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FEEDING PREFERENCE OF *PTERONARCYS PICTETII* (PLECOPTERA: INSECTA) FROM A SMALL, ACIDIC, WOODLAND STREAM[1]

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ABSTRACT

Laboratory experiments evaluated leaf preference, using the stonefly, *Pteronarcys pictetii* Hagen, and differentially incubated red titi leaves, *Cyrilla racemiflora* L., from naturally acidic streams in northwestern Florida. Leaves were incubated from 0-8 weeks in the field and provided as food to *P. pictetii* nymphs in the laboratory. Incubated leaves were strongly preferred over non-incubated leaves, with a tendency for increased preference with increased incubation length. This tendency paralleled a decrease in leaf ash-free dry weight, an increase in fungal colonization of the leaves, and an increase in leaf protein through microbial colonization. Plate counts revealed low numbers of bacteria on leaf surfaces throughout the study. These findings generally agree with those from similar studies in aquatic systems of higher pH.

RESUMEN

Experimentos de laboratorio evaluaron la preferencia de hojas por el "stonefly", *Pteronarcys pictetii* Hagen, de hojas de *Cyrilla racemiflora* L., diferencialmente incubadas, que ocurren naturalmente en arroyos ácidos en el noroeste de la Florida. Las hojas fueron incubadas de 0-8 semanas en el campo y dadas de comida a las ninfas de *P. pictetii* en el laboratorio. Hojas incubadas fueron mucho más preferidas que las que no fueron incubadas, con una tendencia de aumento de preferencia con el aumento del período de incubación. Esta tendencia es paralela a una reducción en el peso seco sin ceniza de la hoja, a un aumento de colonización por los hongos de las hojas, y a un aumento de proteina en la hoja a causa de la colonización microbial. Estos resultados están generalmente de acuerdo con estudios similares en sistemas acuáticos de más alto pH.

The importance of energy input into lotic systems by allochthonous materials, the majority of which is abscised leaves, has been well documented (Nelson and Scott 1962, Minshall 1967, Kaushik and Hynes 1971, Cummins 1974). Once these leaves have entered an aquatic system, they are degraded by the interaction of a number of biotic and abiotic factors.

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One of the most important steps in the degradation of leaf litter is the feeding process of shredders (Petersen and Cummins 1974). These aquatic macroinvertebrates use detrital food particles greater than 1 mm in size, feeding predominantly on large leaf particles by chewing or scraping (Cummins 1973). This process produces smaller particles which, in turn, can be further used by fungi and bacteria, as well as by collectors, those macroinvertebrate consumers which use food particles less than 1 mm in size (McDiffet 1970, Cummins 1973, Cummins et al. 1973, Short and Maslin 1977).

In recent years, several studies have explored the feeding behavior of shredders. These experiments have strongly suggested that shredders are actually feeding on the rich growth of microbial periflora, especially fungi, that colonize the allochthonous leaf material (Triska 1970, Kaushik and Hynes 1971, Barlocher and Kendrick 1973a, 1973b, 1974, Anderson and Grafius 1975). These studies have typically been food preference experiments using leaves colonized by fungi and bacteria (conditioned) and those not so colonized (non-conditioned). Shredders generally prefer conditioned material, presumably because microbial colonization augments and enhances its nutritional value (see review by Cummins and Klug 1979).

This study evaluated food preference of a shredder from streams in the northwestern region of the Florida Panhandle. These streams are characterized by a consistently low pH resulting from 2 major factors: 1) a large humic and tannic acid input from plant litter decomposition occurring on the vast floodplains common to this region; and 2) insufficient buffering due to the virtual non-existence of carbonaceous bedrock and parent materials in the soil.

Most of the previous food preference experiments were conducted in more northern regions where streams are well buffered and of near-neutral pH. In such systems, artificially lowered pH negatively affects the decompositional activities of microbes on coarse organic detritus (see review by Hendrey et al. 1976). Thus, studies in naturally acidic streams could allow important comparisons and contrasts. Such studies are especially important in the light of existing and potential acidification of aquatic communities from mine-waste, acid rain, or other processes.

MATERIALS AND METHODS

The leaves of the red titi, *Cyrilla racemiflora* L., were used in this study. In a preliminary incubation study using 5 species, *C. racemiflora* leaves showed the greatest weight loss in a 6 week period (Moshiri et al., unpublished data). *Cyrilla racemiflora* leaves lack a thick waxy cuticle (present to a greater extent in the other leaves studied), and thus are rapidly colonized and degraded by fungi and bacteria.

Near-abscision red titi leaves were collected, dried at 60°C for 48 h, and stored in polyethylene bags. Approximately 2.5 g of whole, similar-sized, titi leaves were selected from the bags and formed into leaf packs simulating the natural leaf packs that occur in streams. Leaf packs were then placed in incubation chambers constructed from 10.0 cm lengths of thin-walled PVC pipe, 10.0 cm diameter, sealed at both ends by 1.4 mm mesh fiberglass screening. These chambers allowed for microbial colonization and water flow, but excluded larger aquatic macroinvertebrates. One leaf pack was placed into each chamber.

Leaf packs were incubated in Pond Creek, Santa Rosa County, Florida. This is a small, second order stream characteristic of those found in the northwestern region of the Florida Panhandle. Pond Creek has pH values ranging from 4.0 to 6.0. The incubation site was located 6.5 km below the creek's spring-fed headwaters, in an area relatively free from human interference. At this site, the stream was 4 m wide and 1 m deep, with an average current velocity of ca. 0.25 m/sec. The mean stream pH and temperature during the incubation period were 5.6 (range 5.4-5.9) and 21.6°C (range 20.0-23.5°C), respectively. In addition, dissolved oxygen levels averaged 9.7 ppm (range 9.0-11.0 ppm) and total alkalinity averaged 4.3 mg/l $CaCO_3$ (range 4.0-5.7 mg/l $CaCO_3$).

To differentiate between leaves of different incubation periods, all leaves used in the feeding preference experiments were color-coded on the petiole tip with fingernail polish. Beginning on 8 July 1980, 3 chambers were placed in Pond Creek per week for 8 consecutive weeks. Three chambers were leached in the stream water for 48 h prior to the start of the leaf preference experiments and were used as 0-week incubation chambers. Bricks anchored the incubation chambers to the substrate. All chambers were collected on 2 September 1980, thus providing incubation periods from 0-8 weeks. The chambers were placed in ice chests filled with stream water and transported to the laboratory for processing.

Nymphs of the stonefly *Pteronarcys pictetii* Hagen (Plecoptera: Insecta) were chosen for the preference experiments. This shredder, which inhabits Pond Creek, is particularly suitable for such studies because of its high rate of leaf utilization and relatively low mortality in laboratory cultures (personal observations). The 20 *P. pictetii* used in my study ranged from 17-22 mm in length and were collected from Canoe Creek, Escambia County, Florida, an acidic stream similar to Pond Creek. Collecting was done at Canoe Creek (located 27.5 km from the Pond Creek incubation site) due to the presence of large numbers of *P. pictetii* at this location. Nymphs were collected 2 weeks prior to the initiation of the food preference experiments and maintained in an aerated aquarium.

Polystyrene feeding trays (29.0 cm x 18.5 cm x 13.0 cm), each containing 3 liters of water from Pond Creek, were used for the leaf preference experiments. To maintain pH within 0.5 units of the stream water, the tray water was changed daily during the experiments with freshly-collected Pond Creek water. Air stones connected to a common air supply provided aeration to each tray. The intensity of the flourescent lighting provided was decreased by placing smoke-colored plastic sheets in between the light source and the trays. This simulated light filtration caused by the dense tree canopies and heavily stained waters of the streams of this region.

A 12 h light-12 h dark photoperiod was maintained in the laboratory during the experiment. This photoperiod was interupted briefly twice each night during the experiment when sampling necessitated lighting.

One leaf from each incubation period was wet-weighed according to the method of Iversen (1974) and randomly introduced into each feeding tray. In addition, leaves were used to determine a wet-weight:dry-weight relationship for the subsequent determination of the dry weights of all wet-weighed leaves.

One *P. pictetii* nymph was placed in each of 20 trays. Three trays received no nymphs and served as controls. The location of each nymph was then recorded every 6 h for a total of 20 observations. This time period was assumed ample for the insect to locate and to attach to the preferred leaf. After each observation, the nymphs were removed from the leaves and returned to the centers of the trays. The centers were devoid of leaves. At the end of the 20th observation, all leaves were removed and wet-weighed. Comparisons were then made with the pre-experimental leaf weights and the differences were expressed as percentage of leaf weight lost. Any weight loss differences between the control and the experimental leaves were then attributed to the feeding activities of the shredder organisms.

The general fungal succession on the leaves was determined by the plating method used by Kaushik and Hynes (1968) and Suberkropp and Klug (1976), with 3 replicates for each incubation period. Bacterial succession was followed in the same fashion with the use of PYG agar. One set of the PYG plates was acidified with concentrated HCl to the prevailing stream pH (5.6-5.7) and one non-acidified set was retained as a control (6.9-7.0), with 3 replicates for each incubation period. For both the fungi and the bacteria, one appearance on a plate was recorded as one colony-forming-unit.

To examine the possible nutritional enhancement of the leaf from microbial colonization, the amount of leaf protein present was measured for each incubation period. Protein content was estimated by Lowry's method (Lowry et al. 1951), following techniques for leaves described by Kaushik and Hynes (1968). In addition, ash-free dry weights were determined by ignition in a muffle furnace at 550°C for 2 h for subsequent conversions of all dry weights to ash-free dry weights.

Results

Of the 400 observations, nymphs were observed on leaf substrates 377 times. Friedman's test rejected randomness in the feeding process (Friedman 1937). This test considers individuals by the non-parametrical method of ranking each nymph's preference according to percent preference. When the individual nymphs' data were pooled and plotted as percentage nymphal preference (Fig. 1), the results indicated a clear preference for conditioned leaves over non-conditioned leaves (x^2, $P < 0.05$), with a generally increasing preference with increasing incubation time. An exception was a substantial increase in preference for the 5-week incubated leaves followed by a large decrease for the 6-week incubated leaves. When Page's post-hoc statistical analysis was performed on the Friedman rank sums, this tendency for increasing preference with increasing incubation length was significant ($P < 0.001$). This trend was generally supported by the percentage dry-weight loss of leaves recorded in the feeding trays during the 5-day leaf preference experiments (Pearson's correlation, $P < 0.001$) (Fig. 2).

Colony-forming-units (CFU) of fungi as determined by plate counts ranged from a minimum of 1.47×10^3 per leaf disc for the first week plating to 6.36×10^3 per disc for the 6th week plating (Fig. 3). A simple regression analysis yielded a significantly increased fungal count with increased leaf incubation ($P < 0.001$). For this regression, the 0-week data were omitted. The elevated counts from the 0-week period may have been due to a large

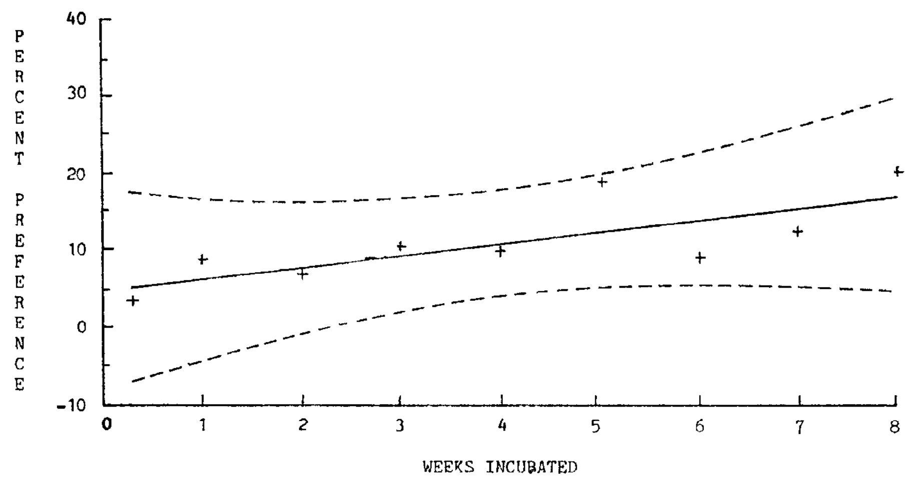

Fig. 1. Percent feeding preference as a function of incubation period for pooled data. Simple regression analysis yielded the regression line (—) and the 95% confidence bands (- -).

amount of spore fragmentation occurring during homogenizing, with each fragmentation giving rise to a CFU. This hypothesis was supported by the presence of numerous spores on the O-week leaves as determined by SEM and light microscopy. These spores were not evident on the leaves incubated 1-8 weeks and were most probably terrestrial fungal spores that were present on the leaves prior to their placement in the stream. Barlocher and Kendrick (1973a) reported that the majority of spore germinations occurring on elm and maple leaves incubated for 14 days in nutrient-enriched stream water were of terrestrial origin.

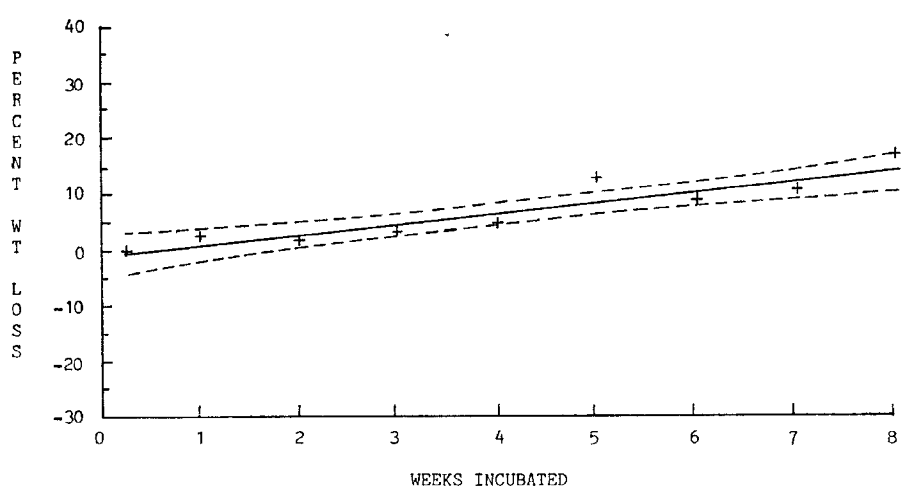

Fig. 2. Percentage dry weight loss of leaves as a function of incubation period for pooled data. Simple regression analysis yielded the regression line (—) and the 95% confidence bands (- -).

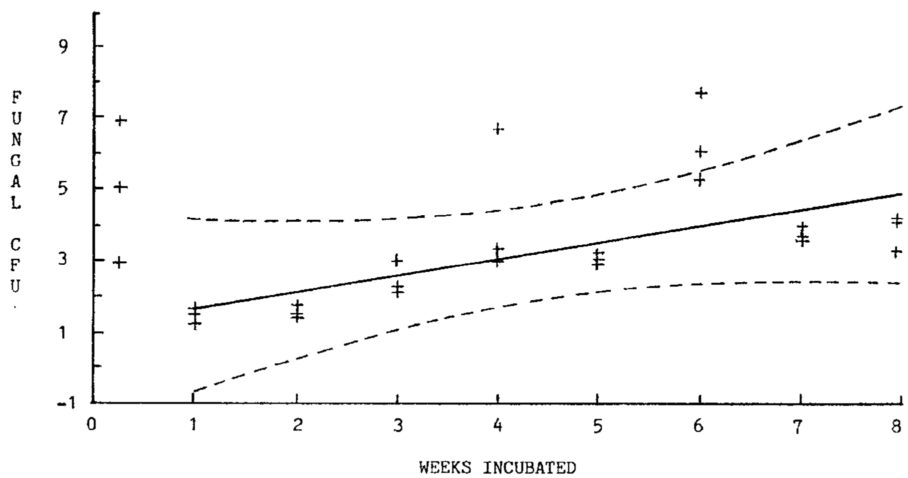

Fig. 3. Colony-forming-units (CFU) of fungi x 10^3 per leaf disc as a function of incubation period for raw data. Simple regression analysis yielded the regression line (—) and the 95% confidence bands (- -).

Bacterial CFU's were similar for the neutral and acidic media (Fig. 4). The highest reading of 13.5 x 10^4 CFU/disc occurred on the acidic medium during the first week, while the lowest reading of 2.7 x 10^4 CFU/disc occurred on the neutral medium during the second week. For both media,

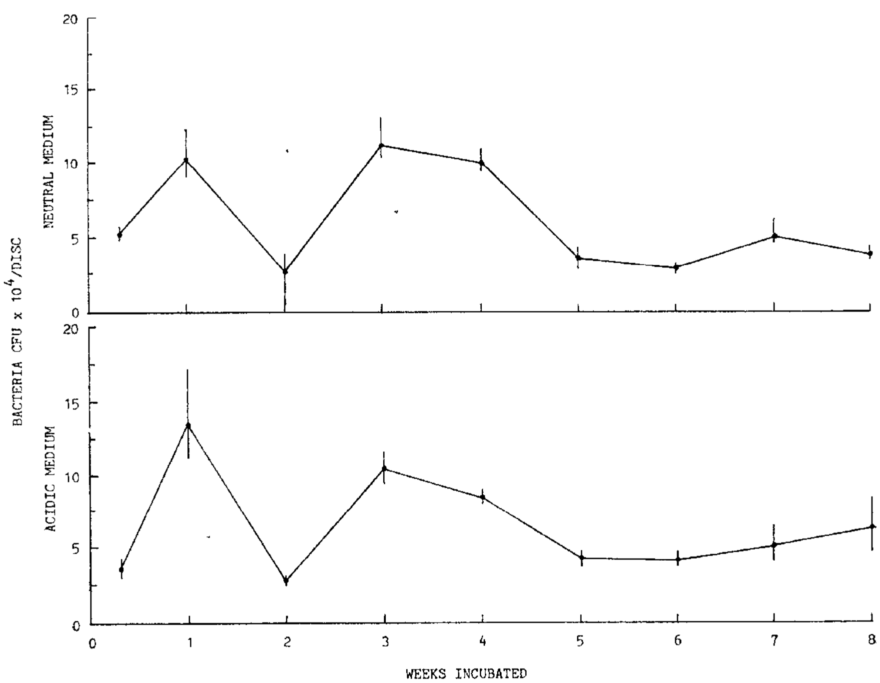

Fig. 4. Mean and range (n = 3) for the colony-forming-units (CFU) of bacteria x 10^4 per leaf disc as a function of incubation period. pH = 6.9-7.0 and 5.6-5.7 for the neutral and acidic media, respectively.

CFU's oscillated sharply from 0 to 3 weeks, decreased sharply from the 3rd to the 5th week, and then somewhat stabilized.

The percentage protein content of the leaves ranged from 1.12% for freshly picked leaves (not incubated in the stream) to 3.42% for 8-week leaves. This increase was generally linear, with a small decline in the protein content in the 6th week, coinciding with the decreased preference for the 6-week incubated leaves.

Leaf ash-free dry weight as a percentage of dry weight decreased from 95.5% for the 0-week leaves to 87.5% for the 8-week leaves, revealing a general increase in the organic content with increasing incubation length.

Discussion

These results strongly indicate that a feeding preference for incubated leaves over non-incubated leaves exists for *P. pictetii* and that this preference increases with increased incubation time. A similar pattern has also been reported in neutral streams for the trichopteran shredders *Lepidostoma quercina* (Anderson and Grafius 1975) and *Sericostoma personatum* (Iversen 1973).

The general increase in fungal CFU's with incubation time in Pond Creek agrees with data collected from neutral pH stream systems (Triska 1970, Kaushik and Hynes 1971, Suberkropp and Klug 1976). In addition, since there were similarities between the neutral and acidic media in the number of bacterial colonies observed, it is possible that the bacterial species present on the leaves do not benefit from the rise in pH (Fig. 4). This may indicate some acid-tolerance in the bacterial community. Bacterial plate counts from leaves incubated in Pond Creek were low compared with similar studies in neutral streams (Kaushik and Hynes 1971, Suberkropp and Klug 1976). However, media tend to be selective, and while the media used in this study were chosen for their general capabilities, the numbers of colonies observed may not have been representative of the true leaf microbial populations (Barlocher and Kendrick 1974, Suberkropp and Klug 1976).

Kaushik and Hynes (1968) have shown that the increase in leaf protein with increasing incubation time is due primarily to fungi. Results from my study suggest this was also the case in Pond Creek. With the steady increase of fungal biomass, there was a concurrent gradual increase in protein per unit weight. Due to the very low assimilation rates by shredders of leaf material, this increase in protein content is very important to shredders (Hargrave 1970, Barlocher and Kendrick 1974).

Leaf preference in Pond Creek by *P. pictetii* seems to be related to the fungal concentration and therefore to the protein content on incubated leaves. The existence of this typical leaf-microbe-shredder relationship in a naturally acidic environment is in contradiction to studies from other acidic streams. Those studies have examined the consequences of increased acidity on the decompositional processes in aquatic systems directly acidified through man's activities. In Swedish lakes, where decreased pH values have been caused by atmospheric fallout in the form of acid rain, coarse organic detritus has accumulated. This detrital accumulation has reportedly been accompanied by a decrease in bacterial biomass and an increase in fungal biomass (Hendrey et al. 1976).

Traaen (1976) measured birch leaf decomposition in experimental tanks over a 1 year period and found a significant decrease in weight loss of leaves in water of pH 4.3-5.6 versus water of pH 6.0-6.5. Minshall and Minshall (1978) observed a similar pattern with oak leaves in streams with values of 4.6-5.4 and 5.6-7.1. Barlocher and Kendrick (1976) reported that most bacterial species and many fungal aquatic hyphomycete species reportedly involved with leaf decomposition are inhibited by acidity.

Acidity also has been shown to have direct effects on the shredder populations. TLm^{50} tests (tests which determine the pH at which 50% of the organisms die after 96 h of exposure) with 2 stoneflies, *Pteronarcys dorsata* and *P. californica*, revealed these shredders were only moderately tolerant to acidic conditions (pH 4.25 and 4.60, respectively) (Bell and Nebecker 1969 and EPA 1973, respectively).

In Pond Creek there were no noticeable detrimental effects as a consequence of the natural acidity. Except for the probably lower bacterial biomass in the present study, the overall leaf-microbe-shredder relationship is very similar to those in more neutral systems. Thus, these naturally acidic systems offer excellent opportunity for the examination of how decomposer systems function with increased acidity. The information from such studies could aid our understanding of the problems associated with the ever-increasing acidification of aquatic systems through cultural processes (Hendrey et al. 1976, Wright et al. 1976).

Acknowledgments

This project was completed for partial fulfillment of the requirements for a Master of Science degree in biology at the University of West Florida. I wish to express my sincere gratitude to my major advisor, Dr. G. A. Moshiri, for continuous support and guidance throughout this project. I also extend my appreciation to Dr. M. A. Hood, whose help and guidance were invaluable in the area dealing with microbial interactions; to Dr. P. V. Hamilton, who kindly provided much help in the careful scrutiny of the original manuscript; and to Dr. O. E. Walton, whose aid with the revision of the manuscript was very helpful. I also extend my appreciation to Mr. T. Gaetz, of the Institute for Statistical and Mathematical Modeling of the University of West Florida, for assistance with the statistical analyses.

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