area. Rocks were collected from local streams and ranged in diameter from 1.2 cm to 3.8 cm. Moss (*Fontinalis* sp.) was gathered with planarians, sand and silt were obtained from nearby streams, and each of these types was placed in the trough to a depth of 6 mm. Leaves of oak, maple, redbud, and elm were collected from ground litter. To remove any food, each type of substrate was washed thoroughly before use, and water from collection sites was placed in the trough to a depth of 1.2 cm. During the substrate experiment water temperature was maintained at approximately 20°C, water depth was 2 cm, and all trials were conducted under constant illumination from fluorescent ceiling light. Water in the trough was aerated at the beginning of each trial.

Three series of tests, each of three trials, were conducted for each species on the five substrate types, 100 planarians were used in each trial, and the sequential arrangement of substrate was changed for each series of tests. At the end of each 24-hr trial, location and numbers of worms on each substrate were recorded, and worms were removed from the trough. In each trial of the first series, 25 worms were placed in the middle of each quarter of the trough. In each trial of the second series, 20 worms were placed in the middle of each type of substrate, and in the third series, all 100 worms were placed on the substrate that was least preferred as revealed by the preceding two series.

A temperature gradient of 3.5 to 38.5°C was established by packing one end of the trough in ice contained in the bottom half of a cut-away styrofoam cooler and by heating the opposite end of the trough with a hot plate. Temperatures were measured with a calibrated mercury type laboratory thermometer, and numbers of worms were recorded for each 0.5°C interval at the conclusion of a 24-hr trial.

The same trough was used for the temperature experiment, and depth of water and illumination were the same as in the substrate experiment. The substrate used for a species was the preferred type as indicated by results of the substrate experiment. In each trial of each species, 100 planarians were used, with 25 worms initially placed in each quarter of the trough.

A temperature preference for each species was established: each temperature reading at which worms were found was multiplied by the total number of occurrences of all three trials for that temperature, and the results were summed and divided by 300.

RESULTS

Dugesia dorotocephala. The percentage occurrences on rocks (35.7) was slightly greater than for leaves (28.2), sand and moss were chosen about equally (18.5, 15.9), but silt was least preferred (1.7) (Table 1). In the temperature gradient, combined results of three trials showed 209 occurrences (69.7%) in the range of 27 to 34°C and only 84 occurrences (28%) below 27°C (Fig. 1, A). The temperature preference for *D. dorotocephala* was 27.5°C.

Phagocata gracilis. Out of 900 possible occurrences, 636 or 70.6% were on rocks and moss (Table 1). Occurrences on leaves were 148 and on silt, 93, whereas sand was chosen only 23 times. Three temperature trials were conducted with rocks and three with moss as substrates. For all trials with rocks the greatest number of occurrences, 273 (91%), was in the 7-22°C range, but a few were recorded up to 30.5°C (Fig. 1, B). The temperature preference on rocks was 14.8°C. For all three trials with moss the range of 4.5 to 21°C had 289 occurrences (96.3%) with two major peaks at 8.5°C and 11.0°C (Fig. 1, C). The temperature preference for *P. gracilis* on moss was 12.6°C.

TABLE 1. *Total number of occurrences and percentages of three planarian species on five substrates for three series of three trials each.*

Species	Total number of occurrences and (percentages) on: Rocks	Moss	Leaves	Sand	Silt
D. dorotocephala	321 (35.7)	143 (15.9)	254 (28.2)	167 (18.5)	15 (1.7)
P. gracilis	319 (35.4)	317 (35.2)	148 (16.4)	23 (2.6)	93 (10.3)
P. velata	729 (81.0)	74 (8.2)	60 (6.7)	17 (1.9)	20 (2.2)

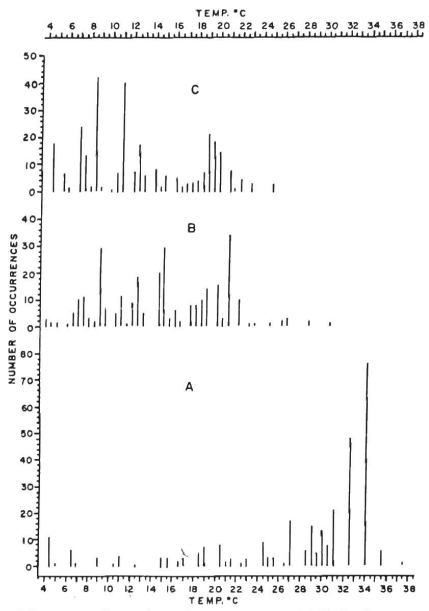

FIG. 1. *Total number of occurrences of (A)* D. dorotocephala, *(B)* P. gracilis *on rocks, and (C)* P. gracilis *on moss at various temperatures.*

TABLE 2. *Substrate types occupied by three species of planarians, showing mean numbers of occurrences (± standard error) and results of t-test (significant difference between means at 0.05 (*) and 0.01 (**) levels of confidence).*

Substrate	*D. dorotocephala*	*P. gracilis*	*P. velata*
Rocks (R)	35.7 (8.360)	35.4 (7.418)	81.0 (3.153)
Moss (M)	15.9 (6.447)	35.2 (4.092)	8.2 (2.278)
Leaves (L)	28.2 (6.069)	16.4 (4.586)	6.7 (1.236)
Sand (Sa)	18.5 (4.353)	2.6 (0.445)	1.9 (0.512)
Silt (Si)	1.7 (0.408)	10.3 (4.556)	2.2 (1.176)
t-test:	R *vs* M, Si** R *vs* Sa*	R *vs* L,Sa,Si** M *vs* L,Sa,Si**	R *vs* M,L,Sa, & Si**

Phagocata velata. This species clearly demonstrated a preference for rocks over all other substrates since 729 or 81% of all occurrences were on rocks (Table 1). Sand and silt were least preferred (1.9%, 2.2%), and moss and leaves accounted for 134 or 14.9% of all occurrences. Combined results of all trials with the temperature gradient indicated the highest occurrence, 262 (87.3%), in the range of 16-20°C with 70 occurrences at 19.5°C (Fig. 2). The temperature preference of *P. velata* was 17.8°C.

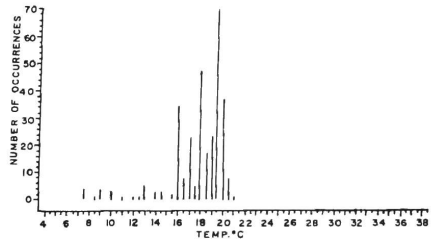

FIG. 2. *Total number of occurrences of* P. velata *at various temperatures.*

DISCUSSION

Dugesia dorotocephala demonstrated less preference for a particular substrate than either of the other species. Statistical analysis (t-test) revealed that rocks were significantly preferred to moss and silt (0.01 level), and at the 0.05 level, rocks were also preferred to sand (Table 2). Except for silt, percentages of occurrences on various substrates, were not widely different. This substantiates the observation that *D. dorotocephala* occurred on a variety of substrates in New Mexico streams (Chandler and Darlington, 1975). Interestingly, 18.5% of occurrences were on sand, which is normally a rather barren area for triclads. Moreover, when collecting *D. dorotocephala* for this study, many were found aggregated on the stream's sandy bottom.

Dugesia dorotocephala seems to be a warm water species that prefers temperatures from 27 to 34°C, since 69.7% of occurrences were in this range and 76 were at 34°C. This is further evidenced by its temperature preference of 27.5°C, which is considerably higher than either *P. gracilis* or *P. velata*. On the other hand, *D. dorotocephala* occurred nearly over the entire gradient, a condition not shown by either of the other species. These results differ somewhat from those of Mast (1903), who found that *D. dorotocephala* aggregated in the range of 17 to 26°C. This species has been considered as one of the most widely distributed planarians in the U.S. and has been found in water with wide temperature variation, *e.g.*, Darlington and Chandler (1979) reported a range of 11 to 29°C in Arkansas. Based on the present study, *D. dorotocephala* is definitely the most euryecious as regards substrate, and the most eurythermal.

Apparently, *P. gracilis* preferred either rocks or moss to other substrates, since a highly significant difference was shown between these two types as compared to

leaves, sand, and silt (Table 2). At the collection site for this study *P. gracilis* was found on many types of substrates but most abundantly on rocks and in moss. Furthermore, it often inhabits headwaters of streams where eroding substrates prevail.

That *P. gracilis* mainly occupied a range of 4.5 to 22°C and had temperature preferences of 12.6°C and 14.8°C indicates it prefers comparatively cooler waters than *D. dorotocephala.* Chandler (1966) found *P. gracilis* at the headwaters of an Indiana stream which had year round cool temperatures and showed little annual variation, whereas *D. dorotocephala* occurred only downstream at warmer and less stable temperatures. Darlington and Chandler (1979) found some *P. gracilis* in Arkansas at 28°C, perhaps near the maximum temperature for this species. In previous temperature gradient experiments of other workers, *P. gracilis* aggregated at 0 to 10°C (Mast, 1903) and 0 to 9.5°C (Eddy and Gleim, 1932).

Phagocata velata exhibited the most clear cut preference for a particular substrate and the narrowest temperature range of the three species. Results indicate a highly significant preference (0.01 level) for rocks (Table 2), which is not in keeping with the observation that moss and silt were much more abundant than rocks at our collection site for *P. velata.*

In the temperature tests, *P. velata* preferred a range of 16 to 20°C. Although its temperature preference of 17.8°C is higher than *P. gracilis, P. velata* seems to be the most stenothermal of the three species. Field observations indicate that *P. velata* is often found in waters with small annual temperature fluctuation (Kenk, 1944; Darlington and Chandler, 1972; Kenk, 1974).

ACKNOWLEDGMENTS

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TRANSPLANTABLE LEUKEMIA IN GUINEA PIGS

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ABSTRACT

Ten examples of leukemia in control or irradiated inbred guinea pigs were tested for transplantability. Nine were transplantable with 1 failure. Six of the original leukemias were classified as lymphatic and 4 as stem cell. As has been frequently observed in transplantable tumors, the latent period from transplant to death of the recipient from leukemia shortened with increasing numbers of transplant generations in those cases where the tumor was continued as a transplant for extended periods. The anatomical resemblance of lymphatic leukemia to the chronic disease in man was readily apparent. The stem cell leukemias closely resembled human acute leukemia.

INTRODUCTION

In an earlier paper we reviewed the evidence for radiation induction of leukemia in guinea pigs (Van Pelt and Congdon, 1972). The study was based on 48 cases observed in the experiments performed by Egon Lorenz and his colleagues at the National Cancer Institute during the 1940's and 1950's. Included in the 1972 study were the unusual and rapidly growing radiation induced stem cell leukemias of bone marrow