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OBSERVATIONS ON PREDACIOUS MIRIDAE

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ABSTRACT

An investigation was undertaken to determine the predatory habits of four species of Miridae associated with ornamental trees. *Deraeocoris albigulus* preyed on *Cinara* aphids; *Deraeocoris nubilis* preyed on *Cinara* aphids, gall aphids and pine needle scale; *Deraeocoris nebulosus* preyed on lacebugs; and *Pilophorus perplexus* preyed on globular scale.

The study indicated that mirids could prove to be important controls of ornamental plant pests.

INTRODUCTION

The food habits of the Miridae, or plant bugs, have not been studied extensively. Originally it was thought that mirids were largely, if not entirely, phytophagous. Saunders (1) summarized the feeding habits of mirids as "subsisting on the juices of leaves". Further investigation revealed that some species are predacious. Knight (2) stated that the majority of mirid species are plant feeders, but he also indicated that a large number of species were known to be chiefly predacious.

The importance of this fact is that certain of these predacious mirids have been utilized successfully in biological control. For example, *Cyrtorhinus mundulus* Breddin was introduced into Hawaii in 1920 to control the sugar cane leafhopper. Feeding on the leafhoppers' eggs, it quickly reduced the infestation to a non-economic level. Also. *C. fulvus* was successfully introduced into Hawaii in 1938 to control the Taro leafhopper (3).

Most of the predacious mirid species have been studied in association with field crops such as sugarcane (3), cotton (4), and alfalfa (5). Since few studies have been made on mirids, associated with ornamental plants, this study was undertaken to determine which species of mirids are predacious, in order to determine their value as biological control factors.

METHODS

Field and laboratory observations were made during the summer of 1973. The insects were collected on 25 cm x 30 cm beating trays. Mirids nymphs were reared to maturity in plastic boxes containing the host plant and prey. The boxes were then placed in a controlled temperature and humidity chamber.

RESULTS

The following is a series of observations on four species of Miridae. The data is of a qualitative nature, and is part of a preliminary attempt to determine the extent of predation in this family of insects.

Deraeocoris albigulus - Knight (6) indicates that many species of *Deraeocoris* were predacious, though their specific food habits were unknown.

Wheeler and Henry (personal communication) observed that *D. albigulus* was frequently found in the presence of heavy aphid infestations. Based on this information, both *D. albigulus* adults and nymphs were placed on their known host, Scotch pine, *Pinus sylvestris,* infested with *Cinara* aphids. Observations indicated that *D. albigulus* nymphs and adults fed readily on dead aphids.

Deraecoris nubilis Knight (2) lists the species as "probably predacious", feeding on soft-bodied insects such as aphids; in one instance, the pine bark aphid, *Chermes pinicortus* Fitch.

D. nubilis, as in the case of *D. albigulus* was also obsrved by Wheeler and Henry to be abundant in the presence of large aphid populations. *D. nubilis* nymphs were placed on Scotch pine infested with the aphid *Cinara pineus.* The nymphs attacked only injured or dead aphids by inserting their beaks and withdrawing the liquid contents from the aphid.

On Douglas fir, *Pseudotsuga menziesii, D. nubilis* nymphs were abundant on areas of the branches infested with Cooley spruce gall aphids, *Adelges cooley.* The *D. nubilis* nymphs were observed to pierce the species' egg masses and destroy the mass in five to ten minutes.

Fourth-instar nymphs were separated into two cages — one containing Douglas fir branches infested with egg masses of *A. cooleyi* and one containing non-infested Douglas fir branches. After seven to ten days most of the nymphs feeding on the aphid eggs developed to maturity. Nymphs not having access to egg masses died within a few days. This suggested that predation was necessary for survival of this species.

In locals where *D. nubilis* was collected on Scotch pine (*Pinus sylvestris*) and White pine (*Pinus strobus*) nymphs and adults were observed to be more abundant on branches infested with pine needle scale, *Phenocapsus pinifoliae,* possibly indicating a predator-prey association.

D. nebulosus Knight (6) lists *D. nebulosus* as being predacious on *Phylloxera rileyi* on oak. Morrill (7) mentions a small heteropterous nymph probably *D. nebulosus* as destroying oak tingid (lacebug) nymphs. Also, Lord (8) lists *D. nebulosus* as a minor predator of the European red mite *Panonychus ulmi* on apple.

D. nebulosus, which was observed by Wheeler and Henry to be predacious on lacebugs on deciduous trees, could prove to be an important predator species. Evidence for this is obtained from the mirid *Stethoconus praefectus* (Distant), which successfully controlled the lacebug *Stephanitis typicus* (Distant), a pest of the coconut palm in India (9).

D. nebulosus nymphs and adults were collected on Washington thorn *Craetagus phaenopynum,* which was heavily infested with the lacebug *Corythucha cydoniae.* When placed on leaves infested with the lacebugs, the nymphs and adults fed readily on the *C. cydoniae* nymphs.

D. nebulosus was also collected on trumpet creeper (*Campsis radicans*), and various species of oak, indicating a wide range of hosts.

Philophorus perplexus, Douglas and Scott-Fulton (10) found *P. perplexus* cited as *P. walshii* to be a valuable predator of aphids on apple, but also observed it feeding on San Jose scale.

P. perplexus nymphs were found on a sample of purple plum, *Prunus cerasipera,* infested with globular scale, *Licanium prunastri.* They were observed to probe and insert their beaks into the globular scale and remain there five to ten minutes as if feeding. *P. perplexus* was also seen to pierce scale crawlers. Five to six crawlers were fed on during fifteen minutes of observation.

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DISCUSSION

The significance of these observations of mirid predation is the recognition of these species as important natural biological control factors and also their possible utilization in economically applied biological control. This possibility has been virtually unexplored. The literature cites few observations of mirid predation and no literature citations were found for mirid predation on the Cooley spruce gall aphid eggs, lacebug nymphs found on oak and hawthorn, and scale crawlers.

Most of the *Deraeocoris* species seem to be chiefly predacious (2) as exemplified by *D. nubilis* associated with the Cooley spruce gall aphid. If *D. nubilis* is isolated from the aphids, death usually results, again suggesting that predation is not supplementary.

D. nebulosus has been collected on many species of deciduous trees and appeared to have as wide a host range as *D. nubilis* does on conifers. Because of this association with injurious aphids, scales and lacebugs on various hosts, both species could be important in controlling populations of hemipterous pests.

Further investigation into the predatory habits of mirids should include controlled experimentation in which statistical analysis is employed to determine the extent to which certain mirids are predacious.

ACKNOWLEDGMENTS

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THE EFFECTS OF SODIUM CYCLAMATE ON THE GROWTH OF CHLAMYDOMONAS REINHARDI (WILD TYPE)

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ABSTRACT

The growth of *Chlamydomonas reinhardi* (wild type–) was curbed corresponding to the increased amount of sodium cyclamate in the culture medium. Some inhibition of growth occurred at 0.00001% (0.1 ppm) cyclamate with increased inhibition at higher concentrations of cyclamate. Concentrations of 0.1% (1000 ppm) sodium cyclamate and above were excluded because of the osmotic effects that these concentrations imposed. The effect of cyclamate on the growth curve of *C. reinhardi* was a delayed rather than an immediate one. As cells in the logarithmic phase of growth were used as inocula for growth curves, the primary effect of cyclamate was neither the onset of a lag phase nor a decrease in logarithmic growth rate, but a decrease in total cell crop. In an effort to explain this type of growth inhibition, assays were done on both the chlorophyll content and cyclohexylamine content of cultures. (Cyclohexylamine is a known, injurious metabolite of cyclamate.)

INTRODUCTION

Viewing the vast array of literature concerning the effects of cyclamates and cyclamate metabolites on animal tissues, there are but few references concerning the effect of cyclamates on plant tissues. Various workers have shown that cyclamates induce chromosome breakage and mutation in animals (1, 2, 3, 4). There is some evidence that cyclamate has a similar effect on plant tissue (5). Others have shown cyclamate-related developmental abnormalities in animal tissues (6, 7, 8), while there is sparse investigation of cyclamate-related developmental aberrations in plants (9, 10). Since there is very little published information concerning the effect of cyclamate on microorganisms, this initial study was undertaken on the green algae, *Chlamydomonas reinhardi*.

MATERIALS AND METHODS

An auxenic culture of *C. reinhardi* (wild type–) was obtained from Carolina Biological Supply Company (11). Sager's Medium I (Table 1) was prepared according to Sager and Granick (12). Ferric chloride was added separately just before autoclaving the medium. Controls contained no cyclamate. The initial pH of the medium as measured with pH/mV Electrometer was 6.8 to 7.0.

Calculations for sodium cyclamate concentrations (13) were routinely done in percentage. Cyclamate being relatively heat and pressure stable, a measured amount of cyclamate stock solution was added directly to each flask of medium before autoclaving.

Cultures in triplicates were grown in 100 ml of medium swirled in 250 ml, wide-mouth Erlenmeyer flasks by a shaking table. The optimum rotation rate of 70 cycles/minute was employed.

Cultures were constantly illuminated by four 48" G.E. cool-white F400W lights immediately above the flasks. Air temperature in the controlled environment room was held constant at 25°C.

Carbon dioxide levels in the growth chamber were elevated to a maximum of 0.15% CO_2 (or 5 times that found in ambient air) by slowly releasing hydrochloric acid under the surface of a $CaCO_3$ slurry.

Cell populations were counted directly in a Neubauer haemocytometer. Cell populations were also followed using optical density (O.D.) readings of the culture. Optical density readings were made at 750 mμ using a Bausch and Lomb Spectronic 20 fitted with a red filter and a CEA–30 long wavelength phototube. Direct counts were usually taken at the time of each O.D. reading (Figure 1). The accuracy of direct counts was improved by immobilizing these motile organisms with a small amount of carbon tetrachloride. One drop of carbon tetrachloride was added to each 3 ml aliquot of culture immediately after O.D. reading and just prior to haemocytometer count. This low level of carbon tetrachloride immobilizes the organisms without undesirable side effects, such as clumping.

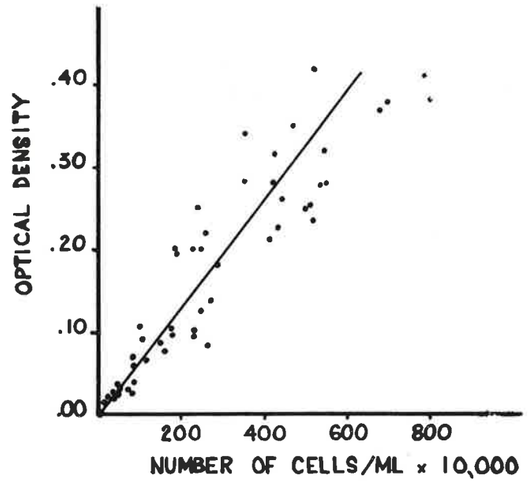

FIGURE 1. Linear relationship between optical density and cell concentration.

Chlorophyll determinations were made by first counting cells of a given algal culture. A specific volume of said culture was centrifuged at 4000 rpm for 5 minutes. The supernatent was poured off and the algal pellet was quantitatively transferred to a Potter-Elvehjem homogenizer using a total volume of 2 ml of an 80% aqueous ethanol solution. The suspension was homogenized for two minutes. Cellular debris (tan in color) was separated from the green supernatent by centrifugation. One ml of the supernatent was placed in a cuvette and brought to 5 ml volume with acetone. Using a Spectronic 20 equipped with a red filter and CEA-30 phototube, optical density readings were recorded at 645 mμ and 663 mμ with 80% acetone as the reference solution. Incorporating the absorption coefficients of chlorophylls a and b (14) and the calculations based on the absorption coefficients (15), the amounts of chlorophyll a plus b were recorded as μg/million cells.

Thin-layer chromatographic procedures (16) were used to determine a possible conversion of sodium cyclamate to cyclohexylamine. Prepared silica gel strips (17) of 500μ thickness demonstrated a minimum sensitivity of 1 μg cyclohexylamine when chromatogramed in ethyl acetate: 80% formic acid: water (4:1:2) and visualized by heating the chromatogram to 80°C after spraying with 0.5% ninhydrin in butanol. Chromatograms run in duplicate were visualized by iodine vapor as well as the ninhydrin method. Initially, chromatograms were spotted with 20 μl aliquots directly from growth experiments. Later, many mature cyclamate-grown cultures were pooled and centrifuged. The supernatent was brought to pH 11.5 with 1N NaOH and extracted with chloroform ($CHCl_3$). Concentrated $CHCl_3$ extracts were chromatogramed.

Statistical methods such as analysis of variance, correlation and Student's *t*-test (29, 30) were employed at appropriate places to determine statistical relationships.

TABLE 1

Growth Medium used for C. reinhardi

Sager's Medium I		*Trace Metal Solution**	
	g/l		*mg/l*
K_2HPO_4	0.1	H_3BO_4	100
KH_2PO_4	0.1	$ZnSO_4 \cdot 7H_2O$	100
NH_4NO_3	0.3	$MnSO_4 \cdot 4H_2O$	40
$MgSO_4 \cdot 7H_2O$	0.3	$COCl_2 \cdot 6H_2O$	20
$CaCl_2$	0.04	$Na_2MoO_4 \cdot 2H_2O$	20
$FeCl_3 \cdot 6H_2O$	0.01	$CuSO_4$	4
Sodium Citrate$\cdot 2H_2O$	0.5		

* 10 ml of trace metal solution per liter was used.

RESULTS AND DISCUSSION

The results of growth experiments showed inhibition of population growth for *C. reinhardi* corresponding to the amount of cyclamate present in the growth medium. Analysis of variance study on the means of cell counts from the control and the treated groups revealed significant differences ($F = 7.7$, $F_{05} = 6.59$; $p < .05$). The use of comparative amounts of NaCl in growth cultures indicated osmotic effects at concentrations of 0.1% and above. Therefore, data for concentrations of cyclamate above 0.01% were discarded.

Optimum growth conditions were carefully controlled throughout each of the last three experiments (Figure 2 & Table 2). Illumination was constant and adequate, and CO_2 generation continued throughout the experiments. Also, gas exchange was facilitated by continually swirling cultures at the optimum rate, and growth temperature was held constant. However, the growth conditions of the first four experiments were suboptimal (19) in that (a) illumination was below 500 foot-candles, and (b) the CO_2 levels of ambient air were not augmented (Figure 3 and Table 3).

At first, the linear relationship between cell numbers and corresponding optical densities (O.D.) at 750 mμ was established (Figure 1). Using 19 random samples, the calculated correlation coefficient (r) value was +.97. This indicated a strong relationship, that is, an increase in cell number was closely associated with an increase in optical density. To determine whether the obtained r value was significantly different from zero, Student's *t* test was employed (30). The test indicated a significant difference ($p << .001$) and thus, a positive correlation between cell numbers and corresponding optical densities was assumed. Turbidity measurements of the cultures at 750 mμ offset the possibility of measuring growth as a function of chlorophyll concentration (12). A direct relationship did exist within the range of 2×10^5 to 5×10^6 cells/ml. This compares favorably with the results of Sager and Granick (12).

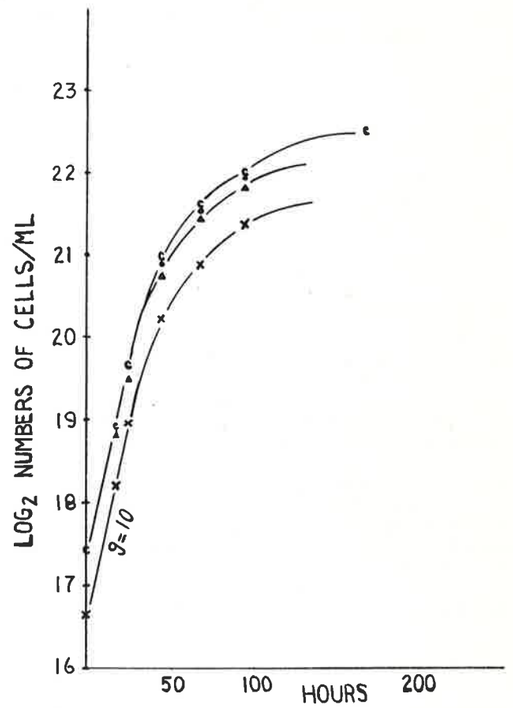

FIGURE 2. Effect of sodium cyclamate on the growth of *C. reinhardi*. (c) control; (0) 0.00001% cyclamate; (Δ) 0.001% cyclamate; and (x) 0.01% cyclamate. (For clarity, data points that nearly coincide do not appear on the Figure.)

Results are finally presented as logarithms to the base 2 of the cell concentration (Figs. 2 and 3; Tables 2 and 3). The growth of *C. reinhardi* was essentially a process of doubling, which concurs with the growth of microbes in general (12). The population growth plotted in this manner facilitated interpretations (18). In addition, generation times were determined directly from the Figures 2 and 3.

Figure 2 demonstrates the growth curves of a typical experiment. Very small amounts of sodium cyclamate curbed the total cell crop, as a result a series of successive curves could be drawn below the control curve depending on the cyclamate concentration of the medium. For reasons of clarity the curve corresponding to the 0.00001% cyclamate is not drawn in Figure 2. However, the curves for 0.001% and 0.01% cyclamate are shown. Table 2 shows more clearly the relationship of cyclamate concentration with cell concentration and O.D., that is, cell numbers decrease as cyclamate concentrations increase. The data of Table 2 correspond to the data of Figure 2 at 43 hours of population growth. Suboptimal growth conditions (19) emphasize the inhibition of cyclamate on *C. reinhardi* (Figure 3 and Table 3). An increase of generation time of the organism from 10 to 25 hours was noted during suboptimal growth conditions, however, the general shape of the growth curves was similar.

Both optimal and suboptimal growth conditions produced growth curves that clearly showed the presence of cyclamate depressed the total cell crop as populations reached the stationary phase of growth. Also, both optimal and suboptimal growth conditions produced growth curves that showed the presence of cyclamate did *not* induce a lag phase or otherwise interfered with the logarithmic rate of population growth. Generation times of the logarithmic phase of growth did not vary with the cyclamate concentration, but were similar to the control situation. The growth responses, then, suggested

TABLE 2

*The Effect of Sodium Cyclamate on the Growth of C. reinhardi Populations**

Regime	Number of cells/ml x ($1x10^4$)	Log_2 Number of cells/ml	O.D.
Control (no cyclamate)	200	20.96	0.132
0.00001% cyclamate	185	20.82	0.121
0.001% cyclamate	174	20.75	0.116
0.01% cyclamate	120	20.20	0.078

* Optimum growth conditions prevail as described in Methods. These data correspond to Figure 2 after 43 hours of growth (or 4 generations).

that cyclamate did not interfere with immediately essential physiological processes, but rather processes that were gradual or latent. Therefore, a preliminary search for overall differences between control organisms (and resultant media) and experimental organisms (and resultant media) was carried out. Detection of a difference in chlorophyll content (19) and change in chloroplast structure (20) was undertaken to possibly explain these results. In addition, the possible breakdown of cyclamate into a known metabolite—cyclohexylamine, a compound linked with harmful side-effects was monitored in some growth experiments.

The chlorophyll content of *C. reinhardi* (Chl a & b) did not show significant differences between control and experimental populations. Assays for chlorophyll content were made both at the onset of and well into the stationary phases of population growth. These values did agree favorably with the extensive work of Hudock, Levine et al (21, 22). The slight difference in Chl a & b content between values obtained by Hudock et al and this work is attributed to the greater light intensity used by the former (23). An average value of 1.40 μg Chl a & b per 10^6 cells was found for control populations while the Chl a & b content of experimental cyclamate populations varied between 1.51 and 1.00 μg per 10^6 cells. The slight trend of decreased Chl a & b content in cultures containing increased amounts of cyclamate may be real or, more probably, a function of cell counting procedure. Likewise, electronmicrographs of *C. reinhardi* (—) grown under the same experimental conditions showed no difference in chloroplast structure between control organisms and those organisms grown in the presence of the lower cyclamate concentrations (20).

Studies by several investigators have shown a low level conversion of cyclamate to cyclohexylamine in man, dog, and spore-forming anaerobes (12, 24, 25, 26). This metabolite of cyclamate has been shown to produce chromosome aberrations (1, 27) as well as tumors (8) in animals.

The presence of this specific metabolite was checked in both growing and mature cultures of *C. reinhardi* by using thin-layer chromatography with a minimum sensitivity of 1μg CHA. Solvent system VI used in this study provided Rf values of 0.7 for CHA while the cumulative contents of cultures with and without cyclamate provided Rf values of 0.28 to 0.40.

Initially, chromatograms were spotted with 20 μl of growing cultures in order to detect possible conversion of cyclamate to CHA by the organisms. These results did not show a detectable conversion. In addition, controls containing cyclamate in water and cyclamate in uninoculated medium showed no endogenous conversion of cyclamate to CHA.

From the conversion rates described by other investigators (16, 24, 26), it became apparent that these initial chromatograms represented detection levels at the lower sensitivity range for CHA. Therefore, cyclamate-grown cultures were pooled, estracted, and the concentrated extracts were then chromatogramed. With 30 mg as the initial amount of cyclamate in the pooled cultures and with the conversion rates described for other organisms, the expected CHA output would range from 250 to 200 μg —an overwhelmingly detectable amount.

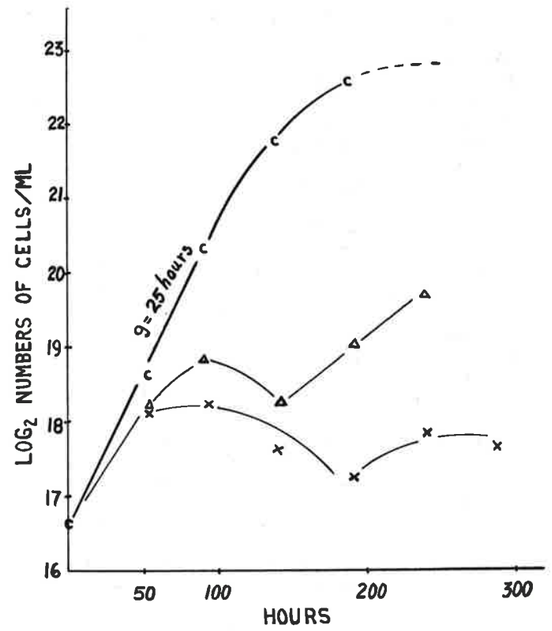

FIGURE 3. Effect of sodium cyclamate on the growth of *C. reinhardi*. (c) control; (Δ) 0.001% cyclamate; and (x) 0.01% cyclamate. (Growth conditions are not optimum [19]).

TABLE 3

*The Effect of Sodium Cyclamate on the Growth of C. reinhardi Populations**

Regime	Number of cells/ml x ($1x10^4$)	Log_2 Number of Cells/ml
Control (no cyclamate)	169	20.63
0.001% cyclamate	40	18.60
0.01% cyclamate	28	18.00

* Suboptimal growth conditions (19); note generation times of 25 hours for control cultures. These data correspond to cell concentrations after 100 hours of growth (4 generations).

As controls, both a dilute CHA solution and a cyclamate solution were taken through the extraction procedure. In each case, all of the concentrated extract was chromatogramed. There was no endogenous CHA recovered from this treatment of 10 mg cyclamate. Both 8μg of untreated CHA and an extracted solution containing 8μg of CHA produced purple-blue spots at the appropriate Rf value. On the other hand, extracted cultures did not produce a corresponding spot for CHA. It is, therefore, unlikely that CHA plays a role in curbing population growth of *C. reinhardi*. This does not exclude, however, low level conversion of cylcamate to CHA which can be further oxidized to some other compounds, undetectable using this assay procedure (28). Procedures to check such a reaction sequence were not carried out as part of this work.

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THIN LAYER CHROMATOGRAPHIC ANALYSES OF NEUTRAL LIPIDS OF UNIONICOLA CAMPELOMAICOLA (HYDRACARINA), A SYMBIONT OF CAMPELOMA DECISUM (GASTROPODA)

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ABSTRACT

Thin layer chromatographic analyses were made on neutral lipids of *Unionicola campelomaicola* (Hydracarina), a symbiont of *Campeloma decisum* (Gastropoda). The major neutral lipid fraction detected in this mite was triglyceride. Glyceryl ethers were detected in the triglyceride fraction. Lesser amounts of free fatty acids, cholesterol esters, and free sterols were detected as neutral lipid constituents of mite tissue. Argentation-TLC revealed that the major free sterol had a chromatographic mobility identical with cholesterol. Neutral lipid fractions of *U. campelomaicola* were compared with those reported for *Leucochloridiomorpha constantiae* (Trematoda) metacercariae, another symbiont of *C. decisum.*

INTRODUCTION

Water mites (Hydracarina) in the genus *Unionicola* are symbionts of fresh water gastropods and bivalves (1), and the nature of the symbiosis is obscure. During studies on *Leucochloridiomorpha constantiae* (Trematoda) metacercariae from *Campeloma decisum* snails (2), we observed *Unionicola* sp. in snail tissue. Recently, (3) we reported observations on neutral lipid fractions in *L. constantiae* metacercariae. To compare our trematode data with another organism associated with *C. decisum,* we initiated lipid studies on *Unionicola* sp. Based on descriptions in Marshall (4), we have tentatively identified the mite from *C. decisum* as *Unionicola campelomaicola.*

Von Brand (5) has reviewed the scant literature on lipids in parasitic arthropods. From his review it is apparent that there are no studies on lipids of mite symbionts of molluscs. The purpose of this study is to report our obsevations on neutral lipid fractions of *U. campelomaicola.*

MATERIALS AND METHODS

Female mites, approximately 1 mm in body length, were dissected from the gill and adjacent tissue of *C. decisum* snails, collected from Jenning's Pond, Medford Lakes, New Jersey (2). Mites were washed in three changes of Locke's solution prior to use, and neutral lipids from the mites were subsequently analyzed by thin layer chromatography (TLC) as described in this paper.

Solvents used for TLC analysis were of analytical grade purity, and glassware was washed prior to use in chloroform/methanol (2/1). TLC analysis was performed on predeveloped (chloroform/methanol, 1/1) and dried, 20 x 20 cm silica gel sheets (Baker-flex IB2, J.T. Baker Chemical Co., Phillipsburg, N.J.). Mites were placed 10 per Pyrex test tube, and mite lipids were extracted with 2 ml chloroform/methanol (2/1) according to Folch et al. (6). This extraction procedure was repeated four times, and the combined extracts were placed in a shell vial. The sample was dried under nitrogen and stored at −20°C. Immediately prior to TLC, the sample was thawed and reconstituted with 100 μl of chloroform/methanol (2/1). Various amounts of sample, along with lipid standards were spotted or streaked 2.5 from the lower edge of the plate using calibrated, disposable micropipettes (Microcaps, A.H. Thomas Co., Philadelphia, Pa.).

Chromatograms were developed in duplicate in a glass rectangular tank (Chromaflex, Kontes Glass Co., Vineland, N.J.) which was saturated with 100 ml of solvent prior to development. Unless otherwise stated, neutral lipids were visualized by spraying or dipping the chromatograms in 5% phosphomolybdic acid (PMA) in ethanol. Such chromatograms when heated at 110°C for 5 to 20 min showed neutral lipids as purple spots on a yellow background.

To detect neutral lipids, samples were developed in petroleum ether/diethyl ether/acetic acid (80/20/1) according to Mangold (7), along with a neutral lipid standard (18-4A, Nu-Chek-Prep, Inc., Elysian, Minn.) containing equal amounts of cholesterol, cholesterol oleate, triolein, oleic acid and methyl oleate. Since this solvent system does not separate all commonly occurring neutral lipids, additional samples were chromatographed in the dual solvent system of Skipski et al. (8) composed of isopropyl ether/acetic acid (96/4) followed by a second development in the same direction using petroleum ether/diethyl ether/acetic acid (90/10/1). Lipid standards in addition to 18-4A spotted along with samples in the second solvent system were as follows: 18-1A (Nu-Chek-Prep, Inc., Elysian, Minn.) containing equal amounts of monolein, diolein, triolein and methyl oleate; a 1:1 cholesterol-cholestane standard (Supelco, Inc., Bellefonte, Pa.); and TLC-A (Supelco, Inc., Bellefonte, Pa.) containing equal amounts of cholesterol, phosphatidyl ethanolamine, lecithin and lysolecithin.

To determine the presence of glyceryl ethers, the triglyceride fraction was isolated preparatively from some samples and rechromatographed along with lard (lard cochromatographs only with triolein in the solvent system used) in hexane/diethyl ether (95/5) according to Snyder (9). Free esterols were occasionally isolated preparatively and rechromatographed along with a cholesterol standard (Medical Research Council, Steroid Reference Collection, London, England) on sheets impregnated with 5% Ag NO_3 according to Morris (10). These sheets were developed in chloroform/acetone (95/5) according to Ditullio et al. (11) and sterols were visualized by charring with 50% H_2SO_4 (7).

RESULTS

The solvent system of Mangold (7) detected triglycerides as the major neutral lipid fraction in *U. campelomaicola* (Fig. 1). Lesser amounts of free sterols, cholesterol esters and free fatty acids were also detected. A distinct band migrated with the solvent front and was identified tentatively a a hydrocarbon fraction.

The Skipski et al. (8) procedure confirmed the presence of triglycerides, free sterols, cholesterol esters and free fatty acids in *U. campelomaicola* (Fig. 2). The cholestane standard cochromatographed with the least polar lipid detected in the mite, indicating the presence of the hydrocarbon fraction mentioned previously. A large phospholipid fraction remained at the origin. This spot cochromatographed with phosphatidyl ethanolamine, lecithin and lysolecithin contained in the TLC-A standard. Mono- and diglycerides were not detected with certainty following analysis according to Skipski et al. (8). A trace substance (X_2) had a chromatographic mobility slightly greater than the monoglyceride standard, monolein. A trace substance (X_3) with a chromatographic

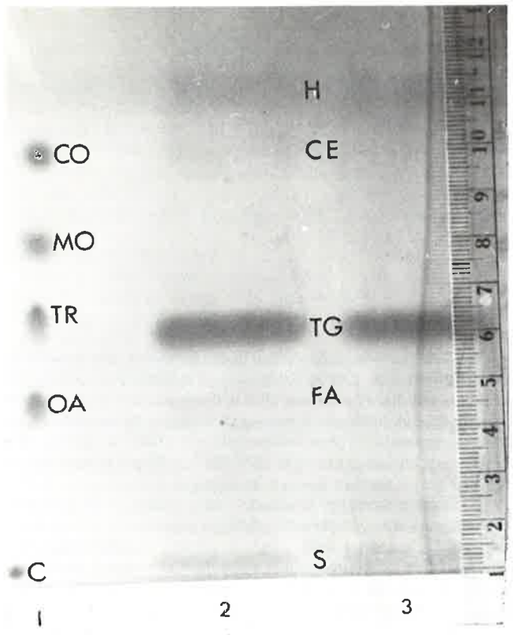

FIGURE 1. Photograph of a chromatogram showing neutral lipid fractions in *Unionicola campelomaicola.* The silica gel sheet was developed 13 cm from the origin in petroleum ether/diethyl ether/acetic acid (80/20/1) and neutral lipids were visualized by dipping the sheet in PMA to 1 cm from the origin. Lane 1 contains 1 μg of neutral lipid standard 18-4A (see text), whereas Lanes 2 and 3 each contain 50 μl of reconstituted sample. Abbreviations: C = cholesterol; OA = oleic acid; TR = triolein; MO = methyl oleate; CO = cholesterol oleate; S = free sterols; FA = free fatty acids; TG = triglycerides; CE = sterol esters; H = hydrocarbons.

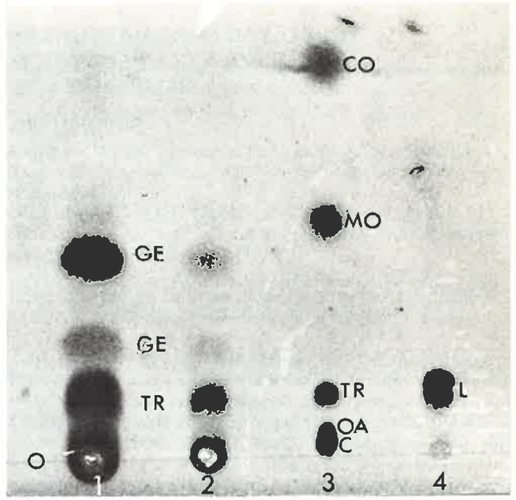

FIGURE 3. Photograph of a chromatogram showing glyceryl ethers of *U. campelomaicola.* The mite triglyceride fraction was isolated preparatively and rechromatographed in hexane/diethyl ether (95/5). The sheet was developed 13 cm from the origin (O), and neutral lipids were visualized by spraying the sheet with PMA. Lanes 1 and 2 represent 30 and 15 μl of reconstituted mite triglycerides, respectively. Lane 3 represents 1 μg of neutral lipid standard 18-4A (see text), whereas Lane 4 contains 10 μg of lard. Abbreviations: TR in Lanes 1 and 2 represent mite triglycerides which cochromatograph with TR in Lane 3 which is triolein; L = lard which cochromatographs with triolein; GE = glyceryl ethers; MO = methyl oleate; CO = cholesterol oleate.

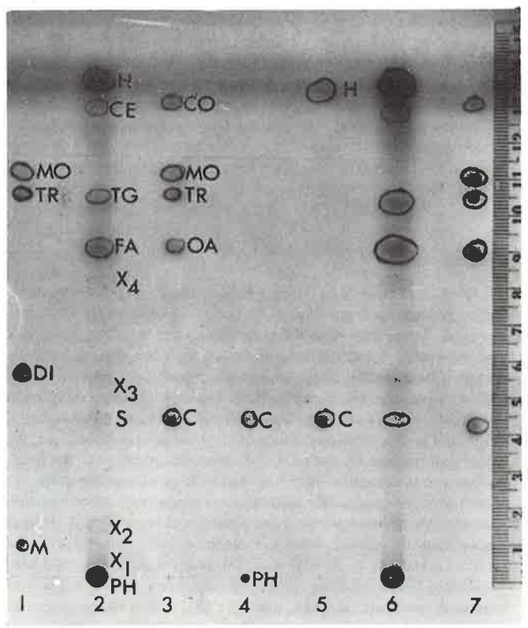

FIGURE 2. Photograph of a chromatogram showing lipids in *U. campelomaicola* following development in a dual solvent system. The silical gel sheet was first developed 14 cm from the bottom of the sheet in isopropyl ether/acetic acid (96/4) followed by a second development in the same direction 19 cm from the bottom of the sheet in petroleum ether/diethyl ether/acetic acid (90/10/1). Lipids were visualized by spraying the sheet with PMA. Lanes 1, 3, 4, 5 and 7 each contain 5 μg of various lipid standards (see text), whereas Lanes 2 and 6 contain 10 and 20 μl of reconstituted sample, respectively. Abbreviations: M = monolein; DI = diolein; TR = triolein; MO = methyl oleate; C = cholesterol; OA = oleic acid; CO = cholesterol oleate; H = cholestane in Lane 5 and hydrocarbon fraction in Lane 2; PH = phosphatidyl ethanolamine, lecithin, and lysolecithin in Lane 4 and phospholipid fraction in Lane 2; X_1, X_2, X_3, X_4 = unknowns; S = free sterols; FA = free fatty acids; TG = triglycerides; CE = cholesterol esters.

mobility slightly less than the diglyceride standard, diolein, was detected. An unidentified pigment (X_1) with a mobility slightly greater than phospholipid was observed. Attempts to identify this substance based on pigment analysis chromatography in Strain and Sherma (12) revealed that the material was neither carotene or xanthophyll. Trace amounts of an additional unknown (X_4) with a chromatographic mobility less than free fatty acids was also detected.

Analysis of the triglyceride fraction according to Snyder (9) indicated the presence of glyceryl ethers (Fig. 3). By comparison with chromatograms in Snyder (9), spots located between triolein and methyl oleate were considered glyceryl ethers.

Argentation-chromatography according to Morris (10) and Ditullio et al. (11) revealed that the major sterol fraction present in the mite was cholesterol.

DISCUSSION

In accord with previous observations on neutral lipids in arthropods (13), the major neutral lipid fraction in *U. campelomaicola* is triglyceride. In this mite, glyceryl ethers are contained in the triglyceride fraction. Further studies are needed to determine the distribution and significance of glyceryl ethers in animal tissue (14).

In a study on *L. constantiae* metacercariae from *C. decisum* snails, free sterol was detected as the major neutral lipid fraction, and only trace amounts of cholesterol esters, triglycerides and free fatty acids were found (3). In the present study, triglycerides were detected as the major neutral lipid of *U. campelomaicola,* along with lesser amounts of free fatty acids, cholesterol esters and free sterol. Although both organisms are symbionts of *C. decisum,* the neutral lipid profiles of each are decidedly different. The significance of these differences is obscure at this time.

ACKNOWLEDGEMENTS

This work was supported, in part, by funds from the Committee on Advanced Study and Research, Lafayette College.

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TERATOLOGIC EVALUATION OF HEXACHLOROPHENE IN MICE

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ABSTRACT

Hexachlorophene was administered subcutaneously to pregnant STS mice on days 3-8, 7-12, or 11-16 of gestation at levels of 0 (DMSO control), 12.5 and 25 mg/kg/day. The females were sacrificed on the 19th day and the fetuses were examined for viability and external abnormalities. Implantations and resorbtions were recorded. All the concentrations of the chemical were found to increase fetal resorbtion and mortality. Examinations of fetuses from the control groups and the experimental groups failed to reveal any teratogenic effects of hexachlorophene.

INTRODUCTION

Hexachlorophene (HCP) has been used as an antibacterial additive to soaps, shampoos, deodorants, and other cosmetics (1). It has also been occasionally used as a broad-spectrum fungicide and bactericide on food crops (2).

The deaths of 35 apparently healthy newborn French babies in 1972, were linked to the babies being accidentally exposed to heavy doses of talcum powder containing 6% HCP (3). Wear *et al* (4) reported the accidental ingestion by ten hospital patients of an emulsion of 3% hexachlorophene, which was mistaken for Milk of Magnesia. Lustig (5) reported the fatal case of a six year old boy who ingested four or five ounces of a hexachlorophene emulsion which contained approximately 250 mg of hexachlorophene per kg of body weight.

Several studies were undertaken in 1971 to determine the possible health hazards of continued hexachlorophene use. In one study HCP was fed to rats (6). After two weeks, the rats developed leg weaknesses which progressed in several weeks to paralysis. The brains of these HCP fed rats contained excess fluid and were heavier than those in the control group. Studies using light and electron microscopes detected histological changes and damages in the brain cells of rats.

In the second study fifty newborn human babies were washed daily in an HCP containing solution. This study revealed that the babies had absorbed HCP into the blood through normal, unbroken skin, and the elevated HCP levels were close to those that were toxic in animals (7, 8). In another investigation newborn monkeys were bathed daily for five minutes in a 3% HCP solution, in a fashion that simulated the bathing of babies in hospitals. Histological abnormalities in the monkeys' brain cells were detected (9).

These findings thus questioned the safety of HCP and suggested that a regulatory measure ought to be taken to control the massive exposure of the public to HCP. On September 22, 1972, the FDA recognizing the HCP's dangerous effects ordered to limit the indiscriminate use of this chemical (9).

HCP is synthesized from the herbicide 2, 4, 5-T which is a known teratogenic, feticidal and mutagenic agent (10, 11). However, no information is available on the teratogenic potential of hexachlorophene. Therefore, this study was undertaken to evaluate the teratogenic effects of HCP in mice.

MATERIALS AND METHODS

Hexachlorophene, 2, 2'-methylene bis (3, 4, 6-trichlorophenol) was administered subcutaneously as a solution in dimethyl sulfoxide (DMSO).

An inbred laboratory strain white mouse (STS) was used. The mice were fed a diet consisting of Purina Laboratory Chow and tap water given ad libitum. Groups of two young females and one male were placed in cages and allowed to breed. The females were checked each morning and each afternoon for the presence of a vaginal plug, representing the congealed contents of the seminal vesicles. The day the plug was observed was considered to be day 1. These impregnated females were divided into three groups, depending on what days the hexachlorophene was to be administered. All mice received the drug subcutaneously once a day for six consecutive days. Those in Group 1 received HCP from day 3 to day 8 of gestation; those in Group 2 received HCP from day 7 to day 12 of gestation; and those in Group 3 received HCP from day 11 to day 16 of gestation. Each group was then subdivided as to the concentration of hexachlorophene received. The concentraitons were: 0, 12.5, and 25 mg of HCP/kg of body weight. At the zero concentration (control) only DMSO was injected. A "no treatment" control group of mice was also studied and the results produced no significant differences when compared to the DMSO control. The experiment was continued until results were obtained for twenty mice per concentration, per group. The concentration of HCP at 50 mg/kg level produced 70-90% maternal lethality. Shortly after the administration of the chemical, the mice suffered from paralysis of the hind legs. A day or two after paralysis was observed, the mice were found dead. The maternal lethal effect prevented us from the compiling of any significant amount of data to be incorporated in this study.

The females were sacrificed on the nineteenth day of pregnancy, with the number of implants, the number of live, and the number of resorbed and dead fetuses noted (Figs. 1, 2, 3). An implant was considered to be any site on the uterus where a scar was observed which was identified using the method developed by Soares (12). Lastly, fetuses were checked for visible abnormalities.

The data were analyzed for statistical comparisons using the Student's "t" test.

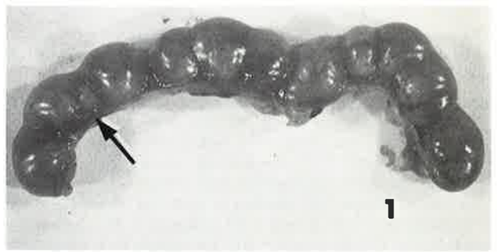

FIGURE 1. A typical control uterus showing a resorbed fetus (arrow).

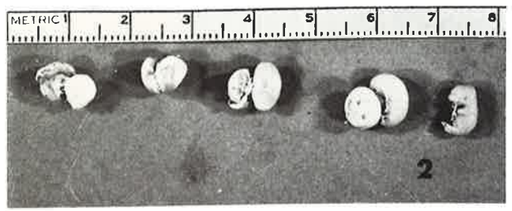

FIGURE 2. Resorbed and dead fetuses obtained from 25 mg/kg of HCP treated mouse, (7 — 12 day group).

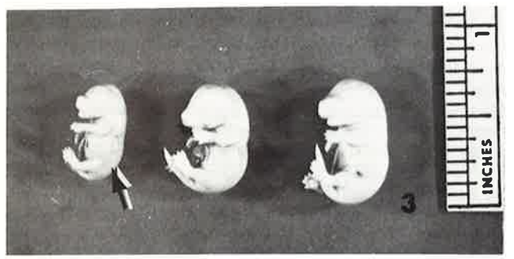

FIGURE 3. Dead (arrow) and live fetuses removed from a pregnant mouse injected with 25 mg/kg of HCP, (11 — 16 day group).

RESULTS AND DISCUSSION

The numbers of implants, live fetuses, and resorbed and dead fetuses, for each gestation period are shown in Tables 1, 2, and 3.The standard error of the mean, as well as percentages are included. Where statistical significance has been observed is noted.

Group 1: Mice injected during the 3rd-8th day of pregnancy (Table 1):

The mean number of implants for each dose level was relatively constant, ranging from 12.6±.51 at 12.5 mg/kg to 13.1±.59 at 25 mg/kg. However, both the mean number and percentage of live fetuses showed a definite trend; the largest values occurred at the zero concentration level (DMSO), and the values gradually decreased as the concentrations increased. Comparing the dose 12.5 mg/kg with the control, produced a statistical significance with $P < .05$. A comparison of 25 mg/kg versus the control showed P to be $< .01$, indicating a high significance. A pattern can also be seen when the resorbed and dead fetuses group is examined. In this case, the greatest value for the mean and the percentage was yielded by the 25 mg/kg concentration, and the lowest value by the DMSO control. The concentrations 12.5 and 25 mg/kg produced significant results at $P < .05$ and $P < .01$ respectively.

GROUP 2:

Mice injected during the 7th-12th day of gestation (Table 2):

The results in Group 2 are very similar to those observed in Group 1. The average number of implants was relatively constant and there was no significant difference among the concentrations used. With the number of live fetuses, the greatest number and percentage were obtained at the control level, decreased as the concentrations increased. Once again, when comparing 12.5 versus the control, there was a significant difference ($P < .05$) and 25 versus the control produced a highly significant result ($P < .01$). The results obtained for resorbed and dead feutses were similar to Group 1, i.e., mortality increased with increased concentrations. However, at both the 12.5 and 25 mg/kg dose levels, the differences were highly significant when compared to the control.

GROUP 3:

Mice injected during the 11th-16th day of pregnancy (Table 3):

Again, the numbers of implants were rather constant with no statistical difference. The mean number of live fetuses gave more or less the same trend as the previous two groups, although there was no significant difference between the 12.5 mg/kg and the control. However, the difference obtained at 25 mg/kg was highly significant ($P < .01$). With respect to resorption and numbers of dead fetuses the trend was toward increasing fetal mortality with increasing concentrations. However, only the 25 mg/kg concentration produced a significant difference at the 0.5 level.

It should be emphasized that hexachlorophene concentrations used in this study produced no visible malformations.

These findings indicate a definite embryolethal effect of HCP. This is evident both in decreasing fetal viability and increasing fetal mortality, with increasing concentrations. A comparison among the three gestation groups showed some differences, but these were not great enough to be significant except for the numbers of resorption and dead fetuses obtained at 12.5 and 25 mg/kg during 3-8 and 11-16 gestation periods as shown in Table 4.

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TABLE 1

Effects of hexachlorophene in mice (Group 1) 3rd — 8th day of gestation.

	Implants		Live		Resorbed and dead fetuses	
Treatment	Total	Mean	Mean	%	Mean	%
DMSO	256	12.8±.50	10.1±.76	78.9	2.7±.50	21.1
12.5 mg HCP/ kg body wt.	252	12.6±.51	7.9±.77*	62.7	4.7±.66*	37.3
25.0 mg HCP/ kg body wt.	260	13.1±.59	7.1±.82**	53.5	6.05±.94**	46.5

± the standard error of the mean
* $P < .05$ when compared to DMSO control
** $P < .01$ when compared to DMSO control

TABLE 2

Effects of hexachlorophene in mice (Group 2)
7th — 12th day of gestation

	Implants		Live		Resorbed and dead fetuses	
Treatment	Total	Mean	Mean	%	Mean	%
DMSO	245	12.2±.58	10.9±.60	88.6	1.4±.32	11.4
12.5 mg HCP/ kg body wt.	238	11.9±.53	8.8±.76*	73.9	3.1±.49**	26.1
25.0 mg HCP/ kg body wt.	248	12.4±.51	7.8±.76**	62.9	4.6±.76**	37.1

± the standard error of the mean
* $P < .05$ when compared to DMSO control
** $P < .01$ when compared to DMSO control

TABLE 3

Effects of hexachlorophene in mice (Group 3)
11th — 16th day of gestation

	Implants		Live		Resorbed and dead fetuses	
Treatment	Total	Mean	Mean	%	Mean	%
DMSO	240	12.0±.48	10.3±.54	85.4	1.75±.36	14.6
12.5 mg HCP/ kg body wt.	238	11.9±.64	9.75±.72	81.9	2.15±.35	18.1
25.0 mg HCP/ kg body wt.	235	11.75±.58	8.1±.60**	68.5	3.7±.51*	31.5

± the standard error of the mean
* $P < .05$ when compared to DMSO control
** $P < .01$ when compared to DMSO control

TABLE 4

Comparison of three gestation periods using student's t-test

	Concentration (mg HCP/kg body wt.)	3 — 8 versus 7 — 12		3 — 8 versus 11 — 16		7 — 12 versus 11 — 16	
	O (DMSO)	0.85	$P > .05$	1.22	$P > .05$	0.20	$P > .05$
Implants	12.5	0.96	$P > .05$	0.86	$P > .05$	0.00	$P > .05$
	25	0.89	$P > .05$	1.63	$P > .05$	0.84	$P > .05$
	0 (DMSO)	0.82	$P > .05$	0.21	$P > .05$	0.74	$P > .05$
Live	12.5	0.83	$P > .05$	1.76	$P > .05$	0.90	$P > .05$
	25	0.66	$P > .05$	0.98	$P > .05$	0.34	$P > .05$
Resorbed	0 (DMSO)	2.10	$P > .05$	1.05	$P > .05$	1.36	$P > .05$
and	12.5	1.95	$P > .05$	3.41*	$P < .01$	1.51	$P > .05$
dead	25	1.20	$P > .05$	2.20*	$P < .05$	0.98	$P > .05$

*Significant

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A FIVE-YEAR MACROINVERTEBRATE STUDY WITH DISCUSSION OF BIOTIC AND DIVERSITY INDICES AS INDICATORS OF WATER QUALITY, CODORUS CREEK DRAINAGE, YORK COUNTY, PENNSYLVANIA

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ABSTRACT

This study, the second in a series on the ecology of the Codorus Creek system, reports on the distribution and relative abundance of macroinvertebrates. A minimum of 132 taxa are recorded in 44 collections at 15 stations from November 1970 to April 1975. Species diversity and biotic indices are presented and compared with fish data collected from 1970 through 1973. Total taxa, mean taxa, indices and dissolved oxygen indicate lowered water quality below Spring Grove, partial to total recovery of some parameters, and more extensive deterioration below York. These studies constitute base-line data and to date place emphasis upon the West Branch and Main Branch of Codorus Creek.

INTRODUCTION

Studies of macroinvertebrates in Codorus Creek began November 1970 in conjunction with a survey of fish distribution. The objective was base-line data for assessment of change with time and of the impact from construction and stream improvement efforts in the future.

Macroinvertebrates are an essential part of the aquatic ecosystem. They serve as fish food, and because of relative immobility are readily affected by environmental stress. Some macroinvertebrates have delicate respiratory mechanisms and may be among the first organisms to respond to change due to industrial, agricultural or municipal effluents (Ingram, 1966). A decline in particular species or changes in community composition may lead to disruption of established food chains. Determination of normal species composition from year to year will facilitate recognition of change and possible causes of variation.

Literature relative to Codorus Creek was reviewed in Denoncourt and Stambaugh (1974). In addition three studies (Academy of National Sciences Philadelphia: 1969, 1973, 1974) were brought to our attention. These three plus Brezina (1969) and Kaeufer (1972) contained information relative to macroinvertebrates.

The purpose of this paper is to record a preliminary checklist of macroinvertebrates from Codorus Creek, to discuss longitudinal variation and to compare macroinvertebrate results with companion data on fishes.

Codorus Creek drains approximately one-third of York County in south central Pennsylvania (Figure 1). It flows northerly from near the Maryland border to enter the Susquehanna River below Codorus Furnace. Several small communities, Spring Grove, and York, influence its quality with industrial and domestic wastes. Seven major sewage treatment plants discharge into the system (Penn Township, Spring Grove, Glatfelter, Glen Rock, Red Lion, York and Springettsbury). Codorus Creek in the vicinity of these is under study to determine effects upon water quality and faunal communities.

METHODS

Fishes and macroinvertebrates were sampled, and selected physical and chemical parameters were measured at 15 stations in the Codorus Creek drainage from November 1970 to April 1975 (Figure 1). Stations were chosen on the basis of accessability, variety of habitats and location relative to major industrial/municipal centers. The results of macroinvertebrate studies and the physical data are reported here, with emphasis upon Stations 1 to 6 and 8 to 10.

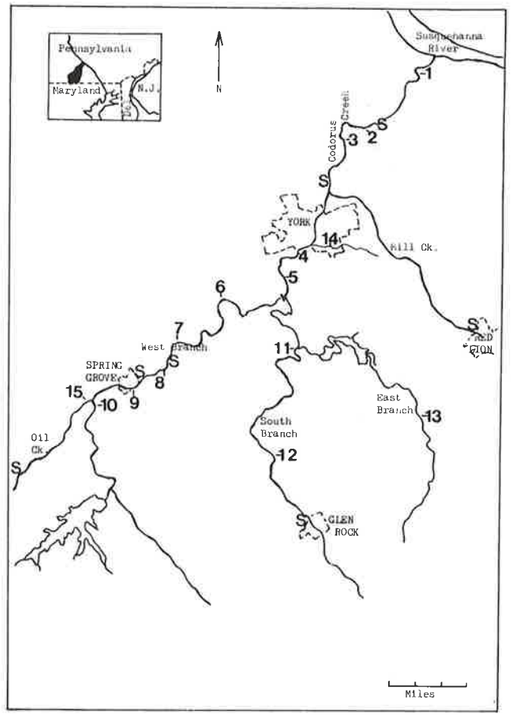

FIGURE 1. Location of stations sampled in 1970-1975 in the Codorus Creek drainage, York County, Pennsylvania. S indicates locations of sewage treatment plants.

Macroinvertebrates were collected with plankton-net (0.5mm mesh) seines and dip nets and were hand-gleaned from rocks, logs and other debris. They were preserved in 60% isopropanol and returned to the laboratory. Identification was made to the generic level when possible, and the number of species noted, with the aid of Ross (1944), Pennak (1953), Burks (1953), Edmonson (1959), Leonard and Leonard (1962), Parrish (1968), and Usinger (1971).

Data were organized by collection, and stations compared longitudinally by season and year, and organized for an overall check list (Table 1).

TABLE 1

Checklist of macroinvertebrate taxa collected from all stations 1970-1975, in the Codorus Creek drainage, York County, Pennsylvania[1]

Stations	1	2	3	4	5	6	8	9	10	11	12	13	14	15
Planariidae	x		x				x	x						x
Dugesia sp.								x						
Lumbricidae I	x	x	x	x	x	x	x	x	x		x		x	x
Lumbricidae II	x			x				x						x
Tubificidae	x		x	x	x	x	x							
Branchiura sowerbyi	x				x	x								
Enchytraeidae											x			
Hirudinea I	x	x	x	x		x		x						x
Hirudinea II	x	x	x	x										
Hirudinea III			x			x		x						
Hirudinea IV	x		x											
Asellidae														
Asellus sp.	x	x	x	x	x	x	x	x			x			
Gammaridae														
Gammarus sp.	x		x	x	x	x	x	x	x		x		x	
Astacidae														
Cambarus sp.						x			x			x		
Orconectes sp.	x		x	x	x	x		x		x	x			
Perlidae														
Acroneuria sp.						x						x		
Neophasganophora sp.												x		
Paragnetina I												x		
Paragnetina II												x		
Perlesta sp.			x	x							x			
Perlodidae				x										
Arcynopteryx sp.						x								
Baetidae		x									x			
Ameletus sp.			x		x	x		x	x					
Baetis sp.			x	x		x	x	x		x	x			
Ephemerella I	x			x	x	x		x						
Ephemerella II										x	x	x		
Ephemerella III									x	x		x		
Ephemerella IV									x	x	x			
Isonychia sp.				x		x		x	x	x	x	x		
Ephemeridae														
Ephemera sp.											x			
Hexagenia sp.									x					
Heptageniidae														
Stenonema I	x			x	x	x			x	x	x	x		
Stenonema II				x			x			x	x			
Aeschnidae														
Aeschna sp.					x									
Boyeria sp.				x				x						
Cordulegasteridae														
Cordulegaster sp.												x		
Gomphidae					x									
Gomphus sp.						x								
Ophiogomphus sp.												x		
Libellulidae														
Didymops sp;					x									
Libellula sp.						x		x						
Macromia sp.					x									
Epitheca sp.							x							
Agrionidae														
Agrion sp.		x				x							x	
Coenagrionidae					x									
Anomalagrion sp.				x										
Argia sp.				x	x	x	x	x						
Enallagma I								x						
Enallagma II								x						
Ischnura I	x					x	x							
Ischnura II				x		x								

TABLE 1. (Continued)

Checklist of macroinvertebrate taxa collected from all stations 1970-1975, in the Codorus Creek drainage, York County, Pennsylvania[1]

Stations	1	2	3	4	5	6	8	9	10	11	12	13	14	15
Belostomatidae														
Belostoma sp.				x				x						
Corixidae	x		x	x		x		x	x					x
Gerridae														
Gerris sp.				x		x		x						
Metrobates sp.	x													
Rheumatobates sp.	x							x						
Trepobates sp.	x					x								
Mesoveliidae														
Mesovelia mulsanti	x					x								
Veliidae														
Rhagavelia sp.						x		x						
Corydalidae														
Nigronia sp.			x	x										
Corydalus sp.			x	x	x	x		x		x	x			
Sialidae											x			
Sialis sp.				x	x	x		x						
Hydropsychidae														
Cheumatopsyche sp.	x		x	x	x	x	x	x	x	x	x	x		
Hydropsyche sp.	x	x	x	x	x	x	x	x	x	x	x	x	x	
Limnephilidae														
Neophylax sp.					x									
Philopotamidae														
Chimarra sp.									x					
Trentonius sp.									x					
Psychomyiidae														
Polycentropus sp.											x			
Rhyacophilidae														
Agapetus sp.									x					
Glossosoma sp.									x					
Rhyacophila sp.									x					
Dryopidae											x			
Dytiscidae														
Acilius sp.	x													
Hydroporus sp.						x								
Laccophilus sp.	x		x	x				x						
Elmidae	x		x	x										
Ancryonyx sp.					x	x								
Dubiraphia sp.						x								
Stenelmis sp.			x	x	x	x	x	x	x	x	x			x
Gyrinidae														
Dineutus sp.	x			x				x		x				
Haliplidae														
Haliplus sp.	x					x		x						x
Peltodytes sp.								x						
Hydrophilidae	x		x											
Berosus sp.				x		x	x	x						
Tropisternus sp.				x				x						
Psephenidae														
Psephenus herricki					x	x		x						
Ceratopogonidae	x								x					
Chironomidae	4	2	4	5	4	5	6	7	3	4	4		2	1
Ablablesmyia sp.							x	x	x					x
Coeltanypus concinnus						x		x						
Cricotopus sp.							x	x	x					x
Polypedilum sp.							x	x						
Procladius sp.								x						
Culicidae					x			x	x		x			
Empididae						x				x				
Rhagionidae														
Atherix sp.										x				

TABLE 1. (Continued)

Checklist of macroinvertebrate taxa collected from all stations 1970-1975, in the Codorus Creek drainage, York County, Pennsylvania[1]

Stations	1	2	3	4	5	6	8	9	10	11	12	13	14	15
Simuliidae														
Simulium sp.	x	x	x	x		x		x	x	x	x			x
Syrphidae											x			
Tabanidae					x				x					
Tipulidae			x											
Pedicia sp.									x					
Tipula I					x	x	x	x	x		x			
Tipula II				x		x		x					x	
Ancylidae														
Ferrissia sp.	x	x	x	x	x									
Lymnaeidae														
Lymnaea I								x	x	x				
Lymnaea II											x			
Physidae														
Physa sp.	x	x	x	x	x	x	x	x	x		x		x	x
Planorbidae														
Gyraulus sp.						x	x							
Helisoma sp.	x	x	x	x	x				x					
Sphaeriidae		x												
Pisidium sp.						x		x						x
Sphaerium sp.	x		x		x			x						
Unionidae					x									
Unidentified sp.			1	1	3	1					1			
Turbellaria	1		1											
Hydracarina						1					1			
Plecoptera		1			1				2					
Ephemeroptera		1			1	1		1	1					
Trichoptera									1					
Coleoptera				1					2		1			
Diptera									1					

[1] *Numbers in table indicate total species in that taxa. Genera of chironomidae were identified by James Kennedy.*

Dominant taxa were considered to be those that constituted 10% or more by total numbers.

Species diversity indices were calculated for each collection as suggested in the E.P.A. Environmental Monitoring Series (Weber, 1973):

$$D.I. = \frac{3.3219}{N}(N\log_{10} N - \Sigma\, n_1 \log_{10} n_1)$$

Biotic indices (Beck, 1954 and 1955) were also calculated:

$$B.I. = 2(n \text{ Class I}) + (n \text{ Class II}).$$

The number of species assigned to each "class of tolerance to organic pollution" was based upon the lowest assigned tolerance, or a mean, determined from Weber (1973). If none was given, a tolerance based upon similar groups or personal experience was assigned.

Data were summarized by station (Table 3) to give number of collections; total, range and mean taxa; range and mean for species diversity and biotic indices; and total and mean number of specimens.

Selected physical parameters were measured at the time of each collection. Dissolved oxygen was determined with a model 54 or 57 YSI; pH with Hach kit and Model 507A Orion ionalyzer, conductivity with a Model 103M meter from Ecologic Instruments; and temperatures with the YSI and standard Taylor field thermometers.

LOCATION OF SAMPLE STATIONS

Stations 1 to 14 are described in Denoncourt and Stambaugh (1975). Station 15 is located in Oil Creek approximately 0.15 river miles above its confluence with the West Branch of Codorus Creek and above Co. rt. 66008 bridge at Menges Mill.

RESULTS BY STATION

A total of 7,324 specimens with a minimum of 132 taxa was taken in 44 collections from 1970 to 1975. Graphic summaries of taxa, biotic indices and species diversity indices were organized into Figures 3 and 4.

Station 1.— A total of 38 taxa and 591 specimens were taken in two collections, 24 July 1971 and 12 April 1975. *Hydropsyche, Ischnura, Physa* and *Simulium* predominated in 1971; while *Gammarus, Asellus* and *Chironomidae* predominated in 1975. The biotic indices (8 to 10) suggested a slight increase in water quality while diversity indices (3.12 to 2.89) showed a moderate decrease. Overall, these suggest little change from 1971 to 1975.

Station 2.— Sixteen taxa and 246 specimens were taken in 2 collections, 22 May and 24 July 1971. Lumbricidae dominated in

TABLE 2.

Summary of pertinent data for all stations collected 1970-1975 in the Codorus Creek drainage, York County, Pennsylvania.

Stations	1	2	3	4	5	6	8	9	10
No. Collections	2	2	5	9	3	7	2	6	2
Total Specimens	591	246	916	724	456	1245	863	691	527
Mean No. Specimens	286	123	183	80	152	178	432	115	264
Total Taxa	38	16	34	45	42	55	26	56	40
Range in taxa	22-28	6-14	11-20	9-20	14-22	6-31	5-17	6-29	11-37
Mean taxa	25	10	14.8	14.5	18.7	16.9	13.5	17.2	24
Range of D.I.	2.89-3.12	2.51-2.57	1.90-2.87	2.70-3.44	2.76-3.23	1.88-4.30	2.62-2.96	2.46-3.76	2.62-3.35
Mean D.I.	3.00	2.54	2.55	3.17	3.07	2.74	2.79	3.00	2.98
Range of B.I.	8-10	7	3-10	6-18	14-18	4-20	7-15	6-20	12-44
Mean B.I.	9.0	7	7.6	10.6	15.3	14.1	11.0	15.4	28

Stations	11	12	13	14	15
No. Collections	1	2	1	1	1
Total Specimens	188	452	52	14	359
Mean No. Specimens	-	226	-	-	-
Total taxa	21	34	12	8	13
Range in taxa	21	17-25	-	-	-
Mean taxa	-	21	-	-	-
Range of D.I.	-	2.69-3.24	-	-	-
Mean D.I.	3.18	2.96	3.10	2.84	.84
Range of B.I.	-	12-24	-	-	-
Mean B.I.	21	18	18	6	10

May: while *Simulium, Physa,* and hirudinea dominated in July. There was a decided increase in species and numbers from May to July, but little change in indices. The increase was primarily due to pollution tolerant species. This station had the lowest mean biotic (7.0) and diversity (2.54) indices of stations in the West and Main Branches.

Station 3.— Thirty-four taxa and 916 specimens were taken in 5 collections: 24 July 1971: 3 April, 7 June and 28 July 1972: 7 June 1973; and 12 April 1975. Oligochaetes (primarily hirudinea) and gastropods (primarily *Physa*) predominated from 1972 to 1975, while *Asellus* and *Gammarus* increased in 1973 and 1975. This station had next to the lowest indices, mean biotic (7.6) and mean diversity (2.55).

Station 4.— Forty-five taxa and 724 specimens were taken in 9 collections: November 1970: 15 March and 24 July 1971: 3 April, 28 July and 17 August 1972: and 20 June 1973: and 12 April 1975. Species in the families Hydropsychidae, Gammaridae, Chironomidae and Physidae predominated in most collections. Lumbricidae and *Orconectes* were frequently dominant, while *Stenonema* (2 species) were dominant in 1972. The mean biotic (10.6) and diversity (3.17) indices indicated clean water. However, heavy growths of *Cladophora* and oil slicks were regularly observed.

Station 5.— Forty-two taxa and 456 specimens were taken in 3 collections: November 1970, 4 May 1971 and 12 April 1975. *Cheumatopsyche* and *Ferrissia* predominated in 1970: *Cheumatopsyche, Hydropsyche, Corydalus* and *Stenonema* in 1971: Chironomidae and *Hydropsyche* in 1975. The mean biotic (15.3) and diversity (3.07) indices indicated clean water.

Station 6.— Fifty-five taxa and 1,245 specimens were taken in 7 collections: November 1970: 29 March, 5 and 24 May, and 24 July 1971: 3 April 1972: and 12 April 1975. *Hydropsyche, Cheumatopsyche* and Chironomidae predominated in most collections: *Physa,* Lumbricidae, *Agrion* and *Ischnura* predominated in a few collections. The mean biotic index (14.1) suggested clean waters, while the mean diveristy index (2.74) indicated moderate organic pollution. Extensive beds of *Elodea* and *Cladophora* were consistently present.

Station 8.— Twenty-six taxa and 963 specimens were taken in 2 collections: 27 July 1972 and 13 April 1975. *Baetis, Gammarus,* Lumbricidae, and *Stenonoma* dominated in 1972: Chironomidae, Lumbricidae and *Hydropsyche* dominated in 1975. Mean biotic (11.0) and diversity (2.79) indices suggested moderately polluted waters.

Station 9.— Fifty-six taxa and 691 specimens were taken in 6 collections: November 1970, 22 May and 24 July 1971, 3 April and 27 July 1972, and 13 April 1975. Chironomidae, Hydropsychidae and *Gammarus* were usually dominant: while *Physa, Berosus, Stenelmis, Simulium, Orconectes,* and hirudinea dominated in some collections. The mean biotic (15.4) and diversity (3.00) indices suggested clean water. However the 1975 diversity index (2.46), silt, and slime-covered rubble, indicated moderate organic pollution.

Station 10.— Forty taxa and 527 specimens were taken in 2 collections: 22 May 1971 and 13 April 1975. Corixidae, *Hydropsyche, Stenonema* and *Isonychia* dominated in 1971: *Hydropsyche* and *Stenonema* in 1975. The mean biotic index (28) and diversity index (2.98) suggested clean waters.

PHYSICAL DATA

Physical data were discussed in Denoncourt and Stambaugh (1974). No outstanding variation was found in April 1975.

Figure 2 shows the dissolved oxygen concentrations recorded from 1969 to 1975. These show a consistent drop below Spring Grove, recovery by Stations 5 and 6; and a second drop to the lowest readings at Stations 2 and 3 below York.

SPECIES DIVERSITY INDICES

Species diversity indices have been used for a variety of faunal groups: Wilhm and Dorris (1968) - macroinvertebrates: Poulson and Culver (1969) - cave communities: Hairston et. al. (1968) - bees; Sheldon (1968), Denoncourt and Stambaugh (1974) and Stambaugh and Denoncourt (1974) - fishes; MacArthur and MacArthur (1961) - birds. Their use has been discussed by many authors (Gleason, 1922; Margalef, 1956; Menheniek, 1964; etc.). Dickman (1968) and Greenawald and Haase (1973) showed that the normal form (Shannon-Weaver,

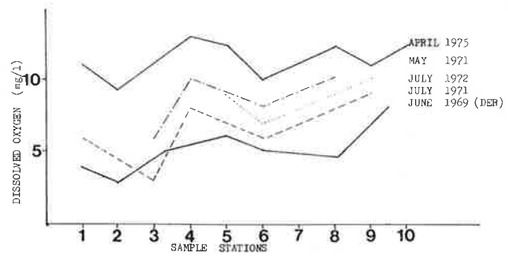

FIGURE 2. Dissolved oxygen in milligrams per liter recorded at sample stations 1 to 10 in the West and Main Branches of Codorus Creek, 1969-1975. DER refers to the Pennsylvania Department of Environmental Resources.

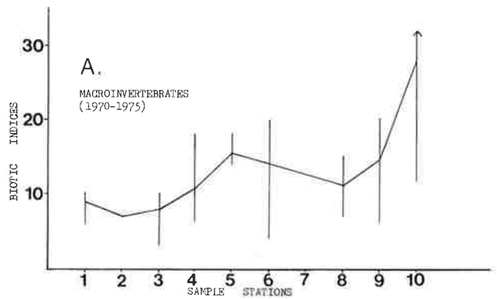

FIGURE 3. Range and mean of biotic indices (A) and species diversity indices (B) for macroinvertebrates collected in Codorus Creek from 1970 to 1975. The mean diversity indices for fishes, 1970-1973, are shown by dashed line in B.

1948) was influenced by large numbers of a few species and affected very little by changes in minor species. Hairston et al (1968) suggested limitations when used as an indicator of stability.

The index used in this paper is widely accepted for aquatic communities and is derived from Wilhm and Dorris (1968). Higher values usually suggest more species with several being abundant, while lower values suggest fewer species or only one or two predominating. The reliability of a mean species diversity index should increase with several collections at the same station over a period of time. But, caution is recommended. A diverse community of "organic" pollution-tolerant species would give high indices. Thus, knowledge of species, their relative abundance

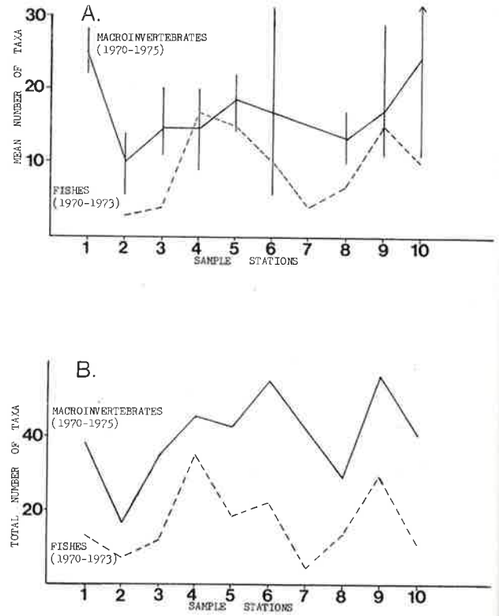

FIGURE 4. Range and mean number of taxa (A) and total number of taxa (B) for macroinvertebrates (solid line) collected in Codorus Creek from 1970 to 1975, and for fishes (dashed line) collected from 1970-1973.

and tolerance to pollution are indispensible for a biological interpretation.

Species diversity indices for Codorus Creek (Table 2 and Figure 3B) clearly show a decrease at Stations 6 and 8 below Spring Grove, recovery from Station 6 to Station 4 as water from the West Branch joins that from the South and East Branches, and then an even greater decrease at Station 2 and 3 below York. A start toward recovery from organic pollution is suggested at Station 1 just above the mouth of Codorus Creek. Examination of data (number species and indices) on a seasonal and year-to-year basis showed little variation. Wilhm and Dorris (1966) compared seasonal results and overall longitudinal trends in dissolved oxygen, number of individuals, number species, redundancy and diversity index. Their conclusion, particularly for number of species (taxa) and diversity indices, was the same as ours.

BIOTIC INDICES

The biotic indices given in Table 2 and shown in Figure 3A demonstrate the same trends as diversity indices. The change is, however, more obvious: a decrease below Spring Grove, partial recovery, and then a drop to less than 10 at Stations 2 and 3 below York.

Beck (1954, 1955) suggested indices above 10 are indicative of "clean" water of low organic load. Two limitations are associated with this index. As more research is summarized, the number of species in category II (moderate tolerance to organic pollution) increases, the number in category I (intolerant) decreases and some evidence is conflicting (Weber, 1973). This results in biotic indices that may approximate species number (Denoncourt, 1975). The second limitation is that the value for each species remains the same regardless of number (1 or 100). A single specimen could be accidental, but 100 is certainly not. Complications also arise in mesotrophic (moderate pollution) conditions which often show increased numbers of species (Patrick, 1970; Mackenthun, 1973).

The biotic index (and the diversity index) is a tool which must be used with knowledge of its limitations, and with knowledge of taxa that are indicative of particular water quality. The research of Goodnight (1973), Hynes (1970), Mackenthun and Ingram (1967), and Mackenthun (1973) suggest the following: Lumbricidae, Hirudinea, Asellidae, Physidae and certain diptera are indicative of polluted waters; while a variety of Plecoptera, Ephemeroptera, and certain Pelecypoda and Trichoptera are indicative of clean waters.

COMPARISON WITH FISH DATA

The macroinvertebrate data were compared with fish data from Denoncourt and Stambaugh (1974) in Figures 3 and 4 (dotted lines for fishes). Similar trends are shown by both fish and macroinvertebrate data with mean diversity index, mean number of species and total taxa. The mean diversity index for fishes and mean number of fish species show decreases to a greater extent below the municipalities than macroinvertebrate data show. Total taxa for fish and macroinvertebrates, on the other hand, show closer correlation. Differences in magnitude are due, in part, to a naturally more diverse macroinvertebrate community. This and the limitations of the diversity index contribute to the less discriminate results with mean taxa and mean diversity index for macroinvertebrates. However, if all macroinvertebrates could be identified to species, the species diversities might have had closer correlation.

It would appear (on the basis of numbers alone) that fish data are more discriminating than macroinvertebrate data as a means to assess water quality. However, once again knowledge of the species involved (fish or invertebrate) is necessary for biological interpretation. We recommend the use of data relative to both fishes and macroinvertebrates for more reliable conclusions. Whether one or the other is adequate requires further study.

CONCLUSIONS

1— A total 7,324 specimens and 132 taxa of macroinvertebrates were taken in 44 collections at 15 stations.

2— Species diversity indices, biotic indices, and total taxa for macroinvertebrates; and dissolved oxygen concentrations show the same trends: decrease below Spring Grove, recovery to Stations 4 and 5, and then a further decrease below York.

3— Comparison with fish data from Denoncourt and Stambaugh (1975) was made. Both fish and macroinvertebrate data show the same trends.

4— The West and Main Branches of Codorus Creek on the whole are moderately polluted. Certain areas appear particularly affected by domestic and industrial pollution from the municipalities of Spring Grove and York. The same conclusion was reached in Denoncourt and Stambaugh (1974). A potential for total recovery is suggested.

5— Data from the study of both macroinvertebrates and fishes should be used to determine water quality and change with time.

6— Diversity and biotic indices are useful tools. However, they msut be used in conjunction with knowledge of the species involved and their relative abundance.

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A REVIEW OF THE LITERATURE AND CHECKLIST OF FISHES OF THE SUSQUEHANNA RIVER DRAINAGE ABOVE CONOWINGO DAM

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ABSTRACT

A total of 71 articles of literature relative to the distribution of 100 fishes in the Susquehanna River drainage above Conowingo Dam in Pennsylvania and New York is given. These were used to produce a checklist divided into three categories: I. — 86 species present or expected, II. — 8 species in the literature but not expected, and III. — 6 species probably based upon misidentifications.

INTRODUCTION

Several publications have listed the fishes of Pennsylvania (Cope, 1881; Bean, 1892; Fowler, 1940; Cooper and Wagner, 1973). Greeley (1936) discussed the fishes of the Susquehanna drainage in New York State and Bielo (1963) fishes of the Susquehanna in Pennsylvania. However, no publication has dealt with the total drainage and no recent review has been made of the literature.

A complete list of species is needed in light of recent and anticipated environmental impact studies and the emphasis upon rare and endangered species. Recent field and museum research has revealed several range extensions in the Susquehanna (Denoncourt, Hocutt and Stauffer, 1975a; Kneib, 1972) and new records for Pennsylvania (Denoncourt, Hocutt and Stauffer, 1975b; Foote, Jacobsen, and Robbins, manus.). Denoncourt, Robbins and Hesser (1975) reviewed recent introductions and reintroductions to the Susquehanna above Conowingo Dam.

The purpose of this paper is to present a review of the known literature and an updated checklist of fish species in the Susquehanna River drainage above Conowingo Dam. Many literature records are not based upon specimen in museums, but upon reports of various investigators. A review of actual field studies and locations of extant specimens will be part of a future work.

METHODS AND MATERIALS

No attempt was made to exhaustively treat all papers related to all species in the Susquehanna waters. Taxonomy/synonymy or life history studies are not included unless they specifically refer to several species at locations within the drainage (examples given below) or to species not clearly given by others (Wallace, 1969, 1973). Species names and order of presentation were organized to follow Bailey et. al. (1970).

The checklist is divided into three sections, all based upon literature or manuscript records and our knowledge of the drainage: I. — Present or expecteed species, II. — Species in the literature, but not expected (primarily due to the presence of Conowingo Dam), and III. — Probably based upon misidentifications (or misinformation).

The literature cited is given alphabetically and numerically. Author citations are given in the text while numbers, and letters from literature addendum, are used in the checklist. An asterisk refers to New York State only.

HISTORY OF LITERATURE

The earliest publications on Pennsylvania fishes were those of Cope (1869, 1881, 1883), Bean (1902) and Ross (1902). Most of these were in annual reports of the Pennsylvania State Fish Commission. Henry W. Fowler (over 40 years with the Academy of Natural Sciences in Philadelphia) published a series of papers relative to fresh-water fishes of Pennsylvania which began in the early 1900s and culminated in 1940. A total of 19 of these were utilized in this review.

Extensive studies were recorded in Greeley (1938) and Bielo (1963) for the Susquehanna drainage in New York and Pennsylvania, respectively. Intensive research in sections of the lower Susquehanna was published by Ichthyological Associates (1972, 1974); Jacobsen, (1973); Potter, (1975); Robbins and Mathur, (1974a, 1974b, 1975). Several specialized studies that relate in part or totally to fishes of the Susquehanna included: behavior (Raney, 1940), trout populations (McFadden and Cooper, 1962; Cooper and Scherer, 1967), water quality (Kneib, 1972; Denoncourt and Stambaugh, 1975), surveys of subdrainages (Hocutt and Stauffer, 1975; Stambaugh and Denoncourt, 1975), and taxonomy (Gibbs, 1963; Ross, 1958; Jenkins, 1970; Raney and Lachner, 1943; Cole, 1965 and 1967; Taylor, 1969).

Popular articles on the fishes of Pennsylvania may be found in the *Pennsylvania Angler* (Buss and Miller, 1965) and in *Pennsylvania Fishes* (Pa. Fish Comm., 1969). Taxonomic keys that cover Susquehanna fishes include Eddy (1969), Blair et. al. (1970), and Denoncourt (1975).

SPECIES PRESENT OR EXPECTED

Amiidae — bowfins

Amia calva Linnaeus. — bowfin (11, 12, 16, 23, 36, 42, 53)

Anguillidae — Freshwater eels

Anguilla rostrata (Lesueur). — American eel (2, 3, 11, 12, 17, 20, 21, 23, 24, 36, 39, 41, 42, 45, 48, 52, 53, 54, 56, 58, 59, 61, 62, A, C)

Clupeidae — herrings

Alosa aestivalis (Wilson). — blueback herring (A)
Alosa pseudoharengus (Wilson). — alewife (2, 11, 12, 16, 23, 29, 36, A)
Alosa sapidissima (Wilson). — American shad (2, 11, 12, 16, 23, 29, 36)
Dorosoma cepedianum (Lesueur). — gizzard shad (16, 52, 53, 54, 61, 62)

Salmonidae — trouts

Coregonus artedi Lesueur. — cisco (Foote, Jacobsen, Robbins, manus., 16)
Coregonus clupeaformis (Mitchell). — lake whitefish (39*)
Oncorhynchus kisutch (Walbaum). — coho (16)
Oncorhynchus nerka (Walbaum). — sockeye (16)

Salmo gairdneri Richardson. — rainbow trout (2, 11, 12, 36, 39, 42, 46, 52, 53, 54, 56)
Salma salar Linnaeus. — Atlantic Salmon (16)
Salmo trutta Linnaeus. — brown trout (2, 9, 23, 36, 39, 40, 41, 42, 43, 45, 46, 48, 52, 53, 54, 58, 59, 61, 62, D)
Salvelinus fontinalis (Mitchell). — brook trout (2, 9, 11, 12, 20, 21, 22, 23, 36, 42, 52, 53, 54, 58, 59, 61, 62, D)
Salvelinus namaycush (Walbaum). — lake trout (36, 39)

Osmeridae — smelts
Osmerus mordax (Mitchell). — rainbow smelt (16, E)

Esocidae — pikes
Esox americanus Gmelin. — redfin pickerel (2, 11, 12, 20, 21, 22, 23, 36, 39)
Esox lucius Linnaeus. — northern pike (16, 42, 43, 52, 53, 54)
Esox masquinongy (Mitchell. — muskellunge (3, 42, 43, 48, 52, 53, 54, 61, 62)
Esox niger Lesueur. — chain pickerel (2, 3, 11, 12, 23, 24, 36, 39, 41, 42, 43, 52, 53, 54, 56, 57, 58, 59, 61, 62, C)
Esox reicheti Dybowski. — amor (16)

Cyprinidae — minnow and carp
Campostoma anomalum (Rafinesque). — stoneroller (2, 3, 17, 19, 20, 23, 32, 35, 36, 39, 40, 41, 42, 43, 46, 52, 53, 54, 55, 57, 58, 59, 61, 62)
Carassius auratus Linnaeus —goldfish (2, 17, 36, 48, 52, 53, 54, 61, 62)
Clinostomus elongatus (Kirkland) — redside dace (36, 50)
Clinostomus funduloides Girard. — rosyside dace (2, 11, 12, 19, 23, 36, 40, 52, 53, 54, 61, 62)
Cyprinus carpio Linnaeus. — carp (2, 17, 20, 36, 39, 41, 42, 43, 48, 52, 53, 54, 59, 61, 62, A)
Ericymba buccata Cope. — silverjaw minnow (15, 17, 63, 64)
Exoglossum maxillingua (Lesueur). — cutlips minnow (2, 3, 9, 10, 11, 12, 17, 19, 23, 25, 35, 36, 37, 39, 40, 41, 42, 46, 48, 52, 53, 54, 55, 58, 59, 61, 62, C, D)
Nocomis micropogon (Cope). — river chub (2, 3, 6, 10, 11, 12, 17, 19, 21, 22, 23, 25, 35, 36, 37, 39, 40, 42, 43, 46, 48, 50, 52, 53, 54, 55, 56, 57, 59, 61, 62, C, D)
Notemigonus crysoleucas (Mitchell). — golden shiner (2, 3, 17, 19, 20, 21, 23, 24, 36, 39, 41, 42, 43, 46, 48, 52, 53, 54, 57, 58, 59, 61, 62)
Notropis amoenus (Abbott). — comely shiner (3, 19, 21, 25, 36, 39, 41, 42, 43, 48, 52, 53, 54, 56, 57, 58, 59, 61, 62, A, C, D)
Notropis analostanus (Girard). — satinfin shiner (3, 6, 17, 21, 23, 36, 38, 39, 40, 42, 57, 59, C)
Notropis bifrenatus (Cope). — bridle shiner (39)*. This species in Pennsylvania is recorded in 23, 36, 58, 59 based upon 19 and one specimen in 59 identified by Fowler. No specimens could be found at the Academy of Natural Science in Philadelphia that were from the Susquehanna drainage. It was felt these latter five references were based upon a misidentification of *N. procne.*
Notropis cornutus (Mitchell). — common shiner (2, 3, 6, 10, 11, 12, 17, 19, 21, 22, 23, 24, 25, 35, 36, 37, 39, 40, 41, 42, 43, 46, 48, 52, 53, 54, 57, 58, 59, 61, 62, C, D)
Notropis heterodon (Cope). — blackfin shiner (39)*
Notropis heterolepis Eigenmann and Eigenmann. — blacknose shiner (39)*
Notropis hudsonius (Clinton). — spottail shiner (2, 3, 10, 11, 12, 17, 19, 23, 35, 36, 39, 40, 41, 42, 43, 48, 52, 53, 54, 56, 57, 58, 59, 61, 62, A)
Notropis procne (Cope). — swallowtail shiner (2, 3, 10, 11, 12, 17, 19, 21, 23, 36, 40, 41, 42, 43, 48, 52, 53, 54, 57, 58, 59, 61, 62, A, D)
Notropis rubellus (Agassiz). — rosyface shiner (17, 39, 40, 41, 42, 43, 52, 53, 54, 57, 59, 61, 62, A)
Notropis spilopterus (Cope). — spotfin shiner (2, 3, 6, 10, 11, 12, 17, 19, 20, 23, 35, 39, 40, 41, 42, 43, 48, 52, 53, 54, 57, 59, 61, 62, A)
Phoxinus eos (Cope). — northern redbelly dace (10, 11, 12, 19, 23, 31, 36)
Pimephales notatus (Rafinesque). — bluntnose minnow (2, 3, 17, 23, 36, 37, 39, 40, 41, 42, 43, 52, 53, 54, 57, 58, 59, 61, 62, A, D)
Pimephales promelas Rafinesque. — fathead minnow (17, 39, 52, 53, 54, 61, 62, G)
Rhinichthys atratulus (Herman). — blacknose dace (2, 3, 6, 9, 10, 11, 12, 17, 19, 20, 21, 22, 23, 25, 35, 36, 37, 39, 40, 41, 42, 43, 48, 52, 53, 54, 56, 57, 58, 59, 61, 62, C, D)
Rhinichthys cataractae (Valenciennes). — longnose dace (2, 3, 9, 10, 11, 12, 17, 19, 21, 22, 23, 25, 35, 36, 37, 39, 40, 41, 43, 46, 48, 52, 53, 54, 56, 57, 58, 59, 61, 62, A, C, D)
Semotilus atromaculatus (Mitchell). — creek chub (2, 3, 6, 10, 11, 12, 17, 19, 20, 21, 22, 23, 33, 35, 36, 37, 39, 41, 42, 43, 46, 48, 50, 52, 53, 54, 57, 58, 59, 61, 62, C, D)
Semotilus corporalis (Mitchell). — fallfish (2, 3, 10, 11, 12, 17, 19, 20, 23, 35, 36, 38, 41, 42, 43, 46, 48, 50, 52, 53, 54, 57, 58, 59, 61, 62, A,G)
Semotilus margarita (Cope). — pearl dace (2, 9, 11, 12, 23, 36, 37, 39, 46, 50, 56, 59)

Catostomidae — suckers
Carpiodes cyprinus (Lesueur). — quillback (3, 11, 12, 23, 36, 41, 42, 43, 48, 52, 53, 54, 59, 61, 62, A)
Catostomus commersoni (Lacepede). — white sucker (2, 3, 6, 11, 17, 20, 21, 23, 24, 25, 35, 36, 37, 39, 40, 41, 42, 43, 46, 48, 52, 53, 54, 56, 57, 58, 59, 61, 62, A, C, D)
Erimyzon oblongus (Mitchell). — creek chubsucker (2, 11, 12, 20, 21, 23, 35, 36, 39, 52, 53, 54, 57, 58)
Hypentelium nigricans (Lesueur). — northern hog sucker (2, 3, 11, 12, 17, 20, 21, 23, 35, 36, 37, 39, 40, 41, 42, 43, 46, 48, 52, 53, 54, 57, 58, 59, A, C, D)
Moxostoma macrolepidotum (Lesueur). — shorthead redhorse (2, 3, 11, 12, 17, 23, 39, 41, 42, 43, 44, 48, 52, 53, 54, 59, 61, 62, A)

Ictaluridae — catfishes
Ictalurus catus (Linnaeus). — white catfish (3, 17, 23, 36, 41, 42, 43, 48, 52, 53, 54, 61, 62)
Ictalurus natalis (Lesueur). — yellow bullhead (3, 17, 41, 42, 43, 48, 52, 53, 54, 57, 61, 62, A)
Ictalurus nebulosus (Lesueur). — brown bullhead (2, 3, 11, 12, 17, 20, 21, 23, 24, 35, 36, 39, 40, 41, 42, 43, 48, 52, 53, 54, 56, 58, 59, 61, 62, A, C)
Ictalurus punctatus (Rafinesque). — channel catfish (2, 3, 11, 17, 40, 41, 42, 43, 46, 52, 53, 54, 61, 62, A)
Noturus insignis (Richardson). — margined madtom (2, 3, 11, 12, 17, 22, 23, 24, 35, 36, 37, 39, 40, 42, 46, 48, 52, 53, 54, 56, 57, 58, 59, 60, 61, 62, C, D)

Gadidae — codfishes
Lota lota (Linnaeus). — burbot (2, 11, 12, 23, 36, 39, F)

Cyprinodontidae — killifishes
Fundulus diaphanus (Lesueur). — banded killifish (3, 11, 12, 21, 23, 36, 39, 41, 43, 52, 53, 54, 56, 57, 59, 61, 62)
Fundulus heteroclitus (Linnaeus). — mummichog (16, 52, 53, 54, 61, 62)

Gasterosteidae — sticklebacks
Apeltes quadracus (Mitchell). — fourspine stickleback (15)
Culea inconstans (Kirtland). — brook stickleback (15, 39)

Percichthyidae — temperate basses

Morone americanus (Gimelin). — white perch (2, 11, 12, 16, 23, 36)
Morone chrysops (Rafinesque). — white bass (B)
Morone saxatilis (Walbaum). — striped bass (2, 11, 12, 16, 23, 36)

Centrarchidae — sunfishes
Ambloplites rupestris (Rafinesque). — rock bass (2, 3, 23, 36, 39, 41, 42, 43, 48, 52, 53, 54, 57, 58, 59, 61, 62, A)
Enneacanthus gloriosus (Holbrook). — bluespotted sunfish (15). A record in Fowler (1919b), but not in subsequent publications (Fowler 1938 and 1940), was considered a mistake. No specimens from the Susquehanna drainage was found at the Academy of Natural Sciences in Philadelphia.
Lepomis auritus (Linnaeus). — redbreast sunfish (2, 3, 11, 12, 17, 21, 22, 23, 35, 36, 39, 40, 42, 43, 48, 52, 54, 56, 57, 59, 61, 62, A, C)
Lepomis cyanellus Rafinesque. — green sunfish (3, 17, 41, 42, 43, 48, 52, 53, 54, 61, 62, A)
Lepomis gibbosus (Linnaeus). — pumpkinseed (2, 3, 6, 11, 12, 17, 20, 22, 23, 24, 35, 36, 39, 40, 41, 42, 43, 48, 52, 53, 54, 57, 58, 59, 61, 62, A, C)
Lepomis macrochirus Rafinesque — bluegill (3, 17, 39, 40, 41, 42, 43, 48, 52, 53, 54, 57, 59, 61, 62, A)
Lepomis microlophus (Gunther). — redear sunfish (16)
Micropterus dolomieui Lacepede. — small mouth bass (2, 3, 17, 23, 24, 36, 39, 40, 41, 42, 43, 48, 52, 53, 54, 56, 57, 58, 59, 61, 62, A, C)
Micropterus salmoides (Lacepede). — largemouth bass (3, 11, 12, 17, 24, 39, 41, 42, 43, 48, 52, 53, 54, 56, 57, 58, 59, 61, 62, A)
Pomoxis annularis Rafinesque. white crappie (3, 17, 36, 41, 42, 43, 48, 52, 53, 54, 57, 58, 61, 62, A)
Pomoxis nigromaculatus (Lesueur). — black crappie (2, 3, 11, 12, 17, 23, 36, 39, 41, 42, 43, 48, 52, 53, 54, 61, 62, A, C)

Percidae — perches
Etheostoma blennioides Rafinesque. — greenside darter (Denoncourt, Potter and Stauffer, Manuscript)
Etheostoma flabellare Rafinesque. — fantail darter (21, 36)
Etheostoma olmstedi Storer. — tessellated darter (2, 3, 11, 12, 17, 20, 21, 22, 23, 24, 35, 36, 36, 39, 40, 41, 42, 43, 46, 48, 52, 53, 54, 56, 57, 58 59, 61, 62, A, C, D)
Etheostoma zonale (Cope). — banded darter (14, 45, 53, 54)
Perca flavescens (Mitchell). — yellow perch (3, 11, 12, 17, 23, 36, 39, 41, 42, 43, 48, 52, 53, 54, 59, 61, 62, A)
Percina caprodes (Rafinesque). — log perch (23, 36, 52, 53, 54, 61, 62)
Percina peltata (Stauffer). — shield darter (2, 3, 11, 12, 17, 22, 36, 39, 41, 48, 52, 53, 54, 57, 58, 59, 61, 62, A, G)
Stizostedion vitreum (Mitchell). — walleye (2, 3, 11, 12, 17, 23, 36, 39, 41, 42, 4^ 48, 52, 53, 54, 59, 61, 62, A)

Cottidae — sculpins
Cottus bairdi Girard. — mottled sculpin (3, 6, 39, 41, 42, 45)
Cottus cognatus Richardson. — slimy sculpin (2, 6, 9, 11, 12, 20, 22, 23, 35, 36, 39, 45, 46, 56, 58, 59)

SPECIES IN LITERATURE,
BUT NOT PRESENTLY KNOWN OR EXPECTED

Petromyzontidae — lampreys
Lampetra lamottei (lesueur). — American brook lamprey (36)
Petromyzon marinus Linnaeus. — sea lamprey (2, 12, 20, 26, 33, 35, 36, 39)

Acipenseridae — sturgeons
Acipenser oxyrhynchus Mitchill. — Atlantic sturgeon (23, 27, 36)

Lepisosteidae — gars
Lepisosteus osseus (Linnaeus). — longnose gar (2, 11, 12, 23, 28, 36)

Salmonidae — trouts
Onchorhynchus tshawytscha (Walbaum). — chinook salmon (2, 11, 12, 36). Eggs were stocked in 1880 (2).
Salmo clarki Richardson. — cutthroat trout (36—eggs and fry stocked in 1898)

Belonidae — needlefishes
Strongylura marina (Walbaum). — Atlantic needlefish (2, 11, 12, 20, 23, 36)

Centrarchidae — sunfishes
Lepomis gulosus (Linnaeus). — warmouth (36).

PROBABLE MISIDENTIFICATIONS

Petromyzontidae — lampreys
Lampetra aepytpera (Abbott). — least brook lamprey (21, 23)

Cyprinidae — minnows and carps
Hybopsis dissimilis (Kirtland). — streamline chub (36, 56)

Catostomidae — suckers
Erimyzon sucetta (Lacepede). — lake chubsucker (58)
Minytrema melanops (Rafinesque). — spotted sucker (36, 56)

Ictaluridae — catfishes
Noturus gyrinus (Mitchill) — tadpole madtom (2).

Percidae — perches
Percina maculata (Girard). — blackside darter (25)

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ISOLATION OF STAPHYLOCOCCUS AUREUS AND STREPTOCOCCUS AGALACTIAE FROM MASTITIS-SUSPECT CATTLE.

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ABSTRACT

Staphylococcus aureus and *Streptococcus agalactiae* were isolated from raw milk samples from cattle suspect for mastitis. The presence of these two species in milk was correlated with their presence on the exterior of the udder prior to milking.

INTRODUCTION

Bovine mastitis has long been recognized as a cause of serious economic loss to dairymen and is probably the greatest single cause of loss from diseases of cattle in the United States (1). This disease has been estimated to cause an annual loss to the dairy industry in the United States of between $225,000,000 and $500,000,000 (2). Mastitis is the reaction of milk secreting tissue to injury resulting from any condition or combination of factors leading to the damage of internal structure of the udder in conjunction with a bacterial infection. Several different genera of bacteria may be involved, including *Streptococcus, Staphylococcus, Pseudomonas,* and the coliforms. However, the genera *Streptococcus* and *Staphylococcus* are the most common invaders of the udder. These organisms enter the udder through the teat canal, and the infected mammary gland serves as a reservoir from which the organism can be transmitted to other cattle by handling and/or milking machine. Once the infection becomes established in an udder, it usually remains there unless it is removed by specific chemotherapy (3). Thus, mastitis may become a chronic problem in dairy herds unless the causative agents are controlled. The diagnosis and treatment of infected cattle are problems for veternarians since they usually are not consulted until the infection becomes chronic. The current study was undertaken with William W. Crawford, D.V.M. and was concerned with the isolation and identification of bacteria from mastitis-suspect cattle.

METHODS

Milk samples from a Holstein herd were obtained from cattle suspect for mastitis flare-ups based on the results of a modified Whiteside test or from evidence of clinical symptoms of the disease. The Whiteside test is used for the rapid screening of mastitis in milk samples. One or two drops of 4% sodium hydroxide solution are mixed with approximately five drops of milk on a glass plate and stirred for 15-20 seconds. The milk separates into particulate matter or into a viscous gel when large numbers of leukocytes and fibrin are present. Therefore, a direct relationship exists between the degree of the reaction and leukocyte count, giving initial information on the presence of a bacterial infectin within the suspect udder. The Hotis test gives a preliminary identification on the type of causative organism within the udder. Eighteen ml of milk added to one ml of 0.33% solution of bromocresol purple and incubated at 37°C will produce green or brown colonies with light-colored centers on the bottom or side of the test tube after 15-20 hours if *Staphylococcus aureus* is present in the sample. The appearance of a yellow coagulant resultin from lactose fermintation after between 20-40 hours or incubation i an indication of *Streptococcus agalactiae* (4).

Prior to the collection of milk samples, the udders and adjacen flank areas were sponged with warm water. The teat and orifice wer scrubbed with cotton gauze and 70% alcohol. The first sample of mil extracted from the udder was collected since it should contain th most bacteria. Milk samples were plated on 5% sheep blood agar an incubated at 37°C for 18-24 hours. Preliminary identification o *Streptococcus agalactiae* and *Staphylococcus aureus* were made o the basis of Beta hemolysis on blood agar plates. The identificatio was further substantiated by subculturing onto nutrient agar plate and microscopic examination. *Streptococcus agalactiae* is characterize by a gram-positive, kidney-shaped cocci usually found in pairs whil *Staphylococcus aureus* is a gram-positive, golden brown cocci foun singularly or in irregular cluster (4-5). Mannitol salt agar was als used for further identification of *Staphylococcus aureus.*

In addition to milk samples, swab samples were also obtained fro the teat opening and surrounding areas of the lower udder. Thes swabs were immediately placed in sterile saline and transported t the laboratory. Swabs were first cultured in nutrient broth for 2 hours at 37°C and then subcultured on blood agar and mannitol sal agar for identification. Final identification was based on a microscopi examination of bacterial cultures.

Data on the presence of bacteria in milk samples obtained fro cattle which did not show clinical symptoms of the disease wer obtained from the examination of cultures and by consultation wit Dr. William W. Crawford, veterinarian, who was responsible for th diagnosis and treatment of mastitis in the Gregg herd.

RESULTS

Mastitis was first observed on the Gregg farm five years ago whe *Streptococcus agalactiae* infected Holstein cattle were probabl introduced into the herd, but the source of *Staphylococcus aureu* remains unknown (4). Five outbreaks of mastitis occurred during th study and the number of cattle infected ranged from 3 to 9 out of herd of 75 animals. This represents an incidence of infection from 4 to 12% of the herd. However, throughout the study a total of 35 case occurred.

The two species of bacteria isolated from these cattle wer *Staphylococcus aureus* and *Streptococcus agalactiae. Staphylococc aureus* was present on the outside of the udder an average of 83% the time, compared with an average of 37% for *Streptococcus agalactia Streptococcus agalactiae* was present an average of 80% in mil samples compared to a mean incidence of occurrence of 47% f *Staphylococcus aureus* (Table 1).

These figures did not indicate which organism was the primar causative agent of the disease nor whether a synergistic affect occurre between these two bacteria. *Staphylococcus aureus* was the onl organism isolated from the exterior of the udder in an average of 53 of the cases examined. *Streptococcus agalactiae* was isolated alone i

TABLE 1
Percentage Incidence of Staphylococcus aureus and Streptococcus agalactiae isolated from Mastitis-suspect Cattles.

Date	No. Cattle	*Staphylococcus aureus* Exterior Udder	*Staphylococcus aureus* Milk Sample	*Streptococcus agalactiae* Exterior Udder	*Streptococcus agalactiae* Milk Sample
Oct. 19, 1972	9	55	44	44	100
Jan. 28, 1973	6	83	50	33	83
Feb. 11, 1973	3	100	0	67	100
Feb. 18, 1973	8	87	37	37	75
April 10, 1973	9	89	55	55	89
Mean		82.3	37.2	47.2	89.4

7.8% of the cattle and both species occurring together were found in 29.7% of the cases (Table 2). In two incidences (9.1%) neither of these bacteria was isolated from the exterior of the udder of a mastitis-suspect cow. In contrast, in raw milk samples *Streptococcus agalactiae* was isolated alone an average of 48% compared to 18.9% for *Staphylococcus aureus,* whereas both species occurring together were isolated in an average of 41.9% of the cases examined (Table 2). In one case, neither of these bacteria was isolated in the milk obtained from a mastitis-suspect cow. These results indicate that *Staphylococcus aureus* is the most prevalent organism on the exterior of the udder, whereas *Streptococcus agalactiae* is more common inside the teat canal. Both of these organisms can be isolated in approximately 20% of the cattle which do not show clinical symptoms (6).

Mastitis re-appeared in five out of the nine cattle which had the disease in October, after a seven-month period. Both *Streptococcus agalactiae* and *Staphylococcus aureus* were isolated from three of these cattle while the remaining two individuals only possessed *Streptococcus agalactiae* in October. *Streptococcus agalactiae* was the only bacteria isolated from these cattle during the re-occurrence of the disease in April. These data suggest *Staphylococcus aureus* may be easier to eliminate from a dairy herd then *Streptococcus agalactiae.*

DISCUSSION

Present-day methods of dairy herd management favor the perpetuation of contagious mastitis caused by *Streptococcus agalactiae* and/or *Staphylococcus aureus.* These organism enter the udder through the teat canal, and the infected mammary gland serves as a reservoir from which these bacteria are spread to other cattle by the milkers' hands or milking machines. Once the infection becomes established in the udder, it usually remains there for the duration of the life of the animal unless it is removed by specific chemotherapy (3). Eberhart and Guss (7) found that intramammary penecillin therapy was effective in eliminating *Streptococci* from infected quarters of the udder. The Federal Food and Drug Administration regulations permits 100,000 units of penicillin-streptomycin be administered to dairy cattle. However, these cattle must be removed from production for at least 96 hours after injection. Veterinarians who are able to prepare their own antibiotics may be administering up to one million units intramammary (6). This will eliminate the source of infection for approximately six months after which the disease may re-appear if the stress condition continues (4). Both *Streptococcus* and *Staphylococcus* may be maintained in cattle without causing clinical mastitis (6).

The exact cause of mastitis flare-ups and the perpetuation of the causative agents in dairy herds can only be theorized at this time. Murphy and Stuary (8) succeeded in infecting 62% of the 444 quarter-exposure to *Streptococcus agalactiae* utilizing the swab technique. These workers further state that there was no correlation between milking rate and infectability. Dodd and Neave (9) suggested that staphylococcal infection is significantly dependent upon the dilatability of the teat canal but Murphy and Stuart (8) demonstrated that *Streptococcus agalactiae* is not dependent upon this characteristic of the teat canal. It has been suggested by dairymen that these bacteria might be transmitted via colostrum to calves, thereby maintaining thses agents in the herd, but data obtained by Klein *et al* (10) and Miller and Heishman (11) does not support this view. The fact remains, however, that once these bacteria become established, they usually perpetuate themselves resulting in mastitis. A reduction in mastitis might be obtained through careful management, thereby reducing the degree of mechanical injury to the udder.

TABLE 2
Percentage Incidence of isolated occurence of Staphylococcus aureus and Streptococcus agalactiae singly or together from Mastitis-suspect Cattles.

Date	No. Cattle	Location	*S. aureus* alone	*S. agalactiae* alone	*S. aureus* and *S. agalactiae*	Neither
Oct. 19, 1972	9	Ext. Udder	33.3	22.2	22.2	22.2
		Milk	0.0	55.6	44.4	0.0
Jan. 28, 1973	6	Ext. Udder	50.0	16.7	33.3	0.0
		Milk	16.7	66.7	16.7	0.0
Feb. 11, 1973	3	Ext. Udder	100.0	0.0	0.0	0.0
		Milk	66.7	33.3	66.7	0.0
Feb. 18, 1973	8	Ext. Udder	50.0	0.0	37.5	12.5
		Milk	0.0	37.5	37.5	12.5
April 10, 1973	9	Ext. Udder	33.3	0.0	55.6	11.1
		Milk	11.1	44.4	44.4	0.0
Mean		Ext. Udder	53.3	7.8	29.7	9.1
		Milk	18.9	47.5	2.5	41.9

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DARK AND LIGHT GROWN ALGAL SPECIES OF THE GENUS NEOSPONGIOCOCCUM

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ABSTRACT

The genus *Neospongiococcum* is a member of the chlorococcalean algae. *Neospongiococcum concentricum, N. excentricum* and *N. mobile* were grown in the light and in the dark on synthetic, semi-synthetic and natural media. Growth of the three algal species on commercially prepared dehydrated media indicates that such media may offer the phycologist an alternative to the media generally employed in studies of heterotrophic microalgae. Dark grown algae remained viable for at least eight months when stored at 5°C and did not show the tendency to become chlorotic as did the light grown algal cultures. An attempt was made to differentiate *N. concentricum, N. excentricum* and *N. mobile* by means of standard physiological tests which are generally used in bacteriological studies. Separation of the three algal species on the basis of physiological reactions alone proved inconclusive. However, when rating of growth was used in conjunction with the physiological reactions, a suitable separation could be made.

INTRODUCTION

The genus *Neospongiococcum* was established by Deason (1) to include the spherical, unicellular chlorococcalean algae with sponge-like chromatophores, pyrenoids and *Chlamydomonas*-type zoospores that do not divide vegetatively according to the concept of vegetative cell division proposed by Brown and Bold (2). Members of this genus have been isolated from a variety of habitats and their morphological characteristics have been thoroughly investigated (1, 3, 4, 5). Traditionally, morphological characteristics have served as the primary basis for phycological classification. More recently, however, the utilization of physiological responses of pure cultures of micro-algae as secondary characteristics in their classification at the species level has gained popularity (4, 6, 7, 8).

In the present study, three species of *Neospongiococcum* were studied (*N. mobile, N. concentricum, N. excentricum*). The objectives of this study were as follows: 1. To compare light and dark growth of the three species. 2. To determine the ability of the three species to be grown on heterotrophic media under both light and dark conditions. 3. To differentiate the three species by their growth in the dark on a number of standard bacteriological media and differences in their physiological reactions produced on these media.

MATERIALS AND METHODS

A. *Autotrophic Medium* — The autotrophic medium (B-Solution) was Bold's medium as modified by Bischoff and Bold (9).

1. *Macronutrient Solutions*

Each macronutrient solution was prepared by dissolving the appropriate weight of salt in 400 ml distilled water:

K_2HPO_4	3.0g
NaCl	1.0g
$CaCl_2 \cdot 2H_2O$	1.0g
$NaNO_3$	10.0g
KH_2PO_4	7.0g
$MgSO_4 \cdot 7H_2O$	1.0g

To 936 ml of distilled water add 10 ml of each stock solution (above) then 1 ml each of the following micronutrient solutions was added to make 1 liter. The micronutrient stock solutions are as follows:

A. EDTA Stock Solution:
50g of ethylenediaminetetracetic acid (EDTA) and 31g Potassium hydroxide (KOH) were dissolved in distilled water to give a final volume of 1 liter.

B. H-Boron Stock Solution:
11.42g of boric acid (H_3BO_4) were dissolved in distilled water to give a final volume of 1 liter.

C. H-Fe Stock Solution:
4.98g of Ferrous Sulfate ($FeSO_4 \cdot 7H_2O$) were added to acidified water to give a final volume of 1 liter. The acidified water was prepared by adding 1 ml concentrated sulfuric acid to 999 ml distilled water.

D. H-H_5 Stock Solution:
8.82g $ZnSO_4 \cdot 7H_2O$, 1.44g $MnCl_2 4H_2O$, 0.71g MoO_3, 1.51g $CuSO_4 \cdot 5H_2O$, 0.49g Ca $(NO_3)_2 \cdot 6H_2O$ were added to acidified water to give a final volume of 1 liter. The acidified water was prepared by adding 1.0 ml of concentrated sulfuric acid to 999ml of distilled water.

The above medium could be used in the liquid state, however, in many of our studies 1.5% agar was added to the Bold's Solution in order to prepare a solid medium.

B. *Heterotrophic Media* — Synthetic as well as semi-synthetic media were used in order to obtain heterotrophic growth. Media employed for heterotrophic studies were as follows:

1. B-Solution with Glucose:
The autotrophic medium was supplemented with glucose (0.5 ml of a sterile 20% glucose solution per each 50 ml of B-Solution) to obtain heterotrophic growth. Agar was added at the rate of 1.5% per liter in order to prepare solid B-Solution and Glucose Medium.
2. Semi-Synthetic Heterotrophic Medium:
A variety of commercially prepared dehydrated media were used to grow the three species of *Neospongiococcum.* Media used included: Tryptic Soy Agar, Potato Dextrose Agar, Mycological Agar, Corn Meal Agar, Lima Bean Agar, Malt Agar, Phenol Red Broths (xylose, rhamnose, raffinose, arabinose, mannose, maltose, fructose, lactose, galactose, glucose, glycerol, sorbitol, mannitol), Urea Broth, Litmus Milk, Gelatin, Starch Agar, Triple Sugar Iron Agar, Lead Acetate Agar, Simmon's Citrate Agar, MR-VP Medium, Nitrate Broth and Cellulose Agar.

Cultural Conditions — Autotrophic cultures (light grown) were grown on B-Solution at 22°C ± 2°C and a 12 hour diurnal light and dark cycle with an illumination of 250 — 300 Ft-C intensity. Heterotrophic cultures (dark grown) were grown at 22°C ± 2°C in total darkness.
Preparation of Pure Cultures — All algal cultures were first grown autotrophically on B-Solution and routine microscopic examinations were made to determine purity. Once the purity of a culture was established, regular transfers on B-Solution were used to maintain active cultures. These active cultures were used as inoculum for heterotrophic studies.
Storage of Cultures — The three species of *Neospongiococcum* were stored at 22°C ± 2 as well as at 5°C. These stored cultures were routinely observed for purity and transferred to detect their viability.
Rating of Growth — A rating system for growth of the three species of *Neospongiococcum* grown in the dark on bacteriological medium

was employed as follows: 1. Growth was rated as (+) Poor growth, (++) Average growth, (+++) Excellent growth, and (−) no growth. 2. Ratings were based on dark grown cultures of the same species grown on B-Solution with glucose for the same time period.

Sterilization of Media — All media were sterilized by autoclaving at 121°C for 15 minutes at 15 psi with the exception of the urea, arabinose, xylose and fructose broths which were sterilized by filtration.

RESULTS

Light and Dark Growth — All three species of *Neospongiococcum* grew well on B-Solution in the light as well as B-Solution supplemented with glucose in the dark. However, it was observed that the best growth for all three species was obtained when these algae were grown on B-Solution with glucose and a 12 hour diurnal photoperiod. However, dark grown cultures proved to remain viable for longer periods of time, especially when they were stored at 5°C—cultures remained viable for eight months. Dark grown cultures did not show the tendency to become chlorotic as did the light grown cultures.

Physiological Tests and Growth on Bacteriological Media — Twenty-three media were used to obtain growth and physiological reactions (See Table 1). Results for the various physiological reactions were negative, however differences in the amount of growth on the various media produced some positive results. Generally, it was observed that *N. concentricum* provided the best growth on all media tested while *N. mobile* and *N. excentricum* exhibited poorer growth. The most striking specific differences were found with *N. excentricum* as indicated by its inability to grow on Litmus Milk, Triple Sugar Iron Agar or on media supplemented with rhamnose or glycerol. Other than the amount of growth obtained, differences between *N. mobile* and *N. concentricum* were less clear. The definitive reaction that could be used to differentiate between these two species was that *N. mobile* did not grow on Simon's Citrate while *N. concentricum* produced poor growth. This indicates that *N. concentricum* can utilize citrate as a sole source of carbon, whereas *N. mobile* can not use it at all.

Growth on Heterotrophic Media in Light and Dark — Figures 1-8 demonstrate the ability of the three organisms to grow in the light and dark on various heterotrophic media. All three species of *Neospongiococcum* grew on the media regardless of the presence or absence of light. However, some of the media supported scanty growth at best. Light grown algae grew best on Tryptic Soy Agar, Mycological Agar, Lima Bean Agar adn Potato Dextrose Agar (not shown in figures). Dark grown algae grew best on Mycological Agar and Lima Bean Agar as well as Corn Meal Agar (not shown in figures).

TABLE 1

Physiological and Growth Characteristics for Dark Grown Cultures of Neospongiococcum mobile, N. concentricum and N. excentricum.

Growth Medium[1]	*N. mobile* Growth[2] (Amount)	*N. mobile* Reaction[3]	*N. concentricum* Growth[2] (Amount)	*N. concentricum* Reaction[3]	*N. excentricum* Growth[2] (Amount)	*N. excentricum* Reaction[3]
Xylose[4]	++	−	+++	−	+++	−
Rhamnose[4]	++	−	+++	−	−	−
Raffinose[4]	++	−	+++	−	+	−
Arabinose[4]	+++	−	+++	−	++	−
Mannose[4]	+++	−	+++	−	++	−
Maltose[4]	+	−	+++	−	+++	−
Fructose[4]	+++	−	+++	−	+++	−
Lactose[4]	+	−	+++	−	+	−
Galactose[4]	++	−	+++	−	+++	−
Dextrose[4]	+++	−	+++	−	+++	−
Glycerol[4]	+	−	+++	−	−	−
Sorbitol[4]	+	−	+++	−	++	−
Mannitol[4]	+	−	+++	−	++	−
Urea Broth	+++	−	+++	−	+++	−
Litmus Milk	+	−	+++	−	−	−
Gelatin Nutrient	+++	−	+++	−	+++	−
Starch Agar	++	−	++	−	++	−
Triple Sugar Iron Agar	+++	−	+	−	−	−
Lead Acetate Agar	+++	−	+++	−	++	−
Simmon's Citrate	−	−	Slight+	−	+	−
MR—VP Medium	+++	−	+++	−	++	−
Nitrate Broth	+++	−	+++	−	+	−
Cellulose Agar	+	−	+	−	+	−

[1] Three replicates for each organism.

[2] (+++) Excellent growth, (++) Average growth, (+) Poor growth, (−) No growth (14 days growth).

[3] Reactions are recorded positive (+) or negative (−) as outlined in Standard Methods (10).

[4] These media contain phenol red as a ph indicator and durham tubes for gas collection. Positive reactions would be indicated as A (acid production), G (gas production), AG (both acid and gas production). Negative reactions recorded as (−). (10)

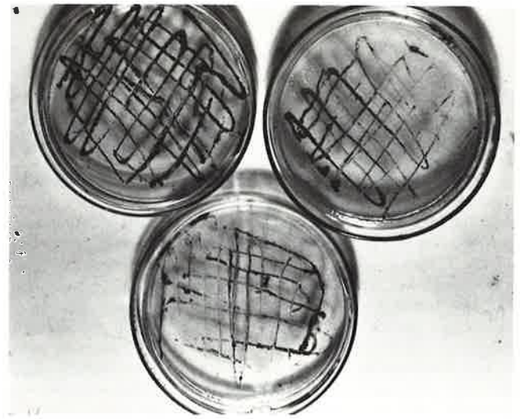


FIGURE 1. Light Grown Cultures on Algae on Tryptic Soy Agar — 7 Days Growth (Upper left — *N. mobile*, Upper right — *N. concentricum*, Bottom center — *N. excentricum*).

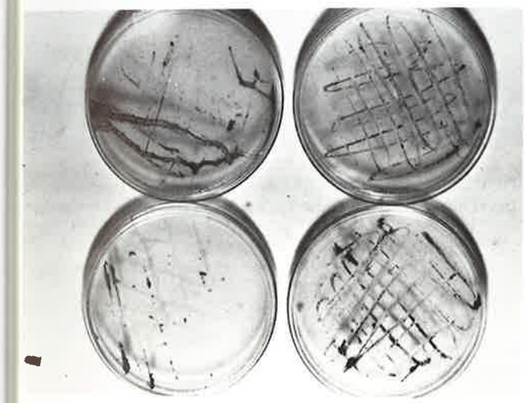
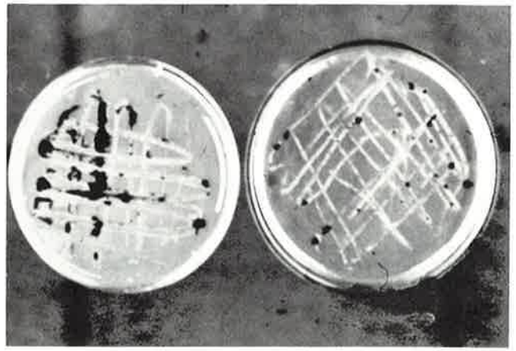


FIGURE 3. Dark Grown Cultures of Algae on Mycological Agar — 9 Days Grown (Left — *N. excentricum*, Right — *N. concentricum*).

FIGURE 2. Algal Growth on Lima Bean Agar — 7 Days Growth (Upper left — *N. excentricum* — Light Grown, Upper right — *N. concentricum* — Light Grown, Lower left — *N. mobile* — Light Grown, Lower right — *N. concentricum* — Dark Grown).



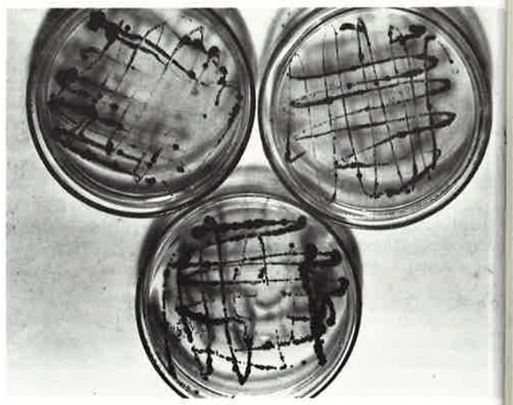


FIGURE 4. Light Grown Cultures of Algae on Mycological Agar — 7 Days Growth (Upper left — *N. excentricum,* Upper right — *N. concentricum,* Bottom center — *N. mobile*).

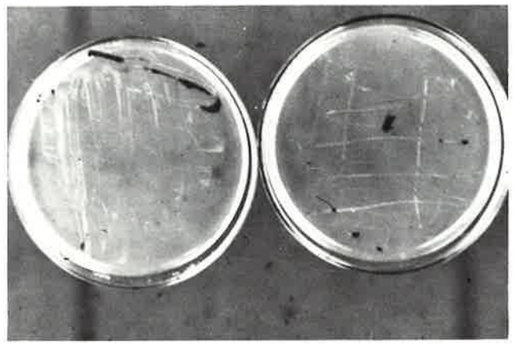


FIGURE 5. Dark Grown Cultures of Algae on Lima Bean Agar — Days Growth (Left — *N. excentricum,* Right — *N. concentricum*).

FIGURE 6. Dark Grown Cultures of Algae on Malt Agar — 9 Days Growth (Upper left — *N. excentricum,* Upper right — *N. concentricum,* Bottom center — *N. mobile*).

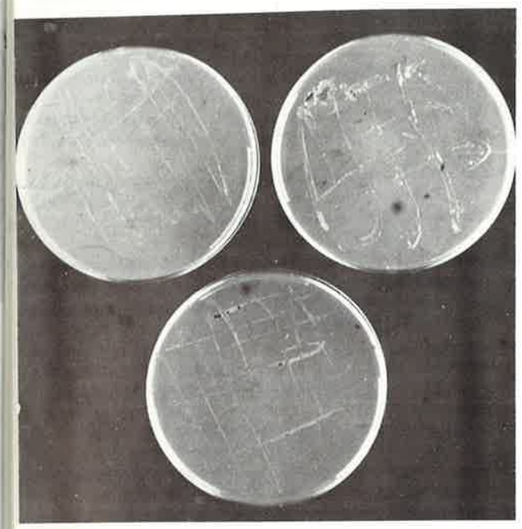


FIGURE 7. Dark Grown Cultures of Algae on Lima Bean Agar — 9 Days Growth (Upper left — *N. excentricum,* Upper right — *N. concentricum,* Bottom center — *N. mobile*).

FIGURE 8. Light Grown Cultures of Algae on Malt Agar — 7 Days Growth (Upper left — *N. concentricum,* Upper right — *N. mobile,* Bottom center — *N. excentricum*).

DISCUSSION

The fact that the dark grown cultures remain greener than light grown cultues requires a careful explanation. Groover and Bold (11) suggested that both nitrogen depletion and dessication were involved in color changes in the plant mass of chlorosarcinalean algae. McClean (12) has implicated the accumulation of secondary caroteniod pigments as the reason for color changes in algae. How much these factors contributed to the color changes in the light grown algae of this study remains uncertain. One interesting facet of coloration in *N. concentricum,* however, deserves comment. When Anderson and Nichols (4) initially described *N. concentricum* they emphasized that the characteristic concentric appearance of the colonies was associated with cell reproduction. Dark green areas in the colony had actively reproducing cells, whereas the light green areas were associated with non-reproducing cells. Furthermore, Anderson and Nichols (4) reported that *N. concentricum* cells grown in dextrose in the dark appear to reproduce more frequently than cells grown in dextrose in the light. Therefore the possibility exists that some color variation in *N. concentricum* can be attributed to increased cellular reproduction in dark grown cultures. There are no data to indicate that a similar situation exists with dark grown cultures on *N. mobile* and *N. excentricum.*

Most phycological studies dealing with microalgae employ media similar to that used by Bischoff and Bold (9). Heterotrophic growth has been promoted in these microalgae by adding various carbon and nitrogen sources to the above mentioned basal medium and incubation in total darkness (4, 6, 8, 11). Results from the present study demonstrate that many comercially prepared media will support the growth of *N. mobile, N. concentricum* and *N. excentricum.* Thus, our results demonstrate that preparation of traditional phycological media may not be necessary for those researchers who wish to grow the three species of *Neospongiococcum* utilized in this study. This does not infer that traditional phycological growth media should be discarded, but perhaps commercially prepared media could be adapted to aid the phycologist in obtaining large quanities of axenic microalgae quickly.

Several authors have used physiological characteristics as supplementary taxonomic criteria for establishing species of microalgae (4, 6, 8, 9, 11). However, with the exception of these few studies only selected physiological tests have been employed. Therefore, one part of the present study was concerned with employing a number of routine bacteriological media to obtain physiological differentiation among *N. mobile, N. concentricum* and *N. excentricum.* Results showed that physiological reactions alone were insufficient to afford differentiation among the three species of *Neospongiococcum.* However, when growth characteristics were considered, e.g. presence or absence of growth, and relative amount thereof, separation of the three species was possible. While such a system of differentiation is not suited for separating numerous species; it can be useful with certain limitations. Furthermore, the inability of the algae to grow on some of the media, i.e. *N. excentricum* did not grow on Triple Sugar Iron Agar, Litmus Milk, Glycerol or Rhamnose media and *N. mobile* did not grow on Simmon's Citrate indicates that greater care in selection of media may be the key to using physiological characteristics as better taxonomic tools in phycological studies.

ACKNOWLEDGMENTS

We are grateful to Dr. Temd R. Deason for supplying us with the species of *Neospongiococcum* used in this study. Also, we wish to acknowledge the technological assistance of Ms. Susan M. LaBute, without whom this work could not have been done.

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BIRD SPECIES DIVERSITY AND FOLIAGE HEIGHT DIVERSITY CORRELATIONS IN A POCONO SPRUCE-LARCH BOG

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ABSTRACT

Bird species diversity (BSD) and foliage height diversity (FHD) studies were made in the Tannersville Bog in Monroe County, Pennsylvania. The correlations indicate that the BSD predictability model used for deciduous forests has applicability to bogs.

INTRODUCTION

MacArthur and MacArthur (1) found, in abandoned fields and deciduous forests, that they could predict the BSD from the FHD. The relationship is expressed by the linear equation BSD = 2.01 FHD + 0.46.

Since that seminal work, additional studies have been made on this continuing problem of diversity prediction. MacArthur (2) found the model workable in the deserts with some modification and MacArthur *et al.* (3) found it to have applicability in the Panama area. Cody (4) noted that from the layering of the grasslands he could predict bird diversity (richness) and Terborgh and Weske (5) found in Peru that the density of the foliage was adequate for predicting BSD. On the New Jersey Piedmont, Kricher (6) demonstrated that the BSD increases through the field-forest successional sequence. The predictability equation was suitable for a Pocono deciduous forest (7). Wilson (8). in a study of fields and deciduous forests in Illinois, had regression slopes of BSD on FHD similar to those of MacArthur and MacArthur. Recher (9) studied the two-diversity relationship in the predominantly evergreen vegetation near Sydney, Australia, and concluded that in southeastern Australia the avifauna habitat subdivision was similar to that of North America.

The paucity of information of bird populations in bogs was pointed out in a review of the topic by Brewer (10). The current study was designed to examine a spruce-larch bog in the context of the MacArthur predictability model and to evaluate its applicability to a different ecosystem.

The study site is located in Pocono Township, Monroe County, about 3.2 Km east of Tannersville. It is designated as the Cranberry Swamp on the Mt. Pocono USGS Quadrangle Map, at 41° 02' 15" N x 75° 16' 00" W, although it is commonly known as the Tannersville Bog. Lafayette College owns the bog and some adjacent land.

The bog itself consists of 12 ha of a *Picea mariana — Larix laricina* association partially bordered by about 90 ha of swamp to the 920 foot contour interval. The bog is dominated by larch and black spruce (combined density of 3 stems/m^2), sphagnum, and ericaceous plants. It is in the Consolidation Phase of development (11).

METHODS

The breeding bird populations were determined for two years by the point-mapping method similar to that of Kendeigh (12). BSD was calculated by $BSD = -\Sigma p_i \ln p_i$, where p_i is the proportion each species is of the whole.

The FHD was determined as described previously (7), with one modification. Because of the density of the lower levels of the vegetation in the bog, it was sometimes impossible to see the point at which the 7.6 m height line became half obscured. In those cases the distance was estimated by the use of aerial photographs taken by the author. The maximum height of the vegetation was determined to be 10.0 m, at which point k = 0.

RESULTS AND DISCUSSION

The results of the foliage density studies are plotted in Figure 1. The extremely high density values at and below the 1 m level are accounted for by the very dense stands of *Chamaedaphne* (average density = 71 stems/m^3) and the sphagnum growth around them, the wide distribution of *Carex,* and other ericads. The shape of the upper portions of the curve reflects the profile expected from the dominant conifers.

From the curve in Figure 1 the FHD was calculated and the data are presented in Table 1. From the table, it is noted that 49.8% of the foliage density is in the lower layer, 49.0% in the middle layer, and only 1.0% in the top layer. The FHD was 0.746.

TABLE 1

Foliage profile data from the Tannersville Bog.

Foliage Layer (m)	Diversity Index (p_i)	FHD
0 — 0.61	0.498	0.746
0.61 — 7.6	0.490	
> 7.6	0.011	
Σ (p_i)	0.999	

The results of the census studies of the nesting avifauna are presented in Table 2. The BSD in 1970 was 1.68 and in 1971 it was 1.66. The catbird (*Dumetella carolinensis*), common yellowthroat (*Geothlypsis trihas*), and song sparrow (*Melospize melodia*) were the most common birds and accounted for approximately 75% of the total population (by territory).

Based on the determined FHD value of 0.746, it could be predicted that the BSD should be approximately 1.9 ± 0.3; the experimental value had a mean of 1.67. The points are within the scatter of points on the original regression line and are presented in Figure 2.

If the BSD-FHD correlation seems applicable for the Tannersville Bog, then other bogs should show similar relationships. BSD analyses were made of the census of 15 other bogs (13, 14, 15, 16, 17) by reducing each area to p/1.6 ha. From the plant community descriptions of the bogs one could then estimate the layering. BSD increased with the change from the open *Chamaedaphne* bogs through the Consolidation Phase bogs. For instance, the bog reported by Kammeraad (16) had a BSD of 2.7 and had a larch average of 6.5" dbh. Such a tree size would readily introduce a fair proportion (p_i) of the vegetation into the upper layer, and hence increase the FHD.

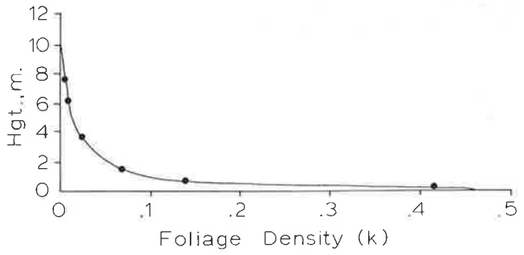

FIGURE 1. Foliage density (k) plotted against height in the Tannersville Bog.

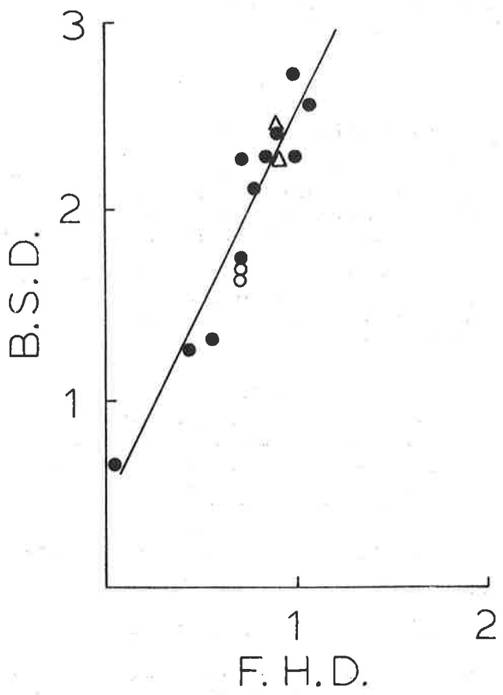

FIGURE 2. Bird species diversity plotted against foliage height diversity. The solid circles and the regression line (BSD = 2.01 FHD + 0.46) are from the initial study (1). The two triangles are from the study of a Pocono deciduous forest (7) and the two open circles are from the study in the Tannersville Bog.

Tramer (19) computed BSD for 267 breeding bird census reports, given in the *Audubon Field Notes* (1937-1966) and demonstrated definite increases in the BSD with the addition of vegetation layers from the essentially one-layered marshes and grasslands through intermediate layering (shrublands) to three-layered vegetation (forests).

Based on (a) the applicability of the model to the Tannersville Bog, (b) the apparent trend from other bogs, (c) the broad studies of the two-diversity relationship (1, 2, 3, 5, 7, 8, 9, 18), and (d) the diversity

TABLE 2

Number of territories of nesting birds per 1.6 ha (4 acres), the diversity index (p_1), and the bird species diversity for the Tannersville Bog.

Species	*1970*		*1971*	
	N	P_1	N	P_1
Catbird	3.25	3.61	4.00	.301
Yellowthroat	3.00	.333	3.25	.245
Song sparrow	1.00	.111	3.00	.226
Nashville warbler	.50	.055	1.75	.132
Cedar waxwing	.50	.055	.25	.018
Veery thrush	.25	.027	.25	.018
Towhee	.25	0.27	.25	.018
Blue jay	—	—	.50	.037
Ch-sided warbler	.25	.027	—	—
Bird species diversity	1.68		1.66	

computations by Tramer (19), it may be concluded that for homogeneo bogs, FHD provides a reasonable prediction of BSD.

If the Tannersville Bog FHD proportions are partitioned as Recher (4), then the fit is even closer (from FHD 0.756 to 0.65 Further field studies on a number of different bogs would be need to determine the most suitable subdivisions of the vertical layering.

Additional studies will, no doubt, continue to refine the model. our accuracy in predicting diversity increases, the more authoritative we can argue for a partitioning of the environment (20) according ecological rather than pragmatic and economic goals.

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POPULATIONS STUDIES OF RED OAK (QUERCUS RUBRA L.) AND NORTHERN RED OAK (QUERCUS RUBRA VAR. BOREALIS (MICHX. F.) FARW.)

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ABSTRACT

Twelve field populations of Red Oak (*Quercus rubra* L. and its variety Northern Red Oak (*Quercus rubra* Var. *borealis* (Michx. f.) Farw.) were studied along a south to north climatic gradient about 400 miles long from Indiana to Northern Michigan. It was found that northern populations tended to have smaller and somewhat narrower acorns. When populations from poor sites were compared to those from better sites, it was found that poor site populations had smaller acorns, had a tendency to evidence pubescence on the twigs, and fringe presence on the acorn cup. They also had a tendency to have uniquely narrow acorns. Good site populations were felt to be expressions of the species (*Quercus rubra*) and poor site populations to be primarily expressions of its variety Northern Red Oak (*Quercus rubra* Var. *borealis*). Poor site populations are thought to be primarily expressions of a gene flow between Red Oak and Black Oak (*Quercus velutina*) in the Michigan area.

"Common garden" studies of seven field populations suggest ecotypic differences between southern and northern populations that are primarily physiological. They also provide some evidence for genetic differences between poor site and better site populations.

INTRODUCTION

To try and understand morphological variations in Red Oak as related to differences in climate and soils, a series of diagnostic morphological characters were scored for several field populations on a south to north climatic gradient approximately 400 miles long. Stands of Red Oak were studied on soils with poor site (nutrient conditions), usually sands, and compared with sites of better nutrient conditions on finer textured soils. It was felt that the comparison of poor site populations and better site populations would lead to some understanding of the relationship between Red Oak and its variety Northern Red Oak.

METHODS

Populations for study were located whenever possible within state parks or preserves for possible future study. Other criteria used in selecting populations were: naturalness of stand conditions, homogeneity of soil type in the stand, and homogeneity of the population itself (avoiding obvious hybrid swarms).

For each population studied, a strip transect was laid out on which approximately 100 mature trees were numbered. The length of the transect depended upon the number of mature trees available within 15 meters of the transect. The specific trees to be studied intensively on the transect were determined by selecting random numbers between 1 and 100 and then locating and studying the proper numbered trees. Twenty-five mature acorns were collected from each tree and from these, one, chosen at random was measured in detail. Inner bark samples were taken at 4½ feet from the ground on the east side of the trees between the bark ridges. Thirteen diagnostic morphological characters were studied and quantitatively assessed by analysis of variance. Eight of the more important characters are presented in Table 1 of this paper.

Acorns from selected field populations were collected during the fall of 1960 and grown under "common garden" conditions in the former Bogue Forest Nursery in East Lansing, Michigan. The nursery planting was one of a randomized block design with six acorns per row replicated four times. On germination the seedlings were scored for morphological differences and a mean obtained for each population for each character. This was then tested statistically by analysis of variance to determine possible significant differences between the populations at the 5% and 1% levels of confidence.

RESULTS AND DISCUSSIONS

Twelve field populations were studied intensively for morphological variation on a south to north transect about 400 miles long. The populations were organized into two general transects for comparison, one in the interior of Indiana and Michigan and the other along the shore region of Lake Michigan northward. Also for comparison two additional populations were studied, one on northern Lake Huron and the other at the northern tip of Michigan at Copper Harbor on Lake Superior.

It was found on both transects (Table 1) that northern populations had smaller and somewhat narrower acorns. The acorn cup tended to cover more of the acorn on the Lake transect but this trend was not clearly developed on the Inland transect.

Populations of Red Oak on poor sites which were tipified by the presence of Jack Pine and/or Red Pine in the stand, showed additional differences. The acorns of these populations were obviously smaller than nearby better site populations. Also, they had a tendency to have uniquely narrow acorns and often pubescence on the twigs and fringe presence on the acorn cup. These poor site populations would best fit the taxonomic description of *Quercus rubra* L. Var. *borealis* (Michx. f. Farw.) (1). The better site populations best fit the description of *Quercus rubra* L. (1). Populations with morphological characters intermediate between the variety and the species are common, as well as, individual intermediate trees in individual populations.

The intermediate nature and relationship between populations is best illustrated by Table 2. Here, the populations by transects are compared statistically for 13 diagnostic morphological characters. When it is realized that the paired poor site and better site populations of the Inland transect, both Northern Lower Peninsula and Upper Peninsula Michigan, and the pair on the Lake transect are less than 25 miles apart,the differences become more significant. The general trend of differences of southern and northern populations is apparent except for the poor site populations.

Poor site populations of Red Oak referred to in this paper as *Quercus rubra* Var. *borealis* show evidence of gene flow with Black Oak (*Quercus velutina*) in the Michigan area. The author has developed hybrid indexes in Michigan for several of these populations, and gave a paper on the subject to the Michigan Academy of Sciences many years ago. This accounts for such typical Black Oak characters that are evident in introgressed populations such as twig pubescence, acorn cup fringe presence, and smaller acorns. I have also observed that

TABLE 1

Population means for selected morphological characters of Red Oak and Northern Red Oak on south to north transects from Indiana to northern Michigan.

	Number trees sampled	Acorn size, length + width, mm.	Acorn shape, width/length x 100	Ratio, acorn cup ht./ total acorn with cup x 100	Acorn cup fringe presence, 10 none, 1 very evident	Inner bark color, 11 orange 8 yellow, 5 pink, 2 tan	Tree form, 9 open growth form, 3 fine branched	Winter bud length, mm. ( ) sample size for this parameter	Twig pubescence, 10 none, 1 abundant
Inland transect									
Northern Indiana	18	45	93	31	10	3.8	8.4	4.7(3)	10
South-central Michigan	25	43	94	28	10	4.1	8.1	5.1(8)	10
Northern Lower Peninsula Michigan	23	39	86	32	9.5	3.9	8.1	4.6(7)	10
Northern Lower Peninsula Michigan, poor site	25	35	81	30	9.3	4.0	8.9	4.6(7)	10
Upper Peninsula Michigan	25	39	87	34	10	4.0	8.7	5.5(11)	10
Upper Peninsula Michigan poor site	18	36	87	32	10	4.4	7.9	——	10
Lake Michigan transect									
Southern Michigan	18	42	91	29	10	3.8	8.9	5.8(5)	10
Central Michigan	20	39	91	30	9.7	4.2	8.5	——	10
Northern Michigan	27	37	89	30	10	4.4	7.0	6.3(9)	10
Northern Michigan, poor site	9	33	79	35	9.4	6.0	6.6	6.8(5)	9.9
Other Populations									
Northern Lake Huron, poor site	21	34	82	36	9.6	4.2	6.0	4.6(14)	9.8
Lake Superior, northern tip of Upper Peninsula Michigan.	19	30	88	38	10	4.4	7.7	——	9.9

TABLE 2

*Number from 13 morphological characters ** in which contrasted pairs of Red Oak and Northern Red Oak populations did not differ significantly at the 1% level (analysis of variance).*

Inland transect	NI	SCM	NLPM	NLPM-P	UPM	UPM-P	SM	CM	NM	NM-P	NLH-P	LS
Northern Indiana (NI)	x	11	8	3	6	7	11	8	4	2	1	6
South-central Michigan (SCM)		x	9	3	7	8	13	10	5	3	2	7
Northern Lower Peninsula Michigan (NLPM)			x	5	8	7	9	10	5	4	5	5
Northern Lower Peninsula Michigan, poor site (NLPM-P)				x	2	4	3	4	7	10	8	3
Upper Peninsula Michigan (UPM)					x	6	7	9	4	1	2	5
Upper Peninsula Michigan, poor site (UPM-P)						x	8	10	10	7	4	6
Lake Michigan transect												
Southern Michigan (SM)	11	13	9	3	7	8	x	10	5	3	2	7
Central Michigan (CM)	8	10	10	4	9	10	10	x	7	4	2	6
Northern Michigan (NM)	4	5	5	7	4	10	5	7	x	10	5	4
Northern Michigan-poor site (NM-P)	2	3	4	10	1	7	3	4	10	x	9	3
Other Populations												
Northern Lake Huron, poor site (NLH-P)	1	2	5	8	2	4	2	2	5	9	x	3
Lake Superior, northern tip of Upper Peninsula (LS)	6	7	5	3	5	6	7	6	4	3	3	x

**These characters were: Total acorn height, acorn length, acorn width, acorn shape, twig brittleness, acorn cup scale shape, twig pubescence, inner bark color, tree form, acorn size, acorn cup height, acorn cup fringe presence, ratio of acorn cup height/total acorn height.

TABLE 3

Population means of selected morphological characters of Red Oak and Northern Red Oak growing under "common garden conditions" at East Lansing, Michigan.

	Number of parent trees sampled	Height, mm. Age 2	Leaf length, mm. Age 2	Leaf width, mm. Age 2	Leaf margin, 2 smooth, 5 wavy, 8 dentate, Age 1	Leaf Angle, Age 1 Degrees	Percent Leaves Green, Nov. 8, 61, Age 1	Percent Leaves Red, Oct. 4 62, Age 2	Percent Leaves Leafing Out, May 5, 62, Age 2	Percent Leaves Lost, Nov. 6, 62, Age 2	Percent Trees with Second Flush, July 5, 61, Age 1
Southern Michigan, along Lake	1	837	165	108	7.5	15°	33	15	60	5	31
Northern Michigan, along Lake	5	737	162	103	6.9	18°	14	20	65	2	69
Northern Michigan, poor site, along Lake	6	712	151	98	6.8	23°	13	21	49	2	50
Northern Michigan, Interior	5	686	162	94	6.2	20°	9	24	55	11	49
Northern Michigan, Interior, near poor site	3	686	162	99	6.1	18°	1	44	85	24	55
Upper Peninsula, Mich.	6	636	156	93	6.4	17°	2	68	90	72	55
Upper Peninsula, Mich.	6	—	—	—	5.8	15°	—	—	—	—	33
Significance, Analysis of Variance, ns — not significant, 5% significant at 5% level, 1% at 1% level.		ns	ns	ns	5%	1%	1%	1%	1%	1%	5%

hybrid populations often show individual trees with unusually narrow acorns. Other species in the Red Oak Group (Erythrobalanus) are also involved in gene exchange with Red Oak in different portions of its range such as Hill Oak (*Quercus ellipsoidalis*), Scarlet Oak (*Quercus coccinea*), and Pin Oak (*Quercus palustris*).

Acorns from seven field populations were collected and planted under "common garden" conditions in East Lansing, Michigan. Seven of the morphological characters measured showed significant differences at the 5% and 1% level of confidence (Table 3). Thirteen additional morphological characters measured showed no significant difference. Three of these non-significant characters are presented in Table 3 which I believe show south to north trends but due to the variability within the individual populations, the differences are not significant. From what has been developed in this paper as to the relationships of the Red Oak populations, this type of variability within populations would not be considered unusual.

Morphological characters showing significant differences were, leaf margin, leaf angle, percent green leaves in November, percent of red leaves in October, percent trees leafing out in spring, percent leaves lsot in November, and percent trees in second flush of leaves in July (Table 3).

The differences between populations under "common garde conditions appear to be primarily physiological which enable southe and northern poulations to be more in harmony with their region environment. The development of these ecotypes in such a wide-rangi species over Eastern United States as Red Oak is expected.

When poor site populations are compared with better site populatio the evidence is conflicting. The Northern Michigan Lake transe populations showed no differences. They were about two miles apa However, the interior populations of Northern Michigan show several differences, namely in percent red leaves, percent leav leafing out in spring, and percent leaves lost in November. The lat two populations were about eight miles apart. Thus evidence fo genetic difference between Red Oak and its variety, Northern R Oak is reasonably supported by the "common garden" results.

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A STUDY OF VARIATION IN BLACK OAK (QUERCUS VELUTINA LAM.) POPULATIONS FROM UNGLACIATED SOUTHERN INDIANA TO THE RANGE LIMITS IN NORTHERN MICHIGAN

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ABSTRACT

Fourteen field populations of Black Oak (*Quercus velutina* Lam.) were studied for morphological variation on a south to north orientation from southern Indiana to northern Michigan. It was found that northern populations as compared to southern populations had smaller, narrower, and less cup covered acorns, lighter yellow inner bark color, a more branching growth form, smaller winter buds, less evident acorn cup fringe, and in certain northern populations, less pubescent twigs. Fifteen populations were studied under "common garden" conditions to give evidence of genetic variation between populations. Significant differences were found in several characters that indicated some genetic differences between southern and northern Black Oak populations. Many differences appear and to be related to acorn size which is postulated to have a genetic basis.

INTRODUCTION

Traveling northward in the Great Lakes area from unglaciated Southern Indiana to the northern limits of Black Oak (*Quercus valutina* Lam), in glaciated Northern Michigan, many morphological differences between populations become evident, in particular acorn size, winter bud size, inner bark color, and general growth form. The objective of this study is to sample and quantify these changes and to probe the question of a possible genetic basis for these differences.

METHODS

Oak stands over much of Northeastern United States were visited in order to understand variation patterns in other parts of the Black Oak range. Many major herbaria were also visited and the oak collections surveyed. The herbaria collections were useful in a broad sense but of limited value in understanding population variation due to the small sample size of local populations.

Several populations near the northern limits of the Black Oak range near Pellston, Michigan were studied intensively in the field. From 37 morphological characters studied, 13 were selected as being diagnostic in measuring variation between and within Black Oak populations. Nine of these characters, though less important, were reported in a statistical treatment of the populations (Table 2).

Fourteen field populations of Black Oak were selected for intensive study during the summer of 1962. These were located on a south—north orientation from unglaciated Southern Indiana to the northern limits of Black Oak in glaciated Northern Michigan. An effort was made to replicate the question of population variability on a south—north basis by establishing three general transects. One was located northward in the central interior of Indiana and Michigan, one on the western side of Indiana and Michigan near Lake Michigan northward, and the third was on the eastern side of Indiana and Michigan and followed Lake Huron northward in Michigan.

Populations were located whenever possible within state parks or preserves and were approximately 50 miles apart. Other criteria used in selecting populations for study were: naturalness of stand conditions, homogeneity of soil type, homogeneity of the population itself avoiding obvious hybrid swarms, and accessibility of the site for future study.

For each population, a strip transect was laid out on which approximately 100 mature trees were numbered. The length of the transect depended on the number of mature trees available within 15 meters of the transect with an average length of about 400 meters. Some stands near the northern limits of Black Oak were very poorly stocked and required a transect over 1000 meters long to obtain a satisfactory sample. The specific trees to be studied intensively on the transect were determined by selecting randon numbers between 1 and 100 and then locating and studying the proper numbered trees. The total number of trees sampled from a population depended on the variation observed within the population but averaged about 20 trees. See Table 1 for each population size.

Whenever possible, 25 mature acorns were collected from each tree studied; of these, one (chosen at random) was measured. Inner bark samples were taken at 4½ feet from the ground on the east side of the trees between the bark ridges.

Acorns from the field populations were collected during two consecutive falls (1960 and 1962) and grown under "common—garden" conditions in the former Bogue Forest Nursery in East Lansing, Michigan. The first fall, 24 acorns were planted from each parent tree in a population. The second fall, 32 acorns were planted from each parent. See Table 3 for the number of parent trees collected in each population.

Following planting all seedbeds were normally mulched for the winter to a ½ inch depth with sawdust. However, in 1961 a portion of the beds were not mulched. The data from these beds were kept separate for possible differences and comparisons. See Table 3.

The nursery planting was of a randomized block design with each row of planted acorns (6 in a row the first fall, and 8 in a row the second fall), replicated four times. On germination the seedlings were scored for morphological differences and a mean obtained for each replication and each parent tree for each character selected for measurement. This was then treated statistically by analysis of variance or in one case by Chi—square to determine possible significant differences between the populations at the 5% and 1% levels of confidence.

RESULTS—DISCUSSION

From personal observations and general study of several hundred field populations which included considerable collections of fruiting and inner bark material through the Great Lakes region and in Eastern United States, a study of relevant literature, visits to selected major herbaria, and an intensive study of Black Oak field populations near Pellston, Michigan, it was possible to develop major morphological criteria for identification of Black Oak populations and their variants. It was found that acorns, especially the acorn cup fringe presence, were the most reliable. The yellow to orange inner bark color was also found to be particularly diagnostic for Black Oak. Winter bud pubescence, twig, and leaf pubscence were also very useful.

Using these and other parameters, fourteen field populations selected along three south to north transects from 200 to over 300 miles long were studied. On all three south to north transcets (Central, West along Lake Michigan, and East along Lake Huron), the following trends were evident (Table 1): northern populations as compared to southern populations had smaller, narrower, and less cup covered

TABLE 1

Population means for selected morphological characters of Black Oak on south to north transects from Indiana to the northern range limit in Michigan.

	Population size, number trees sampled	Acorn size, length + width, mm.	Acorn shape, width/length x 100	Ratio, acorn cup ht./ total acorn with cup x 100	Acorn cup fringe presence, 10 none, 1 very evident	Inner bark color, 11 orange, 8 yellow	Tree form, 9 open growth form, 3 fine branched	Winter bud length, mm.	Twig pubescence, 10 none, 1 abundant
Inland transect									
Southern Indiana	21	31.0	101	60	2	10.7	7.6	7.3 (12)*	1.0
Northern Indiana	18	28.8	94	56	5	9.9	5.5	7.8 (5)	2.2
Southern Michigan	29	28.5	91	56	4	10.4	6.6	7.1 (22)	2.4
Central Michigan	10	29.2	93	50	6	10.2	4.7	5.1 (11)	3.7
Northern Michigan (Indiana River)	29	25.8	81	47	7	8.4	4.2	5.9 (15)	2.0
Following Lake Michigan, transect									
Northern Indiana	25	32.0	99	55	3	10.6	5.3	7.6 (8)	2.1
Central Michigan	21	29.7	91	52	4	11.0	6.5	6.0 (7)	1.9
North-central Michigan	16	26.6	87	47	6	10.5	6.2	5.8 (12)	3.1
Northern Michigan (Honor, Michigan)	29	26.3	82	49	6	9.6	5.0	6.3 (15)	3.2
Northern Michigan (Traverse City)	18	25.8	89	48	6	9.9	5.0	5.2 (12)	2.0
Following Lake Huron, transect and Northeastern Indiana									
Northeastern Indiana	16	30.3	90	56	4	10.8	6.7	6.6 (5)	2.0
Southern Lake Huron Area	26	29.6	83	48	4	10.6	6.1	6.8 (9)	1.3
Central Lake Huron Area	20	27.9	84	45	6	9.8	5.4	6.4 (8)	2.7
Northern Lake Huron Area (Alpena)	19	28.5	81	39	8	8.2	4.9	5.6 (8)	3.4

* Number of trees sampled for winter bud measurements

acorns, lighter yellow inner bark color, a more branching growth form, smaller winter buds, less evident acorn cup fringe, and in certain northern populations, less pubescent twigs.

There was a significant difference at the 1% level between populations of Black Oak for all the characters scored. This means basically that northern populations of Black Oak are significantly different from southern populations at the 1% level in selected diagnostic morphological charters, thus supporting the general field observation of differences between these populations.

In general northern populations are more similar to the other northern populations of Black Oak, and southern populations are more similar to other southern populations of Black Oak. However, populations of Black Oak are not that simple. No two populations are exactly alike, Two populations from the same county may show differences in one or several characters. Table 2 is an attempt to show how individual stands on a south—north orientation can be compared by 13 morphological characters and shows how many of these characters each stand differed from any other stand studied. For example, the southern Indiana unglaciated population differed from the northern Indiana population in 7 out of 13 measured characters, but from the northern Indiana Lake Michigan population in 3 characters. It differed from most northern populations in 10 or 12 characters. I feel this table gives a good picture of the kinds of variability actually found when comparing field populations of Black Oak from localities that differ in climate,soils, and genetic history.

The field studies establish the fact that there are morphologic differences between Black Oak populations and that these differenc tend to follow a south—north trend. The more difficult questio however, is whether these morphological differences also indica genetic differences between and among population

To study and identify genetic trends between the populatio acorns were collected for 15 field populations from southern Indian to northern Michigan and compared under "common garden" conditio in East Lansing, Michigan. The results are summarized in Table

Twenty—four morphological characters were measured comparin Black Oak populations under "common garden". Only those showin signigicant differences, with several exceptions for comparison ar reported in this paper. Those showing significant difference includ leaf angle from stem the first year, the second flush of leaves, bo the first and second year, and leaf and shoot pubescence the secon year. Tree height, leaf width, and leaf length were significantl different between populations the first year but only height growt was significantly different the second year. Seedling emergence tim appeared to be related to seed origin with northern populatio having earlier germination. However, due to individual populatio variability in time of emergence there was no significant differenc between populations. In my opinion, a larger sample would probabl substantiate that these differences are real. In the spring of 1961 th early emerged seedlings were severley frost damaged. Using Chi—squa due to the small size of the populations it was found that Michiga

TABLE 2

*Overall differences in 13 morphological characters * between 14 populations of Black Oak. Each number indicates the number of characters in which one population differed from another at the 1% level (analysis of variance).*

	NII	NIM	NIE	SMI	CMM	NCM	CMI	SMH	CMH	NMM	NMT	NMH	NMI
So. Indiana	7	3	6	6	8	12	10	9	11	12	11	10	12
No. Indiana (NII — Interior)		4	5	2	5	9	6	7	7	10	6	9	11
No. Indiana (NIM — Lake Mich.)			4	4	7	11	9	9	9	11	8	10	11
No. Indiana (NIE — Northeast)				2	3	11	9	8	11	12	10	11	12
So. Michigan (SMI — Interior)					3	10	6	7	9	12	8	9	11
Central Michigan (CMM — Lake Mich.)						12	6 7	7	10	11	11	10	12
Central Michigan (NCM — North Central L. Mich.)							7	10	6	5	3	9	5
Central Michigan (CMI — Interior)								6	7	10	8	7	9
So. Michigan (SMH — Huron)									7	9	10	9	11
Central Michigan (CMH — Huron)										4	6	6	6
No. Michigan (NMM — Lake Mich. Honor)											3	8	5
No. Michigan (NMT — Lake Mich. Traverse City)												9	4
No. Michigan (NMH — Huron, Alpena)													8
No. Michigan (NMI — Interior, Indian River)													

* Morphological characters studied: total acorn height, acorn width, acorn length, acorn size, acorn shape, acorn cup height, ratio of acorn cup height/total acorn height, acorn cup scale shape, acorn cup fringe presence, twig pubescence, inner bark color, twig brittleness, tree form.

trees were less severly frost damaged, 26 out of 68 emerged seedling, as compared to 9 out of 12 emerged seedlings from Indiana. Also that Indiana trees had more tendency to fork after damage, 9 of 9 trees measured, as compared to 12 of 26 trees measured from Michigan. These differences were all at the 5% level of confidence.

The evidence of genetic trends on a south—north basis is not strongly supported by the nursery data. The second flush of leaves and shoot and leaf pubescence differences suggest the possibiliby of trends. The data on height and leaf size appear to be related to acorn size as the differences become less evident the second growing season, and are often no longer significant. This influence of acorn size on seedling differences has been reported by several investigators,(1 2 3). Since acorn size does differ on a south to north basis, and these size differences may have a genetic basis, the trends of height growth and leaf size do substantiate the possibility of a genetic basis for these differences.

In the East Lansing area, due to certain soil conditions, hybrid swarms of Black Oak are quite common. Small fruited trees can be found growing near normal or unusally large fruited trees. I observed and collected fruiting material from a number of these trees for four years and these acorn characteristics were consistent from year to year.

The fact that such a variety of fruiting forms occur and can survive under natural conditions suggest the probability of it occuring through—out the range of Black Oak in the glaciated area of Michigan, and that selective influences have favored these small—fruited forms in the northern part of the range. Other species of oaks in the Great Lake areas appear to show similar small—fruited northern forms and will be reported in future papers.

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TABLE 3

Population means of selected morphological characters of Black Oak populations growing under "common garden conditions" at East Lansing, Michigan.

First Planting, 24 acorns/row, 4, replications	Number of parent trees sampled	Emergence time, days av. for population after first nursery germination	Height, mm. Age 1	Height, mm. Age 2	Leaf length, mm. Age 1	Leaf length, mm. Age 2	Leaf width, mm. Age 1	Leaf width, mm. Age 2	Leaf Angle, Age 1, with horizontal plane	Percent trees with 2nd flush of leaves, July 5, 61; Age 1	Percent trees with 2nd flush of leaves, June 11, 62, Age 2	Leaf pubescence, 10 heavy pubescence 100 no pubescence, Age 2
Southern Indiana	2	18	73	380	88	141	59	89	41	0	30	10
Central Indiana	2	18		360	78	147	50	92	44	0	22	10
Northern Indiana	5	18	59	410	86	145	57	102	52	1	6	10
Southern Michigan (Interior)	2	15		330	79	149	50	93	43	25	3	10
Central Michigan (Near Lake Mich.)	4	9	58	410	79	134	52	88	45	41	35	10
Northern Michigan (Near Lake Mich., Honor)	2	13	50	460	70	136	40	81	39	55	72	24
Northern Michigan (Near Lake Mich. Traverse City)	6	11	38	300	66	131	41	83	39	43	48	11
Northern Michigan (Interior, Indian River)	3	10	39	410	73	124	48	77	36	44	53	20
Significance, Analysis of Variance, ns-not significant, 5% sig. at 5% level, etc.		ns	1%	5%	1%	ns	1%	ns	5%	1%	1%	1%
Second Planting, 32 acorns/row, 4 replications												
Seedbed A, mulched												
Northern Indiana (Near Lake Mich.)	8		127		91		57		38	57		24
Southern Michigan (Interior)	9		102		92		57		44	35		30
Central Michigan (Interior)	6		114		92		58		40	41		41
Northern Michigan (Near Lake Mich. Traverse City)	10		114		89		54		41	48		32
Significance			ns		ns		ns		ns	ns		1%
Seedbed B, unmulched												
Northern Indiana (Near Lake Mich.)	3		146		105		67		38	48		18
Southern Michigan (Interior)	6		95		88		58		32	25		24
Central Michigan (Near Lake Mich.)	5		127		90		56		32	54		30
North-central, Mich. (Near Lake Mich.)	7		108		84		53		33	56		33
Significance			5%		ns		ns		ns	ns		1%

Shoot pubescence.

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THE INFLUENCE OF ENVIRONMENTAL FACTORS UPON THE EXPRESSIVITY OF THE ABNORMAL ABDOMEN MUTANT OF DROSOPHILA MELANOGASTER

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ABSTRACT

The present research was conducted to determine if a range of environmental conditions would produce a range of concentrations of morphogenetic substance in the *Abnormal abdomen* mutant, thereby producing a range of phenotypic expressions which would correlate to a threshold phenomenon as described for the scute mutant. Experimental conditions included temperature and relative humidity.

It was found that both parameters studied produced at least one threshold value for the *Abnormal abdomen* mutant, for which different amounts of protein synthesis were supposedly responsible.

INTRODUCTION

In recent years, extensive research has been conducted on the *Abnormal abdomen* mutant ($A^{53}g$) of *Drosophila melanogaster.* This mutant was recorded first by Morgan (1) as a sex linked dominant showing abnormal pigment banding. This characteristic varied greatly, and Morgan (2) later assumed the expressivity of the trait to be due to the amount of water present in the food source. Hillman (3) showed that this mutant had a reduced expressivity when flies were raised under conditions of environmental stress, such as low humidity, low temperature, and overpopulation. He also showed that the expression of the abnormal condition was controlled by a major gene located on the distal end of the X chromosome, plus modifier genes on the X and third chromosome. Hillman, et al. (4) demonstrated that the expressivity of the mutant was also related to the synthesis of proteins, since higher expressivity and penetrance were exhibited in the presence of a higher concentration of protein in the fly. Rose and Hillman (5) showed that the modifier genes were responsible for producing the enzymes which then activated an increase in tRNA aminoacylation. This, plus unpublished data, led Hillman (3) to conclude either that different environmental conditions of stress stopped the increased protein synthesis by the modifier genes, or that the fly used up the excess protein, the final result being a more normal phenotype under adverse environmental conditions.

Rendel (6) and Sang (7) have shown similar mutations, penetrance, and expressivity to be the product of a threshold phenomenon. If the level of gene activity was above a certain threshold, a normal phenotype resulted; below this threshold, a mutant phenotype resulted. The phenotypic expression was determined by the amount of morphogenetic substance present. This morphogenetic substance, as described by Rendel (6), is any substance which initiates the development of a given phenotype.

Rendel (6) also stated that environmental factors could influence the amount of morphogenetic substance present. If a range of environmental effects produced a range of concentrations of morphogenetic substance, then a range of phenotypic expressivities should be observed. The purpose of the present research was to determine if a range of environmental conditions would produce a range in the expressivity of the *Abnormal abdomen* phenotype. Environmental conditions studied included temperature and relative humidity.

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FIGURE 1. Dorsal view of normal female abdomen; grade zero for Hillman's and author's grading system.

MATERIALS AND METHODS

Abnormal abdomen stocks of *Drosophila melanogaster* were obtained from Mr. Ralph Hillman, Temple University, Philadelphia, Pa. Flies were grown on Carolina Biological formula 4-24 blue Drosophila medium, with active dry yeast added.

The effects of temperature on expressivity of abnormal mutants were studied by culturing $A^{53}g$ flies at different temperatures. Experimental cultures were started with five pairs of flies grown in half-pint milk bottles containing 20 cc each of medium and water. Five populations of flies were begun, with each population composed of three bottles.

FIGURE 2. Dorsal view of female abdomen with part of one tergite missing; grade one for Hillman's and author's grading system.

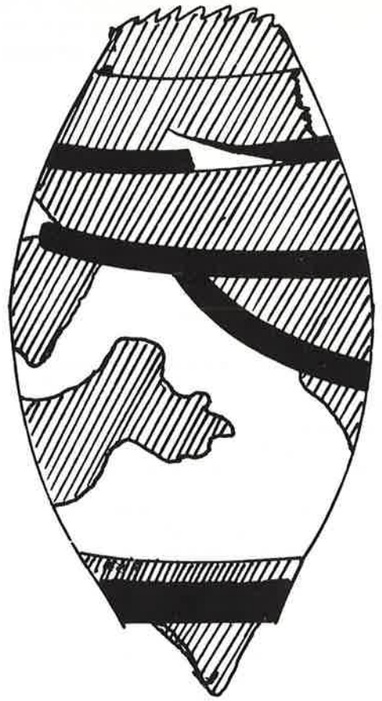

FIGURE 3. Dorsal view of female abdomen showing parts of four tergites missing; grade two under Hillman's grading system, grade four under author's grading system.

The flies were maintained initially at 26ºC for two to three days to assure that egg laying could occur. The flies were moved to incubators set at 32º, 30º, 26º, 22º, and 18ºC. Humidity in the incubators was maintained by placing water under the heating elements. Flies were not scored until complete emergence had occurred, thus avoiding temperature fluctuations.

Each of eight populations of $A^{53}g$ flies was exposed to different relative humidities. The values utilized were 21%, 35%, 43%, 51%, 58%, 66%, 72% and 84% (see sulfuric acid procedure of Buxton and Mellanby [8]). The humidity chambers were tested with a sling psychrometer attached to a mechanical stirrer to verify acutal humidity values. Three pairs of flies were placed in plastic vials 100 mm. by 34 mm. filled with 10 cc. each of medium and water. Five vials were used for each population. Adults were allowed to mate in the vials until eggs or first instar larvae were observed. The adults were then released, the vials were covered with cheesecloth, placed in the appropriate humidity chamber, and maintained at 25 - 27ºC. The next generation was scored after emergence was completed.

Penetrance and expressivity calculations were conducted according to Hillman (3), and the author's modified version. The modified version assigned a grade of zero to five for each fly, according to the number of tergites actually affected (Figures 1, 2, 3, and 4). The phenotypic range for each population was determined by calculating the mean expressivity for abnormal individuals using a frequency distribution of grouped data. A population with a low mean expressivity value was more normal in appearance than a population with a higher expressivity value.

RESULTS AND DISCUSSION

Hillman's stock flies had been grown on cornmeal - karo - agar medium seeded with live yeast. Experimental flies were grown on the Carolina medium. To assure that the medium used would not significantly alter results,penetrance and expressivity values were compared with those published by Hillman (3) for an inbred cross. The two expressivity values were statistically evaluated at the 95% confidence interval by conducting a Z test for the difference of the means; and the two penetrance values were evaluated by performing the same test for the difference of the percentages (Table 1). No significant differences were detected in the penetrance values for the total population, indicating that the medium used would not alter the results. Expressivity values for the males and females did not correspond to those previously published by Hillman (3). It was thought that a misinterpretation of Hillman's grading system was responsible for altering these results.For this reason the author's grading system was employed.

TABLE 1

Comparison of penetrance and expressivity in flies reared on cornmeal vs. instant medium.

Medium	Sex	Total Flies	Penetrance	Expressivity
Corn	M	1711	.95	1.46
4—24	M	203	.96	1.33
Corn	F	2347	.98	2.31
4—24	F	195	1.00	1.89
Corn	M & F	4058	.97	— —
4—24	M & F	398	.98	— —

Temperature effect data is shown in Table 2. No flies emerged from the cultures grown at 30º or 32ºC., although a number of attempts were made to produce results. Emergence of flies was

TABLE 2

Comparison of expressivity values of flies grown at different temperatures.

	No. of Flies			Average Expressivity Values					
				Hillman's System			Modified System		
ºC	M	F	Tot.	M	F	Tot.	M	F	Tot.
18	56	68	124	1.18	1.28	1.23	2.04	2.57	2.33
22	178	192	370	1.10	1.66	1.39	2.11	3.45	2.80
26	457	394	851	1.24	1.66	1.43	2.48	3.48	2.94

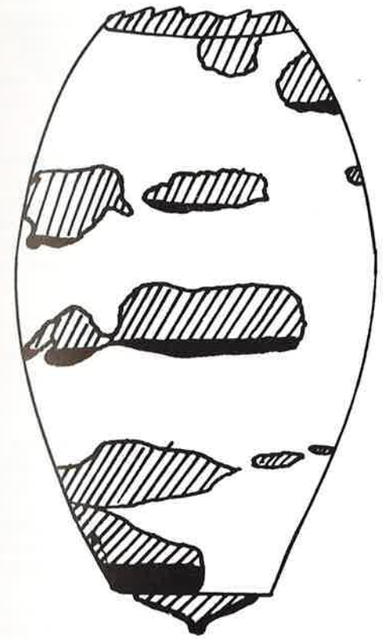

FIGURE 4. Dorsal view of female abdomen showing all five tergites incompletely formed; grade three under Hillman's system, grade five under author's system.

TABLE 3

Comparison of expressivity values of populations grown at different humidities.

	No. of Flies			Average Expressivity Values					
				Hillman's System			Modified System		
RH	M	F	Tot.	M	F	Tot.	M	F	Tot.
21%	156	152	308	.60	.97	.78	.72	1.62	1.16
35%	158	145	303	.75	1.14	.93	1.02	1.83	1.41
43%	156	149	305	1.11	1.69	1.39	1.57	3.46	2.50
51%	126	136	262	1.09	1.62	1.37	1.99	3.34	2.68
58%	97	103	200	1.05	1.44	1.25	1.73	2.52	2.14*
66%	119	126	245	1.02	1.32	1.18	1.89	2.75	2.33
72%	61	56	117	1.07	1.68	1.35	1.87	3.64	2.72
84%	25	32	57	1.00	1.25	1.14	1.64	2.16	1.93

*Flies grown under this humidity demonstrate a low expressivity value due to conditions of crowding in one vial. Expressivity value for the whole population without this vial is 2.38.

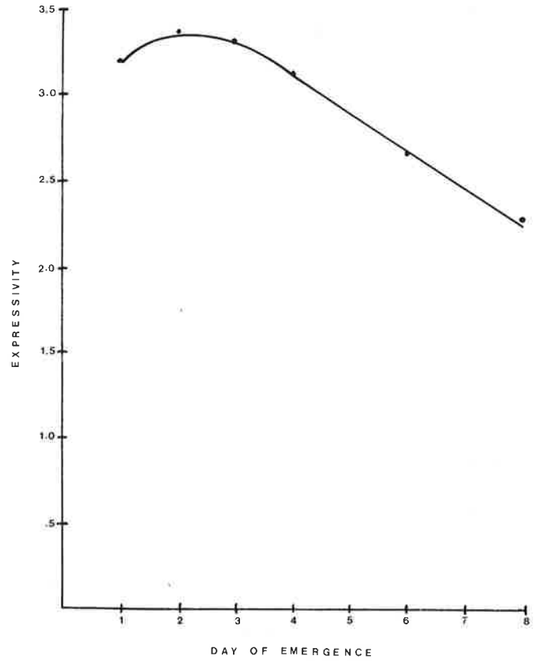

FIGURE 5. Daily expressivity values of a population of flies raised at a normal temperature and humidity.

expected at these temperatures since Powsner (9) reported that development beyond the egg period could not be completed at temperatures below 14º or above 33.1ºC. Cultures raised at 32ºC. were placed in the 26º incubator for a few days in the pupae stage, but no emergence occured at the lower temperature. Possibly a temperature of 30ºC and higher may be lethal to this mutant.

The traits of the 26ºC cultures were recorded daily. These daily observations recorded in Figure 5 reveal that the expressivity of the cultures declines after a few days. These observations correlate with findings of Hillman (3) and Morgan (2). Expressivity dropped as the culture medium aged and dried out.

The 22º and 18ºC cultures had expressivity values lower than the 26º population; the 18º population was significantly lower at the 95% confidence level than the other two, and the 22º population being just barely similar to the 26º population with a Z test value of 1.84 (a value below 1.96 needed to be similar). As the temperature approached 26ºC, expressivity of the mutant condition increased (Figure 6).

If a threshold phenomenon such as Rendel (6) described was expressed through temperature, it could be seen that the lower limit to the threshold would fall at approximately 22ºC. This assumption was made on the basis that the expressivity of the 22º population was just barely similar to that population grown at 26ºC. It was expected that populations grown at 30º and 32ºC would show that 30º was the temperature above which the second threshold lay. These assumptions were made on the basis that the optimum temperature for the development of *Drosophila* had been shown by Ludwig and Cable (10) to be 29.5ºC., while the development rate fell off above 30º regardless of the sex of the flies.

Results of the humidity effects were recorded in Table 3 and Figure 7. Expressivity values rose to 43% RH, remained at this general level until 72% RH, then declined. Statistical analysis at the 95% confidence interval for the difference of the means showed that the expressivity for 21% and 35% relative humidity were similar, whereas the expressivity for 35% and 43% were not. Each humidity value including 72% showed an expressivity value similar to that of 43%.

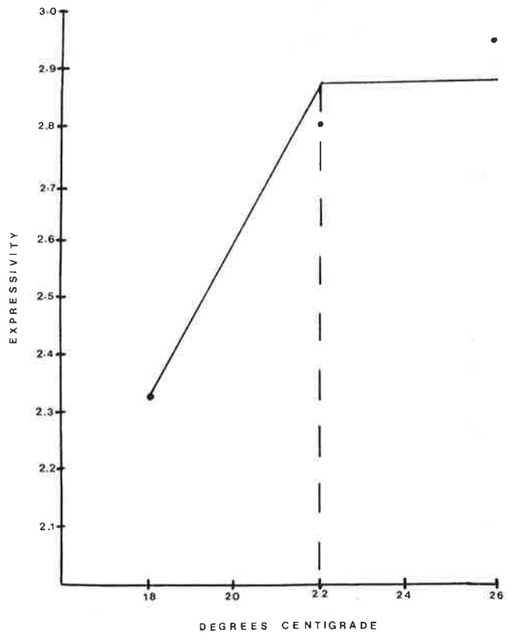

FIGURE 6. Effect of temperatures on expressivity.

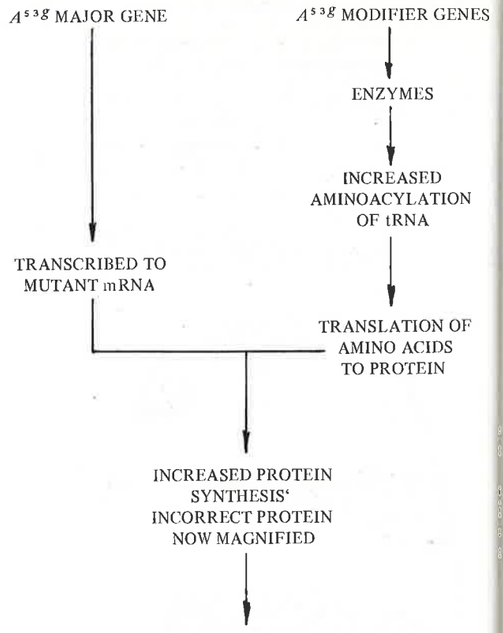

FIGURE 8. Schematic diagram showing how the phenotype is produced. Environmental changes vary the effect of the modifier gene side of the diagram.

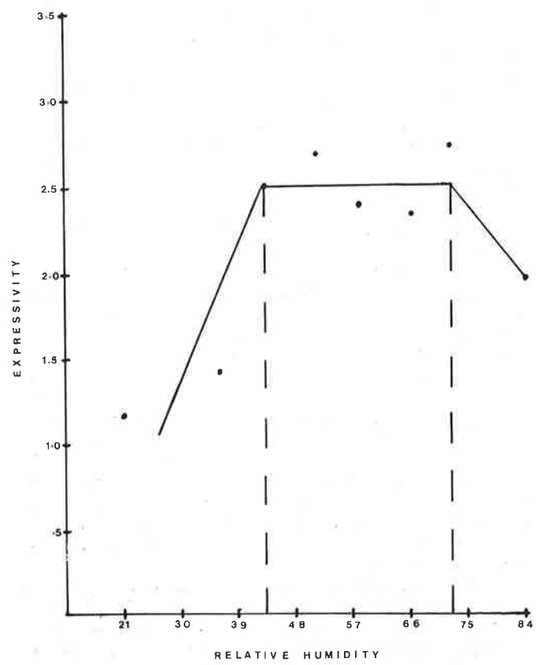

FIGURE 7. Effect of humidity on expressivity.

The population raised at 84% showed a significant difference from the population raised at 43%. If humidities between 40% and 70% were taken as "normal", it could be seen that normal humidity conditions gave expressivity values that were higher than expressivity values shown by populations grown at low or high humidities. It could be surmised from Figure 7 that Rendel's threshold values fall in the vicinity of 40% RH for the first threshold and 75% RH for the second threshold.

Rose and Hillman (5) have shown that the phenotype of the *Abnormal abdomen* mutant is controlled by the modifier genes in conjunction with the major gene. The $A^{33}g$ major gene is a gene for a structural protein in the hypodermal cells, which becomes transcribed in the mutant form into mRNA. Due to the action of the modifier genes, the increased aminoacylation of tRNA acts to produce an excess amount of protein, thus, an excess of the mutated hypodermal protein is produced, and the phenotype is observed (Figure 8). The environment produces changes in expressivity by altering the action of these genes.

Working with the scute mutant, Rendel (6) increased the gene activity by selectively mating flies with higher bristle numbers. The increased gene activity produced an increase in morphogenetic substance. It may be assumed that the morphogenetic substance was either a protein or an enzyme, or both, since genes are responsible for the joining of amino acids into polypeptide chains. Hillman, et. al. (4) have shown that the extremely mutated flies show a higher concentration of protein present, thus, the modifier genes bring about a higher expressivity value in the flies by causing production of an overabundance of protein.

The results of the present research imply that there may be an environmental scale which can be plotted against the expressivity to produce a threshold effect as Rendel (6) has done for gene activity. This first threshold shows a peak of protein synthesis above which phenotypic expression remains relatively constant. Threshold two is the point above which protein synthesis decreases, lowering the expressivity. Only under normal environmental conditions will maximum protein systhesis occur. These normal conditions may be approximated at humidity values between 40% and 75% RH, and the lower threshold for temperature at just below 22°C.

There are a number of possible explanations as to why the expressivity decreases in conditions of environmental stress. Hillman (11) believes that the fly larvae use up the extra protein in excessive movements due to the environment. Another explanation may be in a gene system as described by Rendel (12) for a number of mutated phenotypes. The picture as drawn by Rendel bears similarity to end product enzyme repression as described by Levy, Campbell, and Blackburn (13). If these findings are applied to Rendel's (6) and the present research, then the repressor substance, if released by environmental conditions of stress, produces its effect before the first and after the second threshold level. Between the first and second threshold level the repressor is not present due to normal environmental conditions, the modifier genes again produce an increase in aminoacylation of tRNA, and the operon or gene system again becomes active. If in assuming Rendel (6) to be correct in stating that environmental conditions play as much of a role in phenotypic expression as the amount of genetic products, then the environment may produce its effect through the repressor substance, if it exists.

Although the modifier genes of the $A^{33}g$ mutant do not describe an operon system in that they are not adjacent to each other on the same chromosome,a possible explanation of environmental interaction may be that the environment, under abnormal conditions, triggers a repressor substance which eliminates the protein synthesis by the modifier genes.

ACKNOWLEDGMENTS

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BENTHIC INSECT SPECIES COMPOSITION IN RELATION TO WATER QUALITY IN SINKING CREEK, CENTRE COUNTY, PENNSYLVANIA[1]

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ABSTRACT

Samples of benthic insects and water were taken 3 times in 1973 at 3 sites in Sinking Creek, Centre County, Pennsylvania. Gradients were observed for the chemical parameters pH, acidity, and total alkalinity. The number of benthic insect species present decreased as the pH decreased. The insects most adversely affected were Coleoptera, Ephemeroptera, and Plecoptera. Odonata and Trichoptera were less severely affected. Diptera seemed unaffected, and the number of taxa of Megaloptera increased in the more acidic waters of the stream.

INTRODUCTION

The effect of acid mine drainage upon benthic macroinvertebrates in streams has been studied by several workers. Parsons (1968) reported the effect of acid strip mine effluents on the ecology of a stream in central Missouri. In western Pennsylvania several streams polluted by mine drainage have been studied by Roback and Richardson (1969). Warner (1971) published the result of his study on the effect of acid mine drainage on Roaring Creek, West Virginia. Streams in northern and western Pennsylvania were studied by Weed and Rutschky (1972) and Koryak, Shapiro, and Sykora (1972), respectively.

There are few reported studies of streams displaying acid properties from "natural sources" such as decaying vegetation. Bick, Hornuff, and Lambremont (1953) described the biota of Bayou Lacombe, an acid stream in Louisiana that was later dredged for increased navigability. Geagen (1963) published the result of a study comparing the biota of the water body before and several years after dredging was completed.

The paucity of information on such non-mine drainage acid streams led to the present study of the benthic insects of Sinking Creek, Centre County, Pennsylvania. Sinking Creek is a small stream flowing six miles through forested land from Bear Meadows Bog into an artificial water body, Colyer Lake, before resuming its path through the farmland of Centre County. Underlying Sinking Creek and Bear Meadows Bog is bedrock of sandstone and shale making up the Juniata formation of the Ordovician Period (Kovar 1964).

The purpose of this study was to investigate the benthic insect species composition in acid waters free of high concentrations of ferrous and ferric iron and sulfate, common in acid mine drainage.

MATERIALS AND METHODS

Samples of benthic insects were taken 3 times at 3 study sites in Sinking Creek in 1973. The first collection was secured July 3, 5, and 6; the second September 17-18; and the third October 29 and November 5-6, 1973. Water samples were secured at the time of the insect collection at the particular site.

Each study site was a 15 yard length of stream containing pool an riffle areas, divided into 15 sampling subunits, each 3 yards long an 1/3 the width of the stream. One benthic insect sample was take from each sampling subunit, yielding a total of 15 samples per stud site per sampling date. A grand total of 135 samples was taken.

The benthic insect sampler consisted of 9" x 9" x9" galvanize smoke pipe "tee" with a 1-mm mesh net attached to the side apertur The sampler was placed on the substrate and the substrate within th confined area was disturbed with a stick for 1 minute. Benthic insec dislodged from the substrate and scraped from stones within th sampler were washed into the net by the stream current. Identica methods were employed at all study sites to avoid bias of the sample

The insects were killed in the field using a mixture of kerosene acetic acid, and ethanol (KAA solution). They were preserved in 70% ethanol. When possible, identification was made to species, otherwis to genus, tribe or subfamily. The identification of Elmidae (Coleopter was confirmed by Dr. Harley Brown, University of Oklahoma, an the identification of stoneflies was verified by Ms. Rebecca Surdic of the University of Utah. The specimens were deposited in The Fros Entomological Museum, Department of Entomology, The Pennsylvani State University.

Water samples were collected in 1 liter plastic bottles and transporte to the laboratory for analysis. Three parameters of water quality wer measured within 4 hours of collection of the samples. A Corning Model 5 pH Meter was used to measure pH and end point of acidit and total alkalinity titrations. Acidity was measured to the end poin pH 8.3 and total alkalinity was measured to end points 4.5 and 4. (potentiometric method for low alkalinity), following procedure outlined in *Standard Methods* (Taras *et al.* 1971).

DESCRIPTION AND CHEMICAL CONDITIONS OF STUDY SITES

Each of the study sites in Sinking Creek was selected for ease of access and the opportunity to observe a gradient of chemical conditions. The study sites were within 6 miles of each other, thus weather conditions were essentially similar at each study site. There was a difference of only a few degrees Celsius in water temperature among the study sites.

Figure 1 presents the measured values for acidity, total alkalinity, and pH of the water at the study sites.

STUDY SITE 1

Study Site 1 was selected 0.5 miles below Colyer Lake near a small bridge. Here, Sinking Creek is bordered by grasslands and old fields with trees along its banks. The stream is 7-10 yards wide and 6 inches to 2 feet deep. There are alternate pool and riffle areas with a substrate of silt, sand, gravel, and stones up to 6 inches long.

Study Site 1 most nearly represented what might be called a "normal" stream in that the pH was circum-neutral with the lowest acidity and highest total alkalinity measured. The acidity increased through the sampling dates.

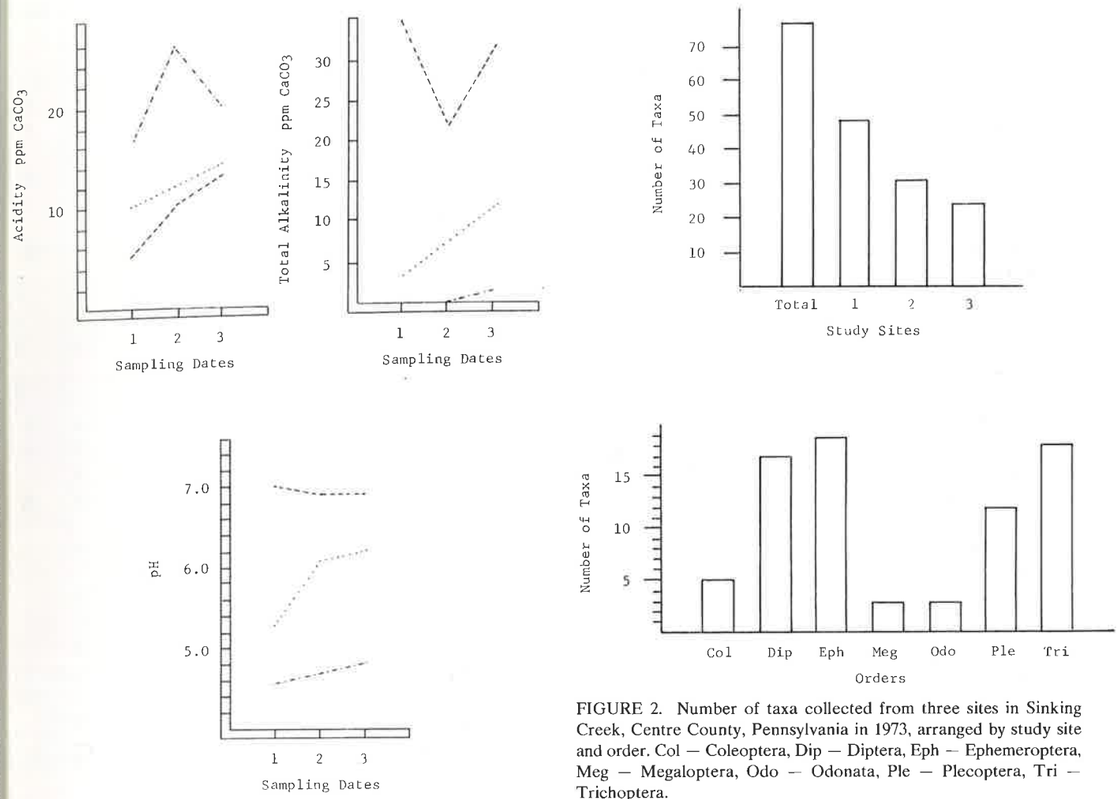

FIGURE 1. Mean values for acidity, total alkalinity, and pH of water collected from three sites in Sinking Creek, Centre County, Pennsylvania in 1973. Sampling dates: 1—7/3, 7/5, or 7/6; 2—9/17 or 9/18; 3—10/29, 11/5, or 11/6. Site 1 - - -, Site 2 · · ·, Site 3 –·–·–

STUDY SITE 2

Study Site 2 was selected where a pipeline crosses underneath Sinking Creek in a forest clearing, 2.8 miles below Bear Meadows Bog. The stream is 5-7 yards wide and 6 inches to 2 feet deep. There are alternate pool and riffle areas with a substrate of silt, sand, gravel, and stones up to 1 foot long.

Study Site 2 displayed values for pH, acidity, and total alkalinity intermediate between those of the other study sites. Values for the chemical parameters rose through the sampling dates.

STUDY SITE 3

Study Site 3 was selected 0.5 miles below Bear Meadows Bog in forest. The stream is 3-5 yards wide and 6 inches to 2 feet deep. There are alternate pool and riffle areas with a substrate of sand, gravel, and stones up to 1.5 feet long.

Study Site 3 displayed the lowest pH and total alkalinity values and the highest acidity values of the study site. Total alkalinity was virtually non-existent, a measurable quantity present only in November. The value of pH rose through the sampling dates.

FIGURE 2. Number of taxa collected from three sites in Sinking Creek, Centre County, Pennsylvania in 1973, arranged by study site and order. Col — Coleoptera, Dip — Diptera, Eph — Ephemeroptera, Meg — Megaloptera, Odo — Odonata, Ple — Plecoptera, Tri — Trichoptera.

BENTHIC INSECT SPECIES COMPOSITION

The data for the number of insect taxa collected at each study site are presented in Figures 2 and 3. Table 1 lists the collected taxa.

Specimens of 77 taxa in 7 orders of Insecta were collected in Sinking Creek. Nineteen ephemeropteran taxa were collected, followed closely by Trichoptera and Diptera, with 18 and 17 taxa, respectively. Plecoptera was represented by 12 taxa, Coleoptera by 5 taxa, and Megaloptera and Odonata each by 3 taxa.

The number of benthic insect taxa collected decreased toward Bear Meadows Bog. Study Site 1, furthest from the bog, yielded 48 taxa, the largest total, predominatly Ephemeroptera, Trichoptera, and Diptera. Study Site 2 provided 31 taxa, mostly Plecoptera, Trichoptera, and Diptera. Twenty-four taxa were collected at Study Site 3, mostly Diptera and Trichoptera. Seven taxa were common to all study sites, 4 of them dipteran.

The insects most affected by the changing conditions of Sinking Creek nearing Bear Meadows Bog appeared to be Coleoptera, Ephemeroptera, and Plecoptera. There were 4 coleopteran species collected at Study Site 1: *Optioservus ovalis, O. trivittatus, Stenelmis crenata,* and *Psephenus herricki.* Further upstream at Study Site 2, only 2 species were collected: *Optioservus immunis* and *O. ovalis.* None were collected at Study Site 3.

The number of taxa of Ephemeroptera declined toward the bog. Sixteen species and genera were collected at Study Site 1, but only 3 species were collected upstream at Study Site 2: *Ephemerella funeralis, Hexagenia recurvata,* and *Stenonema pulchellum.* Only *Ephemerella temporalis* was collected at Study Site 3.

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[2]This study completed as partial fulfillment of requirements for M.S. degree, The Pennsylvania State University, 1974.

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TABLE 1

Taxa collected from three sites in Sinking Creek, Centre County, Pennsylvania in 1973.

	Site 1	Site 2	Site 3
Coleoptera			
Elmidae			
Optioservus immunis		X	
O. ovalis	X	X	
O. trivittatus	X		
Stenelmis crenata	X		
Psephenidae			
Psephenus herricki	X		
Diptera			
Chironomidae			
Chironomus sp.			X
Diamesinae		X	
Eukiefferiella sp.		X	
Orthocladiinae	X	X	X
Pentaneurini	X	X	X
Polypedilum sp.	X		
Procladius sp.			X
Smittia sp.		X	
Tanytarsini	X		X
Tribelos sp.			X
Trichocladius sp.			X
Simuliidae			
Simulium sp.	X	X	X
Tipulidae			
Antocha saxicola	X		
Dicranota sp.	X	X	X
Pedicia sp.	X	X	
Tipula sp.	X		X
Ephemeroptera			
Baetidae			
Baetis sp.	X		
Pseudocloeon sp.	X		
Ephemerellidae			
Ephemerella bicolor	X		
E. cornuta	X		
E. deficiens	X		
E. funeralis		X	
E. invaria	X		
E. temporalis			X
Ephemeridae			
Ephemera guttulata	X		
Hexagenia recurvata		X	
Heptageniidae			
Stenonema bipunctatum	X		
S. fuscum	X		
S. heterotarsale	X		
S. ithaca	X		
S. luteum	X		
S. pulchellum	X	X	
S. vicarium	X		
Leptophlebiidae			
Paraleptophlebia	X		
Siphlonuridae			
Isonychia sp.	X		
Megaloptera			
Corydalidae			
Nigronia fasciatus			X
N. serricornis	X		X
Sialidae			
Sialis sp.	X	X	X
Odonata			
Aeshnidae			
Boyeria vinosa	X		X
Agrionidae			
Calopteryx maculata	X		
Gomphidae			
Lanthus parvulus		X	
Plecoptera			
Nemouridae			
Leuctra sp.		X	X
Nemoura sp. 1		X	X
Nemoura sp. 2		X	
Taeniopteryx sp.	X	X	X
Peltoperlidae			
Peltoperla arcuata	X	X	
Perlidae			
Acroneuria abnormis		X	
A. carolinensis	X		
A. xanthenes		X	
Phasganophora capitata	X		
Perlodidae			
Isogenus sp.		X	
Pteronarcidae			
Pteronarcys biloba	X		
P. proteus	X		
Trichoptera			
Hydropsychidae			
Cheumatopsyche sp.	X		
Diplectrona modesta		X	X
Hydropsyche sp.	X	X	X
Lepidostomatidae			
Lepidostoma sp.			X
Limnephilidae			
Drusinus sp.		X	X
Neophylax sp.	X		
Philopotamidae			
Trentonius distinctus	X		
Phryganeidae			
Oligostomis ocelligera			X
Ptilostomis sp.	X		
Psychomyiidae			
Nyctiophylax species A			X
Polycentropus centralis	X		
P. cinereus	X		
Rhyacophilidae			
Glossosoma sp.	X		
Rhyacophila amicis		X	
R. atrata		X	
R. fuscula	X	X	
R. minora		X	
R. nigrita		X	

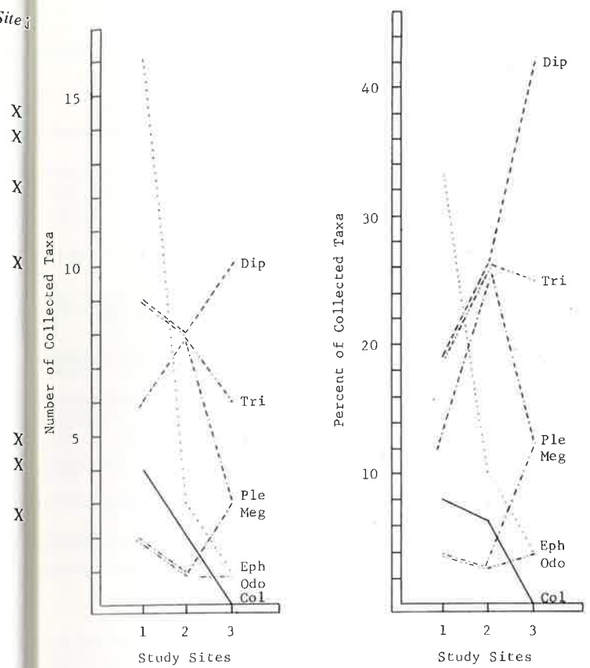

FIGURE 3. Number and percentage of taxa collected from three sites in Sinking Creek, Centre County, Pennsylvania in 1973: ————Col — Coleoptera; - - - -Dip—Diptera; · · · Eph — Ephemeroptera; – – · – · Meg — Megaloptera; – · · – · · – · · Odo — Odonata; – – · – – · Ple — Plecoptera; — · · — · · — · · Tri — Trichoptera.

Although the number of Plecoptera species and genera increased from Study Site 1 to Study Site 2, the number dropped at Study Site 3. Six taxa, the majority Perlidae and Pteronarcidae, were collected at Study Site 1. Eight species and genera, including 4 Nemouridae, were collected at Study Site 2. At Study Site 3, only 3 species and genera were collected, all Nemouridae.

Two other orders, Odonata and Trichoptera, declined in the number of taxa collected toward the bog, but not as dramatically as the Coleoptera, Ephemeroptera, and Plecoptera. Of the Odonata, the species collected at Study Site 1 were *Boyeria vinosa* and *Calopteryx maculata. Lanthus parvulus* was the only species collected at Study Site 2 and *Boyeria vinosa* was the only species collected at Study Site 3. Nine taxa of Trichoptera within 6 families were collected at Study Site 1. But, at Study Site 2, 8 taxa were collected, including 5 species of *Rhyacophila.* At Study Site 3, more equal distribution among families was recorded: 6 taxa within 5 families were collected.

Nearly equal numbers of dipteran taxa were collected at the study sites and the number of taxa of Megaloptera increased toward Bear Meadows Bog. At Study Site 1, 9 taxa of Diptera were collected. Eight taxa were collected at Study Site 2 and 10 taxa were collected at Study Site 3. Two megalopteran taxa were collected at Study Site 1: *Nigronia serricornis* and *Sialis* sp. Only *Sialis* sp. was collected at Study Site 2, but *Nigronia fasciatus, N. serricornis,* and *Sialis* sp. were collected at Study Site 3.

DISCUSSION AND CONCLUSION

The ability of stream water to absorb quantities of acid or alkali with minimal change in pH is governed by the carbonate - bicarbonate buffer system (total alkalinity) of the water. The "strength" of the buffer system (bicarbonate content) depends on two factors: the calcium content of underlying strata and carbon dioxide content of the water (Ruttner 1953). Absence of calcium or low concentration of carbon dioxide will weaken the buffer system. Poorly buffered water will display a large change in pH with addition of acid or alkali.

Bog water is acid from decaying vegetation and low in concentration of dissolved electrolytes (Ruttner 1953). Sinking Creek, with its origin in Bear Meadows Bog, is acid with few dissolved electrolytes (range of specific conductance measured in 1973, 23-92 micromhos/cm @ 25°C. In addition, total alkalinity is low, perhaps due to the absence of carbonate rocks in the vicinity.

Total alkalinity and pH were lowest near Bear Meadows Bog and increased proceeding downstream. Acidity was highest near Bear Meadows Bog and decreased proceeding downstream. Water of non-bog origin flowing into Sinking Creek would dilute acid water from the bog, decreasing acidity and increasing pH and total alkalinity.

Fewer insect taxa were collected as the pH of Sinking Creek decreased nearing Bear Meadows Bog. Dunson and Martin (1973) reported that the number of fish species decreased nearing the bog. In addition, they cited a preliminary study by Dr. D. Hales, in which he collected 14 species of aquatic insects near Study Site 2 and 12 species near Study Site 3, very few species common to both areas. Data from the present study support his work. Of 77 taxa collected from 3 study sites, 9% were common to all study sites. Forty-four taxa were collected from Study Sites 2 and 3, 25% common to both sites.

The hydrogen ion concentration of water is one of those environmental factors that are very strikingly linked to the species composition of communities and their life processes (Ruttner 1953). Although other undetermined environmental factors such as dissolved oxygen or light intensity may be involved, it appears that increasing acidity and decreasing total alkalinity of Sinking Creek nearing Bear Meadows Bog are potent factors in restricting the number of taxa of aquatic insects present. Coleopteran, ephemeropteran, and plecopteran taxa seem to be most sensitive and dipteran and megalopteran taxa least sensitive to differing water quality at several locations in Sinking Creek.

In order to determine the limiting effect of acidity at various locations in Sinking Creek, further studies are possible in two directions. Specimens of selected taxa could be collected and exposed to acid water of differing concentration in the laboratory. Another method, perhaps better, would be to place cages of aquatic insects directly into Sinking Creek and to monitor survival. The second procedure would have the advantage of observing the insects in stream conditions difficult to duplicate in the laboratory. These experiments would clarify the significance of pH as a factor defining benthic insect species composition in Sinking Creek.

SUMMARY

Sinking Creek displayed gradients in the measured quantities of pH, acidity, and total alkalinity and in the number of benthic insect taxa collected from its source in Bear Meadows Bog to below Colyer Lake, a distance of 6 miles.

In its headwaters, Sinking Creek reflected the influence of the acidity of bog water with a pH range of 4.6—4.8, an acidity range of 16—26 ppm $CaCO_3$, and a total alkalinity range of 0—1 ppm $CaCO_3$. Near the bog, the insect species composition was mostly Trichoptera and Diptera, accounting for 16 of the 24 taxa collected.

Midway between Bear Meadows Bog and Colyer Lake, the water of Sinking Creek displayed a pH range of 5.3—6.2, an acidity range of 10—14 ppm $CaCO_3$, and a total alkalinity range of 3—12 ppm $CaCO_3$. These values indicated this part of Sinking Creek to be intermediate in chemical conditions between the acid environment near Bear Meadows Bog and the essentially more "normal" stream environment below Colyer Lake. Thirty-one benthic insect taxa, mostly Trichoptera, Plecoptera, and Diptera, made up the species composition at this location in Sinking Creek.

Below Colyer Lake, the chemical conditions of the water consisted of a pH range of 6.9—7.0, an acidity range of 5—13 ppm $CaCO_3$, and a total alkalinity range of 22—35 ppm $CaCO_3$. Forty-eight taxa of benthic insects were collected here, 16 taxa of Ephemeroptera, 9 taxa of Trichoptera, and 9 taxa of Diptera making up the majority.

The orders which seemed most affected by increased acidity or other undetermined water quality factors in Sinking Creek were Coleoptera, Ephemeroptera, and Plecoptera. Less severely affected were Odonata and Trichoptera. Dipteran taxa appeared to be little affected and the number of taxa of Megaloptera increased in the more acid portion of the stream. Only 9% of the 77 collected taxa were common to the 3 study sites, 4 of them dipteran.

ACKNOWLEDGMENTS

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THE EFFECT OF DEER BROWSE ON SEEDLING REGENERATION IN THE ALLEGHENY NATIONAL FOREST, PENNSYLVANIA

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ABSTRACT

Deer have been found to have a profound effect on the composition of the understory seedlings above one decimeter in height. This can be attributed to browse pressure during winter when these plants are the most readily available food source above the snow. The ground cover composition was found to also be affected with an average percentage ground cover of 60.8 percent within deer exclosures and only 30.5 percent in areas exposed to deer. It was also found that there was a greater species diversity in plots protected from deer with some species completely eliminated from deer exposed areas. The composition of the ground cover species both in and out of the exclosures showed no change during a three month study in the summer of 1974.

INTRODUCTION

It has long been known that animals, especially deer, affect the vegetative composition of forests due to their eating habits and trampling, (1—7). This is important from an economic as well as a forest composition standpoint when the forest is being managed for lumber production. The Allegheny National Forest is such a forest. The deer browse problem is extensive; (7—10), even though the forest has been under a tight management program since it was almost completely cut over during the period of 1890—1920.

This study seeks to measure the effect of deer upon the regeneration of the major tree species in the forest. It also demonstrates the effect of the deer on the quantitative and qualitative composition of the forest ground cover species.

STUDY AREA AND METHODS

A major portion of the Allegheny National Forest is composed of the cherry—maple or Allegheny Hardwood forest type. (12), which comprises some twelve million acres of second growth forest in Pennsylvania and adjacent southwestern New York. The cherry—maple forest type is characterized by *Prunus serotina, Acer rubrum, Acer saccharum,* with *Fraxinus americana, Betula alleghaniensis, Fagus grandifolia,* and *Tsuga canadensis* as common associates. It is the direct result of the extensive cutting of the hemlock—white pine northern hardwoods around the turn of the century.

The Vandergrift Corners Deer Habitat Research Area was established by the United States Forest Service near the center of the Allegheny National Forest in Warren County (Fig. 1). It varies in elevation from 1500—1900 feet (Fig. 2). The area is characterized by long cold winters and rather mild short summers. The growing season is from four to five months.

Within the research area the Forest Service has constructed a series of deer exclosures in various habitat types. This study is confined to eight locations which are representative of the cherry—maple forest type. Each location consists of two 0.05 acre plots selected on the basis of similarities of plot physical characteristics and the composition of overstory and understory species. At each site location one of the plots is indicated by painted markers on the trees while the other has been enclosed in an eight foot deer fence. The result is a series of paired sample plots one exposed to deer browse the other not.

The seedlings from one decimeter to two meters in height within each of the paired plots were recorded as to species and numbers. For the understory evaluation, within each plot a ten meter transect was permanently established crossing the center of the plot. Eleven micro-plots, two decimeters by five decimeters, were established along this transect. From these eleven micro-plots the frequency and ground cover values for all the understory species were determined using a technique originated by Daubenmire (13). These frequency and ground cover determinations were made four times during the 1974 growing season at one month intervals, May through August.

FIGURE 1. Location of Vandergrift Corners Deer Habitat Research Area.

RESULTS AND DISCUSSION

The frequency and ground cover values for the various species at any of the sample plots were compared over the four sample times and found to be identical. This was true to the extent that the

TABLE 1

Comparison of occurrence of groundcover species for eighty-eight micro-plots both outside and inside deer exclosures – outside/inside.

Species	Plot 1	7	12	14	20	22	29	38	Average occurrance of species per sub-plots
Black Cherry *(Prunus serotina)*	10/11	11/11	9/5	5/7	8/11	9/10	11/9	5/11	8.5/9.4
Red Maple *(Acer rubrum)*	10/3	9/3	8/7	"/„	4/4	1/1	5/6	8/1	5.6/3.1
Sugar Maple *(Acer saccharum)*	1/4	"/1	"/„	"/„	"/„	"/2	"/„	"/2	0.1/1.1
Striped Maple *(Acer pennsylvanicum)*	"/1	"/2	"/„	"/„	"/1	"/„	"/„	1/„	0.1/0.5
Fern *(Polypodioceae)*	"/5	"/2	"/„	9/10	1/1	1/„	"/1	11/11	2.8/3.8
Sedge *(Carex* spp.*)*	3/1	1/1	"/„	"/1	1/1	"/„	"/„	"/„	0.6/0.5
Ground Pine *(Lycopodium* spp.*)*	"/8	"/1	2/„	"/„	"/4	"/„	"/„	"/„	0.3/1.6
Northern White Violet *(Viola pallens)*	8/7	5/2	5/2	"/5	1/2	"/1	3/1	"/8	2.8/3.5
Partridge Cherry *(Mitchella repens)*	"/5	"/2	"/1	"/„	"/„	"/„	"/„	"/4	0/1.5
Wild-lily of the Valley *(Maianthemum canadense)*	"/2	1/1	"/4	"/„	"/„	"/„	"/1	"/4	0.1/1.5
Wood Sorrel *(Oxalis montana)*	"/„	"/„	"/„	5/11	"/1	"/„	1/1	9/11	1.8/3.0
False Solomon's Seal *(Smilacina racemosa)*	"/„	"/„	"/„	"/2	"/„	"/„	"/„	"/„	0/0.3
May Apple *(Podophyllum peltatum)*	"/1	"/1	"/„	"/4	"/„	"/„	"/„	"/„	0/0.8
Total number of species per plot	5/10	4/11	5 5/5	3/8	5/8	3/4	4/6	5/8	4.3/7.5

individual cover values within the micro-plots were virtually identical over the four visit time span. The fact that this was true both inside and outside the exclosures indicated that browse was having little if any effect on the understory during this period of the year.

However, when the frequency of the fourteen ground cover species was compared between the paired plots it was found that with the exception of *Acer rubrum* and *Carex* spp. all of the species had a higher average frequency withing the exclosures than without (Table 1). *Mitchella repens, Smilacina racemosa,* and *Podophyllum peltatum* were not found in any of the eighty eight micro-plots outside the exclosures and *Acer saccharum, Maianthemum canadense* were found in only one out of the eighty eight sample locations.

It also can be seen that with the exception of plot 12, where five ground cover species occurred in the exclosures and five outside, there always was a greater number of species in the exclosures than in the paired location outside the exclosure. This would indicate that the overall species diversity of the site is affected by the browsing pressure.

When the percentage ground cover figures are compared from the subplots taken inside and outside the exclosures (Table 2) similar results are observed. Only *Acer rubrum* has a greater average percentage ground cover value outside the exclosures. The overall average percentage ground cover taking all species into account is twice as high inside as opposed to outside the exclosures.

There seems to be a high correlation in the distribution of the *Polypodioceae* and *Oxalis montana.* Plots 14 and 38 had very high frequency and cover values for both these species. These high values could be attributed to the rather moist rocky location of plots 14 and 38. However, this could only be evaluated if there was a greater number of plots containing high values for these two species.

The most striking difference between plots located inside and outside the exclosures can be seen when the number of seedlings from one decimeter to one meter in height are compared (Table 3). Every species encountered was found in greater numbers inside the exclosures. About the only species that was found outside the exclosures in substantial numbers is that of *Fagus frandifolia.* This difference in

TABLE 2

Comparison of the percent of groundcover species for eight study plots both outside and inside deer exclosures — outside/inside.

Species	Plot 1	7	12	14	20	22	29	38	Average Percent Cover of Species
Black Cherry *(Prunus serotina)*	25.6/19.3	11/38.6	11/5	5/9	8/12	10/11	16/9	5/25.6	11.4/16.2
Red Maple *(Acer rubrum)*	1/+	1/+	1/1	"/„	1/+	+/+	1/1	+/+	0.6/0.3
Sugar Maple *(Acer saccharum)*	+/+	"/+	"/„	"/„	"/„	"/+	"/„	"/+	+/+
Striped Maple *(Acer pennsylvanicum)*	"/9	"/2	"/„	"/„	"/1	"/„	"/„	+/„	"/1.6
Fern *(Polypodioceae)*	"/37	"/3	"/„	24/38	1/9	1/„	"/1	60/87	10.8/21.8
Sedge *(Carex* spp.*)*	3/1	1/1	"/„	"/3	1/1	"/„	"/„	"/„	0.6/0.8
Ground Pine *(Lycopodium* spp.*)*	"/25	"/1	3/„	"/„	"/+	"/„	"/„	"/„	0.4/3.2
Northern White Violet *(Viola pallens)*	18/16	5/2	1/1	"/8	1/2	"/1	3/7	"/13	3.5/6.2
Partridge Berry *(Mitchella repens)*	"/6	"/„	"/2	"/„	"/4	"/„	"/„	"/1	"/1.6
Wild-lily of the Valley *(Maianthemum canadense)*	"/„	"/2	1/1	"/2	"/„	"/„	"/1	"/4	.0.1/1.2
Wood Sorrel *(Oxalis montana)*	"/„	"/„	"/„	16/36	"/+	"/„	+/+	9/28	3.1/8.0
Total percent cover per plot	47.6/113.3	18/49.6	17/10	45/96	12/29	11/12	20/19	74/158	30.5/60.8

seedling composition could be attributed to the fact that the seedlings above one decimeter in height would be exposed to deer browse above the snow much of the winter season when other food sources are quite scarse. Such feeding habits have been observed in deer (5).

The number of seedlings from one to two meters in height was very low both in and out of the exclosures. Twelve seedlings were found in the exclosures and one outside. The small number in the exclosures could be attributed to the fact that the exclosures were only erected during the 1971-1972 winter season. Therefore, the seedlings in the exclosures have been protected from the deer browse only two winter seasons. This would not be sufficient time to allow the complete reestablishment of the seedling understory of the enclosed areas, especially in the taller height classes.

The lack of sufficient seedlings to sustain the present forest composition is apparent at almost any location in the area studied (Fig. 3). It likewise seems apparent that if the Allegheny National Forest is going to continue to yield quality wood products and deer habitat an alternative management procedure is necessary.

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TABLE 3

Comparison of one decimeter to one meter tall seedings from eight study plots both outside and inside deer exclosures — outside/inside.

Species	Plots 1	7	12	14	20	22	29	38	Total per Species
Stripped Maple *(Acer pennsylvanicum)*	10/43	"/8	"/„	"/1	"/„	"/„	"/2	"/„	10/54
Black Cherry *(Prunus serotina)*	1/10	"/„	"/„	"/„	"/„	"/„	"/„	"/1	1/11
Red Maple *(Acer rubrum)*	1/2	"/„	"/„	"/1	"/„	"/„	"/„	"/„	1/3
American Beech *(Fagus grandifolia)*	3/10	9/28	"/31	1/1	15/22	"/„	"/31	"/5	28/128
Sugar Maple *(Acer saccharum)*	"/8	1/6	"/1	"/1	"/3	"/1	"/2	"/1	1/23
Yellow Birch *(Betula alleghaniensis)*	7/19	"/„	"/„	"/1	"/„	"/„	"/„	"/„	1/20
White Ash *(Fraxinus americana)*	"/„	"/„	"/„	"/„	"/„	"/4	"/„	"/1	"/5
Total per plot	22/92	10/42	0/32	1/5	15/25	0/5	0/35	0/8	48/244

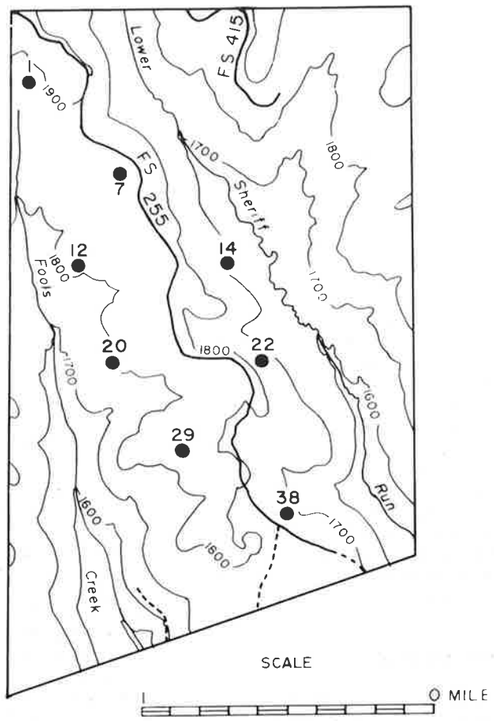

FIGURE 2. Vandergrift Corners Deer Habitat Research Area. Numbers indicate the location of each of the eight paired sample plots.

FIGURE 3. Sample plot located outside deer exclosure. Note lack of understory development.

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CYCLIC FLUCTUATIONS IN ODOR DETECTION BY FEMALE RATS AND THE TEMPORAL INFLUENCES OF EXOGENOUS STEROIDS ON OVARIECTOMIZED RATS

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ABSTRACT

Female rats trained to discriminate the odor of cyclopentanone were tested at concentrations from one to four log units below saturated vapor. Cyclic fluctuations in odor discrimination corresponded to the phases of estrus cycle. Animals were then ovariectomized. After ovariectomy no cyclicity in performance appeared. Sham operated animals continued cyclic performance. Ovariectomized animals received 5.0 μg of estradiol or 2.0 mg of progesterone. Estradiol treatment increased performance; the progesterone depressed it. Two weeks later the groups were reversed and retested with results comparable to the first test series.

INTRODUCTION

The phenomenon of cyclic variations in olfactory sensitivity corresponding to the sexual cycle has been reported by various investigators. Le Magnen (1, 2) has shown and Vierling *et al.* (3) have confirmed that the ability of women to detect Exaltolide fluctuates during the menstrual cycle. The lowest threshold (greatest sensitivity) occuring on or near the day of ovulation. This is when estrogen should be very near peak circulation levels (4). Although in Le Magnen's (2) work the phenomenon was reported to be specific for Exaltolide and covered a remarkable range of five log units of concentration, Veirling *et al.* (3) could not confirm this range but instead reported a magnitude of less than one log unit of concentration.

In contrast to these findings Schneider *et al.* (5) reported higher thresholds in women for citral during menstruation, with no change around the time of ovulation. Furthermore Le Magnen (6) was unable to detect fluctuations in the performance of normal female rats on an odor detection task. However this last point has been challenged by Pietras *et al.* (7). Cyclicity has been observed in normal female rats detecting cyclopentanone (7). In a more recent study (8) these authors have also reported cyclic variations in the performance of normal female rats for the odors of Eugenol, Alpha Ionone, Exaltolide and again for cyclopentanone, with the highest performance (greatest sensitivity) occuring on the day of estrus. When male rats were tested however, no significant variations in performance could be found, and ovariectomy of the female rats eliminated their cyclic fluctuations. Such cyclic variations in performance were also eliminated by inducing pseudopregnancy in normal females, resulting in performances which were surpressed to chance levels.

Despite some apparent inconsistencies, it appears probable that cyclic variations in olfactory sensitivity, corresponding to the sexual cycle, do exist (8, 9). But the magnitude of these variations and the influences of circulating levels of gonadal steroids remains uncertain. In addition there appears to be only two reports dealing with experimentally modified hormone levels (6, 8), and these are somewhat contradictory. Le Magnen's (6) work did not include accurate olfactometric measurements and it may be that such sensitive techniques are required to reveal subtle fluctuations in olfactory acuity.

The series of experiments described in this work were designed to first measure any changing sensitivities of female rats for an olfactory stimulus (cyclopentanone) at a constant concentration; second, to eliminate any fluctuating sensitivities via ovariectomy (removal of the primary source of estrogen and progesterone) and third, to characterize any modified sensitivities in ovariectomized rats due to the temporal effects of a single injection of estradiol or progesterone. The hypothesis is that estrogen facilitates odor detection while progesterone depresses it.

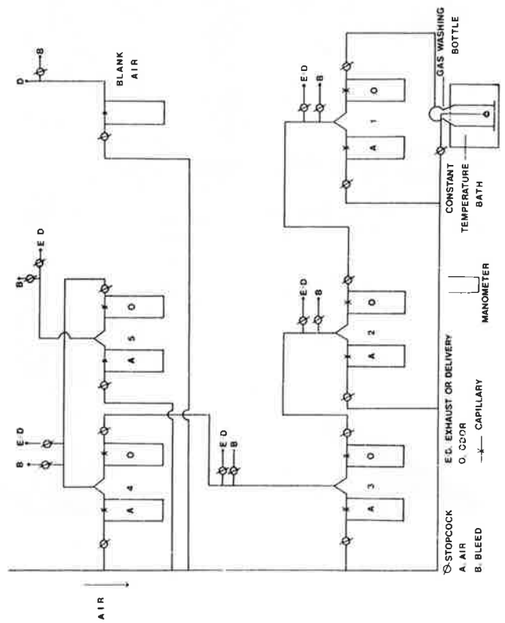

FIGURE 1. Olfactometer.

MATERIALS AND METHODS

Olfactometer. The olfactory stimulus (cyclopentanone) in this study was produced by a five stage air dilution olfactometer (Fig. 1). Compressed air, delivered from a pressure regulator, was purified and dried by passing it through tubes of glass wool, activated charcoal and silica gel. After purification the air was sent into pressure buffering flasks to remove excessive pressure fluctuations. From here the air was directed into the olfactometer, which included a gas washing bottle (containing the liquid stimulus) housed in a constant temperature bath held at 23°C. A blank air meter was also included in the olfactometer. Both stimulus and blank air were delivered to the testing box at 1000 cc per minute, as measured by a flow meter at the points of delivery to the testing box. The olfactometer was constructed of glass and teflon tubing, and the maximum dilution was 10^{-4} per stage. The construction and operation of this olfactometer is similar to that described by Pietras *et al.* (8).

Behavioral testing box. The behavioral testing apparatus (Fig. 2) incorporated features employed by various investigators (8, 10, 11) involved with odor detection tasks in rats, rabbits, oppossums and dogs. The apparatus consisted of a main compartment and two presentation bays which were sealed from the main compartment by glass doors. These doors were raised from above to allow the animal access to the bays. Each bay housed a wind tunnel. Odorized air or "blank" air was introduced through the bottom of each tunnel, and exhausted, to the outside of the building, from the top. A circular port, cut into the center of each tunnel, allowed the rat to sample the gas streams. Located in the floor of each bay, in front of each tunnel, were small ports. Through these ports pyrex glass water dippers could be raised. When the animal chose the correct bay, the associated dipper was raised and delivered 0.2 ml of water.

FIGURE 2. Testing Box.

Light sources, on the outer walls of the two bays, directed light beams horizontally to photcells on the opposite sides of the bays. When the rat projected its head into either tunnel the light beam was interrupted in that tunnel. This caused a small lamp to be lit on the outside of the associated bay. When the interruption was sustained for five seconds it was registered as a choice. An interruption of less than five seconds was considered as mere sampling. A correct choice was reinforced with the delivery of water from the appropriate dipper. An incorrect choice resulted in the immediate closure of both doors. Once the doors were closed the stimulus position was manually changed, if so dictated, according to a Gellerman series. This began a 30 second inter-trial interval.

All of the exposed surfaces of the compartments were covered with aluminum-backed teflon, or were constructed of glass or teflon. Two air inputs at the front of the main compartment provided an air flow to continually flush the main compartment. This was then exhausted via a duct in the roof by the rear wall. The animals could be observed through a one-way glass panel in the roof of the testing box.

All animals were kept in an environmental chamber on a light/dark schedule of 14/10 hours, and the temperature was maintained at 25°C. Food (Purina Laboratory Rodent Chow) was available *ad lib.* but water was presented for only 10 minutes a day at the end of the testing sessions. Each animal was tested once a day, for 10 trials per session, except during the last experiment, when each animal was tested every 6 hours for 48 hours.

Stimulus and hormones. The odorant, cyclopentanone, used throughout this study was purchased from the Aldrich Chemical Company, Inc., lot numbers 041937 and 073037. The steroids estradiol and progesterone were purchased from the Nutritional Biochemicals Corporation. Estradiol control number 3166, progesterone control number 5463.

RESULTS

Correlation of performance to estrus cycle. Eleven female hooded rats (12) were selected for training and testing, and the vaginal aspiration technique was used to monitor the phases of the estrus cycle in each animal. The classification employed by Zarrow *et al.* (13) was used to evaluate the cellular contents of the fluid aspirated from the vaginae. Samples were taken immediately before testing at approximately the same time each day (0600 hrs. to 1000 hrs.) and followed regularly in the group. The cycles ranged from four to eight days in length.

Once the initial training was completed at a stimulus concentration of 10^{-1} of vapor saturation, the stimulus was lowered to 10^{-2} and testing continued until the performance stabilized. This procedure was repeated at 10^{-3} and 10^{-4}. At 10^{-4} fluctuations in performance corresponding to the phases of the estrus cycle were first observed. Testing then continued for 10 consecutive days. Figure 3 shows the data for all animals recombined into one estrus cycle. The data for each phase of the cycle were analyzed by a matched pair t-test (14). The percentage of correct responses on the day of estrus were found to differ significantly from those of proestrus ($p < 0.01$), metestrus ($p < 0.005$) and diestrus ($p < 0.005$). The clear peaking in performance on the day of estrus is seen in figure 3. This represents a fluctuation comparable to at least one log unit of concentration (compare to fig. 4) and is closely related to the cyclic fluctuations reported by Pietras *et al.* (8). It appears that this phenomenon may not be concentration dependent, as much as detectability dependent. These investigators (8) report cyclic changes at 10^{-3}. However their experimental apparatus differed somewhat from that employed in this investigation. In their investigations the stimulus was delivered from above the rats' heads and directed down through the wind tunnel at a presentation flow rate of 600 cc/min.

Elimination of cyclic fluctuations in performance by ovariectomy. Eight of the eleven animals from the previous experiment were randomly selected for ovariectomy. The remaining three animals were sham operated as controls.* All of the operations were performed under Nembutal anesthesia.** Following surgery all of the animals were rested for two months. A short period of retraining was required following the two month rest, however all of the animals retained the requirements of the testing situation remarkably well. On the second day of retraining every animal performed at least 80 percent correct responses to a stimulus concentration of 10^{-1}. Daily vaginal sampling was then resumed. The ovariectomized group remained in the state of continual vaginal diestrus while the sham operated group exhibited the expected continuation of the vaginal estrus cycle. Testing then followed the same pattern, as in the previous experiment, from 10^{-1} to 10^{-4}. Figure 4 indicates the slope of the stimulus reponse curves for the ovariectomized group, the sham operated group and the normal females from the first experiment.

At 10^{-4} the sham operated group again exhibited cyclic fluctuations corresponding to estrus while the ovariectomized group showed no significant changes in performance. Testing continued for 10 days. Figure 5 shows the results of this testing. This represents the mean daily performances of the ovariecomized group, and the sham group. Two of the sham operated controls had syncronized cycles while the third was one day behind. The data for the latter animal were shifted back one day and then combined with that of the other two and analyzed by the matched pair t-test (14). Performance levels on the day of estrus differ significantly from those of proestrus ($p < 0.01$),

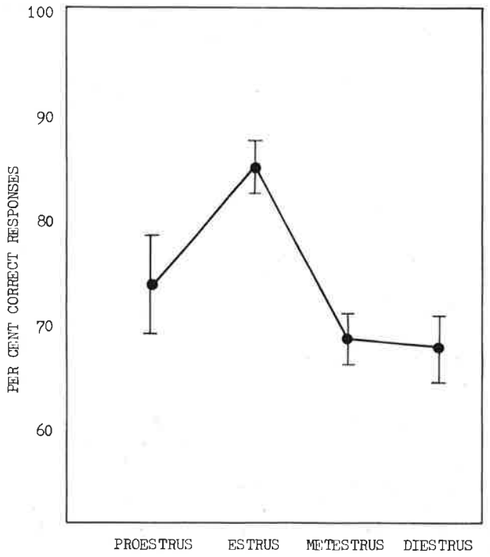

FIGURE 3. Performance of 11 normal female rats detecting cyclopentanone at 10^{-4} of vapor saturation, ± S.E. (The results were actually obtained over 10 successive days but recombined to relate to one estrus cycle).

Each animal therefore served as its own control by comparing their performances from the previous experimental series with the results of this test series.

** Longitudinal incisions were made through the dorsal body wall above each ovary. The ovarian vascular supplies were tied off and the ovaries removed. Both the body wall and the skin were sutured separately. Sham operations consisted of the same procedures except that although the ovaries were handled as in the ovariectomy procedure, the vascular supplies and the ovaries were left intact.

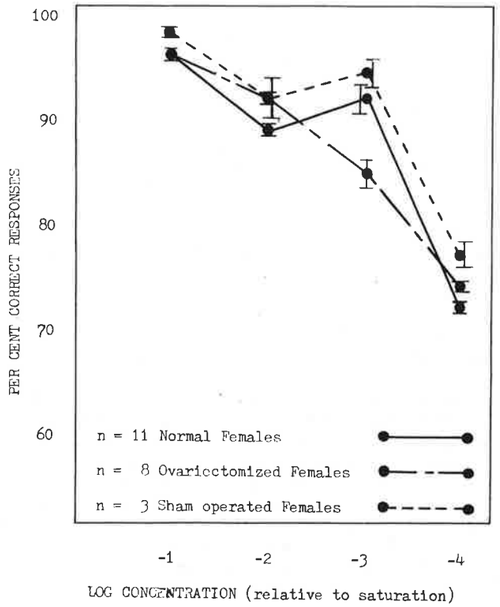

FIGURE 4. Stimulus response curves for cyclopentanone. Included are the experimental group (ovariectomized), the control group (sham operated), and normal females (from first experiment, before ovariectomies or sham operations). The mean performances of all groups were allowed to plateau, at a given concentration, before data were collected for analysis. The points at 10^{-1}, 10^{-2} and 10^{-3} represent the mean performances for the three days following plateaued performances. The points at 10^{-4} represent the mean performances for the 10 days following plateaued performances. S.E. bars are represented as follows: to the left of the point for normal females, through the point for ovariectomized females, and to the right of the point for sham operated females.

metestrus ($p < 0.02$) and diestrus ($p < 0.02$). A clear peak in performance on the days of estrus in the sham control group is seen in figure 5. In contrast to the control group, the experimental group showed no significant fluctuations in performance during the ten days of testing. In addition a "Students" t-test was applied to the mean performances of the experimental and control groups (14). This revealed no significant differences in overall performance between the two groups (ovariectomized group mean was 74 per cent ± 1.77, the sham group was 76 per cent ± 2.8). However, the same test applied to the performance of the control group on the days of estrus versus the mean performance of the experimental group revealed a significant difference ($p < 0.005$). This test showed the control group at estrus outperforming the experimental group. Furthermore, the "Students" t-test showed no significant differences between the control group at proestrus, metestrus and diestrus and the experimental group. This confirms the results by Pietras *et al.* (7, 8).

The influences of estradiol and progesterone on odor detection in ovariectomized rats. The eight ovariectomized animals from the previous experiment were randomized and divided into two equal groups. Group one was tested twice at six hour intervals, and then injected subcutaneously with 5.0 μg of estradiol (15) in 0.1 ml of sesame oil. Group two was also tested twice at six hour intervals, but then injected similarly with 2.0 mg of progesterone (16). (Both dosage levels illicite typical behavioral responses and are within the normal physiological range.) Both groups were then tested every six hours for a total of eight more sessions each. A stimulus concentration of 10^{-4}

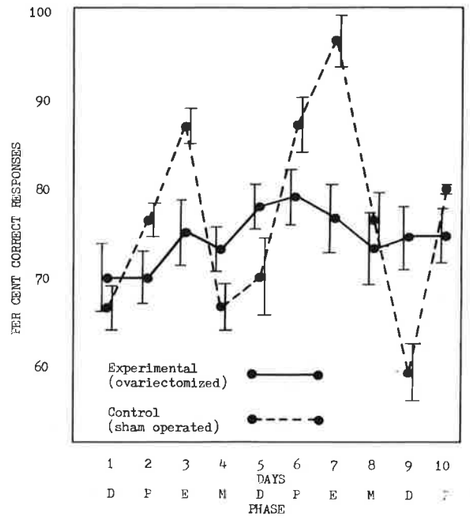

FIGURE 5. The mean per cent of correct responses for the experimental group (n = 8) and control group (n = 3) at 10^{-4} of vapor saturation. Days (1-10) are for the ovariectomized group. Phase is for the sham operated group (D = diestrus; P = proestrus; E = estrus; M = metestrus). S.E. bars are represented as follows: to the left of the point for ovariectomized animals, and to the right of the point for sham operated animals.

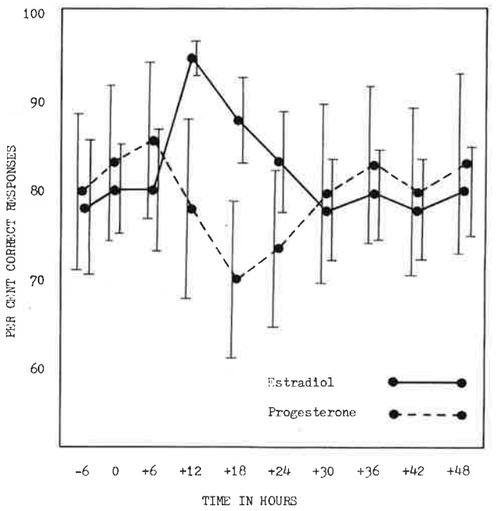

FIGURE 6. Data for estradiol (n = 4) and progesterone (n = 4) treated rats. The points at (−6) and (0) hours are considered baseline performance. Hormone treatment immediately followed the sessions at time (0). (Hormone treatment series number one) S.E. bars are represented as follows: to the left of the point for progesterone and to the right of the point for estradiol.

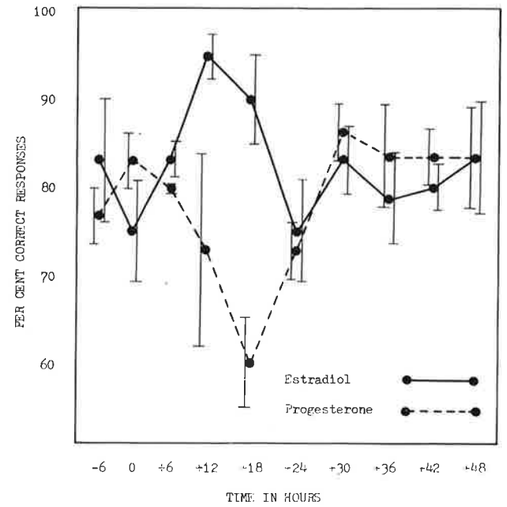

FIGURE 7. Data for estradiol (n = 4) and progesterone (n = 4) treated rats. The points at (−6) and (0) hours are considered baseline performance. Hormone treatment immediately followed the sessions at time (0). (Hormone treatment series number two) S.E. bars are represented as follows: to the left of the point for progesterone and to the right of the point for estradiol.

was used throughout this experiment and water bottles were not made available to the animals until all of the sessions had been completed.

Following the completion of the preceeding work the animals were "rested" (tested daily, however only to maintain performance) for two weeks. After the end of the two week period the same experiment was repeated, however this time the groups were reversed, i.e., group one now received progesterone while group two received estradiol. Once more each group was tested twice, as before, then every six hours for a total of eight additional sessions each.

The data were analyzed by the matched pair t-test (14). In the first series the peak in performance (+ 12 hrs.) following estradiol administration is significantly greater than the pre-treatment baseline performance ($p < 0.05$). Also the depression in performance following progesterone treatment (+ 18 hrs) is significantly lower than the pre-treatment baseline performance ($p < 0.01$) (Fig. 6). The same analysis applied to the second test series produced similar results. The peak in performance following estradiol treatment is significantly greater than the pre-treatment baseline ($p < 0.05$) and the depression in performance following progesterone treatment is significantly lower than the pre-treatment baseline ($p < 0.05$) (Fig. 7).

A comparison of the data from series one and series two indicates that enhancement or depression of performance is not the result of some synergistic effect of estrogen and progesterone, nor does there appear to be any priming required for one hormone by the other. However there is still little evidence as to the site or sites at which these hormones act to produce the observed effects. In addition it must be noted that the changes in performance could be due to titers of hypophyseal gonadotropins which would fluctuate in response to fluctuating levels of estrogen and progesterone; we offer no experimental evidence to support this view.

DISCUSSION

Although the phenomenon of cyclic variations in odor detection

may be explained in terms of circulating titers of estrogen and progesterone, the questions of where and how these hormones affect this cyclicity remain. There are several possible sites of potential hormonal action, but there is insufficient evidence to conclude which sites are actually involved, however some potential targets can be eliminated. The known vasodialator action of estrogen (17) upon the nasal mucosa could not account for the observed performances since such action would restrict the nasal air flow reducing the access of receptor sites to odor molecules. This restricted air flow would contribute to a decrease in performance, the opposite of what is observed in this and a previous study (8). Additionally, elevated levels of estrogen promote release of luteinizing hormone, which among other things induce ovarian histamine secretion (18) which also has a vasodialator action.

Autonomic influences upon the vascular supply to the nasal mucosa may also be eliminated. Bilateral cervical sympathectomy (19) did not have any effect on odor detection performance. Again, the fluctuations in performance apparently are not due to varying odorant access to olfactory receptors.

There are sites within the central nervous system which could be involved in mediating the fluctuating olfactory sensitivity. Stumpf (20, 21) has shown (by subcellular fractionation and autoradiography) that labeled estrogen binds specifically to the olfactory bulb. Evidence is unavailable to demonstrate that these events in the central nervous system are producing the observed fluctuations in odor detection performance.

Along similar lines Anton-Tay *et al.* (22) have shown that in the rat olfactory bulb there is an increase in the synthesis of norepinephrine following castration. They attribute this effect to the increase in levels of follicle stimulating hormone (FSH). The administration of estrogen blocked the increase in norepinephrine synthesis apparently by the inhibition of FSH release. Reports such as these while not proving the relationship between circulating levels of gonadal steroids and cyclic changes in performance of sensory mediated tasks do lend support by relating to specific biochemical changes in the olfactory bulb.

Finally, the work of Kawakami *et al.* (23, 24) correlate well with the results of this study. These investigators found that the administration of progesterone raised the EEG arousal threshold while estrogen depressed it. These results are also consistent with the performance data obtained by Pietras *et al.* (8), although in the latter study experimental manipulation of estrogen was not done and progesterone levels were elevated via pseudopregnancy.

The present study has extended the inferences presented by Pietras *et al.* (8) by showing the temporal effects of exogenous estradiol and progesterone on odor detection performance. Still to be found are the mechanisms by which these steroids produce their observed effects.

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THE CHICKIES QUARTZITE AND SOME TECTONIC IMPLICATIONS

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INTRODUCTION

Prior to general acceptance of the theory of plate tectonics by North American geologists, the sedimentary rocks of the Appalachian Mobile Belt were generally studied in relationship to the geosynclinal model of continental accretion. By the mid 1960's, however, most American geologists recognized the possibility that sea floor spreading could reorient continental blocks and thereby provoke facies changes and create new sites for the deposition of sedimentary rocks.

This acceptance of plate tectonic theory demands a reconsideration of the paleoenvironments and tectonic controls involved in the formation of various segments of the sedimentary prism which makes up the Appalachian Mountains. The purpose of this paper is to discuss some of the pertinent aspects of the Chickies formation of central and southeastern Pennsylvania which may be related to the new global tectonics. The Chickies is particularly interesting in this respect in that it represents the initiation of Paleozoic sedimentation in Pennsylvania along the margin of the Early Paleozoic proto-Atlantic Ocean.

THE CHICKIES FORMATION

The Chickies formation is exposed in a series of outcrop belts extending for approximately 125 miles form Trenton, N.J. to Hanover,Pa. (see fig. 1). It unconformably overlies the Precambrian basement complex, specifically, the Baltimore Gneiss in eastern Pennsylvania and rhyolites, greenstones and volcanic slates west of the Susquehanna River (Hyde, 1971). In the outcrop belt between Langhorne and Trenton, the Wissahickon Formation lies in fault contact above the Chickies. Aside form this Langhorne to Trenton belt, however, the outcrops east of the Schuylkill River are overlain by Cambro-Ordovician carbonates. West of the Schuylkill, the Early Cambrian (?) Harpers Formation is in apparently conformable contact overlying the Chickies. While no definite age can be assigned to the Chickies, it is probably Early Cambrian based on stratigraphic position.

The Chickies formation in its entirety shows a variety of textures, compositions and primary structures. Textures range from pebbly conglomerates to slaty mudstone. Compositionally, the formation varies from pure orthoquartzites to arkosic and micaceous "wackes," and visible primary structures include: imbricate structures, various types of cross bedding and occasional ripple marks. In addition, the biogenic structures *Skolithos* and *Monocraterion* occur in abundance in certain facies.

Hyde (1971) divided the Chickies Formation into six facies which he labeled: (1) the conglomeratic facies, (2) the cross-stratified facies, (3) the burrowed facies, (4) the feldspathic-agrillaceous facies, (5) the argillaceous quartize facies and (6) the mudstone facies He considered facies 1 to represent a braided stream environment, facies 2 and 3 were considered to have resulted from deposition in the intertidal zone and facies 4, 5 and 6 were considered subtidal.

Goodwin and Anderson (1974) examined the physical and biogenic structures within parts of the Chickies and were able to build a strong argument that most of the sandy facies and some of the muddy facies

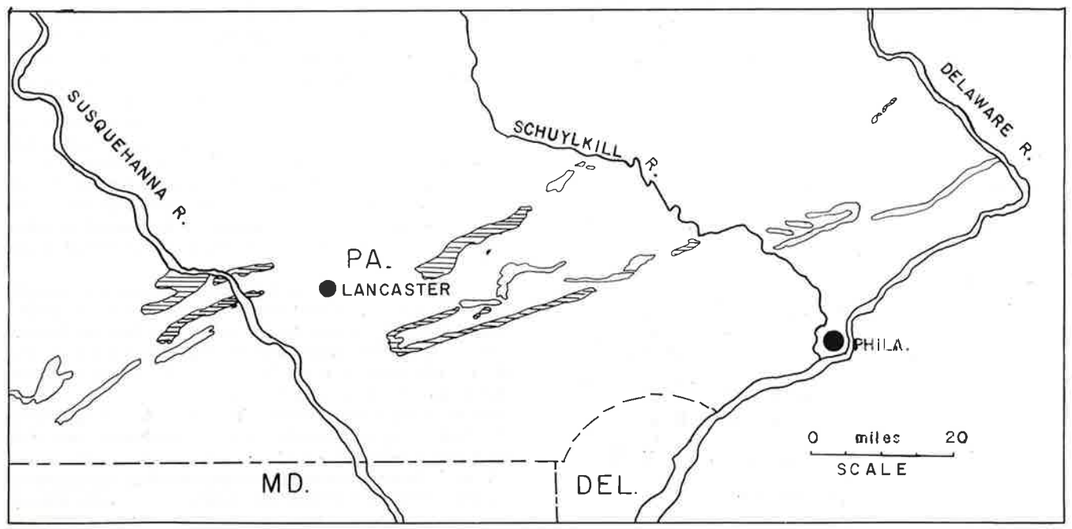

FIGURE 1. Index map showing the locations of Chickies outcrops. The localities which were examined for this paper are shown with the striped pattern.

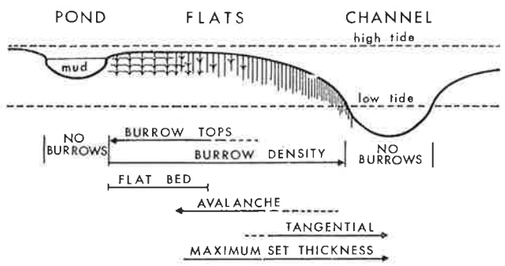

FIGURE 2. Chickies paleoenvironmental model showing relationships of burrow types and sedimentary structures to tide-dominated environments (After Goodwin and Anderson, 1974).

were deposited in environments dominated by strong tidal currents with large tidal ranges. They were able to recognize four facies which were controlled by the hydrodynamic conditions at the time of deposition (fig. 2). These facies included (1) high intertidal sand flats, (2) low intertidal sand flats, (3) subtidal channels and (4) tidal flat ponds. The high intertidal sand flat facies is characterized by widely spaced *Skolithos* burrows and funnel topped *Monocraterion* associated with avalanche cross stratification. The low intertidal sand flat facies shows closely spaced *Skolithos* without *Monocraterion* tops associated with tangential to avalanche type cross stratification. Subtidal channel facies display tangentially cross-stratified dune deposits with an absence of burrows, and the tidal flat ponds appear as non-burrowed gray muds usually overlying channel deposits.

The texture and bedforms revealed in parts of the Chickies formation suggest that current velocities could have been in excess of 80 cm./sec. (see fig. 3). The velocities of tidal currents within bays, lagoons or estuaries may be modified by many factors including size and shape of tidal inlets, size and shape of the tidal basin, distance from open ocean and orientation. However, large tidal current velocities nearly always demand significant tidal ranges because if the range is small the volume of the tidal prism must also be small. Large tidal ranges can only be produced in fully developed oceans with mature dimensions such as the Atlantic rather than in semi-isolated seas such as the Mediterranean, which has a tidal range of approximately 20 centimeters. It therefore follows that the proto-Atlantic Ocean which existed during Chickies time must have had mature dimensions (perhaps a minimum of 10 million square kilometers) in order for the necessary tidal range to develop.

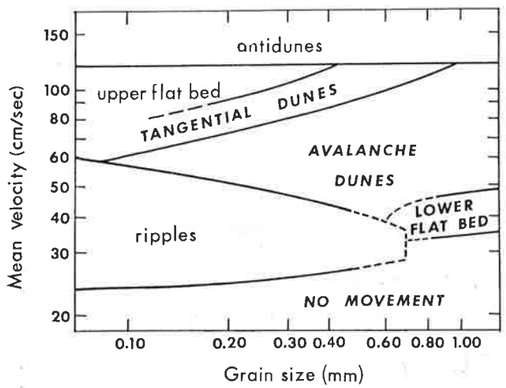

FIGURE 3. Chickies bedforms (capital letters) related to current velocities. (After Goodwin and Anderson, 1974).

DEPOSITIONAL SYSTEMS

Depositional systems commonly found along coastal margins include: the strand plain, barrier bar-lagoonal, deltaic and estuarine. The simple strand plain system develops where a limited supply of sediment is carried parallel to the coast in the long shore drift, or is piled onto the beach by wave action in the surf zone. The typical textures and structures of the strand plain include high energy sheet bed deposits of the fore beach with relatively coarse lag deposits in the breaker zone. There is generally an absence of intertidal flat sediments associated with the simple strand plain system. Therefore, the strand plain system can be rejected as the depositional system which lead to the formation of the Chickies Quartzite.

Lagoonal systems should present evidence of lower energy levels than the cross-stratified units of the Chickies imply. The exception to this of course is in the vicinity of inlets where tidal currents can be strong. However, the near absence of lenticular lagoonal muds and low energy structures such as small scale ripple marks tends to reject the barrier bar-lagoon system for Chickies deposition.

Classical deltaic sedimentation patterns are clearly not in evidence in the Chickies Formation. There is no stratigraphic evidence that the Chickies is part of a progradational sequence. Also there are no progradational coarsening-upwards sequences within the Chickies itself. Thus the typical features of constructional type deltas are absent.

A tide dominated high destructional type delta remains a possibility, however. Tide-dominated type deltas have the sediment which is introduced by rivers reworked by the dominant tidal currents into a series of elongate sand bodies between the tidal channels. The muds may be carried up delta on the flood tide or seaward on the ebb flow. Mud may also be accreted as marginal tidal flats (Fisher et. al., 1969). Modern examples of tide dominated high destructional type deltas include: The Fly Delta in the Gulf of Papua, the Irrawaddy, the Mekong and the Kelang Lanet in southern Malayasia.

Perhaps a better model than a tide-dominated delta for Chickies deposition is that of the estuarine depositional system. In some estuarine depositional systems only small amounts of sediment are provided by discharging streams and sand carried in the long shore drift and into the estuary by tidal currents provide most of the sediment budget (Meade, 1969). The maximum current velocities are developed in the deepest and most constricted parts of the tidal channels. Where the channels are not constricted and the estuary broadens current velocities decrease and sandy shoals and tide delta islands form. There is usually much less mud depositied in such an environment in that the sediment is supplied largely from the high energy surf zone from which the fines had already been winnowed. The small amount of mud associated with the sandy facies of the Chickies suggests that river muds were not being contributed in important quantities. The conclusion therefore derived from the sedimentological characteristics is that the Chickies formation was formed in an estuarine environment. Modern estuarine systems exhibiting bedforms and sedimentary structures comparable to those of the Chickies include New England estuaries (Boothroyd and Hubbard, 1971), estuaries of the Rhine River system (DeRaaf and Boersma, 1971) and the Wash in Great Britain (Evans, 1965).

The Eriboll Sandstone of northern Scotland occupies a stratigraphic interval similar to that of the Chickies as well as showing petrographic and paleontologic similarities to the Chickies. Goodwin and Anderson (1974) compared their depositional facies from the Chickies to those described by Swett et. al. (1971) from the Eriboll. They recognized that the basal Cambrian in both Scotland and Pennsylvania is largely intertidal in origin, but point out a significant difference, namely that the Chickies displays a complex mosaic of interbedded facies while the Eriboll is described as tidal flat sands overlying a subtidal lower unit. the Eriboll therefore seems to display an offlap or progradational sequence while the Chickies may suggest a more stable although dynamic coastal zone. A simple explanation of this difference could simply be that the ratios of rate of depositions (R_d) to rate of subsidence (R_s) is variable. In the case of the Eriboll (R_d:R_s) is greater than one resulting in progradation, but at the Chickies depositional site (R_d:R_s) may have approximated one resulting in stabilization of the coastline for that time. Perhaps the Eriboll site of deposition was closer to a source of sediment supply than was the Chickies site.

GLOBAL TECTONIC CONSIDERATIONS

In the now classic paper "Did the Atlantic close and then reopen?" Wilson (1966) recognized the existance of a proto-Atlantic Ocean recently dubbed "Iapatus," which closed during the mid Paleozoic. Wilson suggested that Ireland and northern Scotland were once joined to the North American craton in the vicinity of Newfoundland. This of course raises the possibility that the Chickies of Pennsylvania and the Eriboll of northern Scotland were deposited along a common coastline, the nature of which may be partly determined by the sediments themselves.

Some of the characteristics of these sediments which may relate to plate tectonic theory are as follows:

1) The sediments show compositional maturity. They are not the product of rapid flysch type or eugeosynclinal type deposition even though the sedimentation rate may have been quite rapid at intervals. The sediments seem to reflect stable shelf sedimentation and, in the case of the Chickies, form part of the foundation for the Appalachian miogeocline.

2) There is an absence of pyroclastics which means that no volcanic island arcs or continental volcanic systems were contributing sediment. Volcanics are usually associated with colliding plate margins; they may be associated with conservative plate margins, but are practically never associated with expanding plate margins.

3) The volume of sediment is small compared to tectonic clastic wedges. This feature also points to the tectonic stability of the continental margin throughout the time of deposition.

4) The primary structures reflect high energy conditions and imply an oceanic basin large enough for high tidal ranges to develop.

All of these observations tend to document the hypothesis suggested by numerous authors that the present eastern margin of North America was part of an expanding plate margin during late Precambrian and Early Cambrian time.

We conclude that the Chickies-Eriboll system and deposits similar to the Chickies and Eriboll have certain unique characteristics and that these characteristics are usually associated with deposits formed on trailing edges of continental margins. On a global scale, similar conclusions have been suggested by other authors such as Dietz and Holden (1966), Dewey (1969) and Curray (1975). The ocean basin associated with such deposits is established and of mature dimensions.

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INTERPRETING WEATHER DATA IN MOUNTAINOUS REGIONS: A CASE STUDY

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ABSTRACT

Synoptic weather conditions in mountainous regions may vary considerably over small distances. For those living in mountainous regions a knowledge and understanding of these differences is important whenever weather data is interpreted for scientific or personal needs. This study documents some of the smallscale weather variations present in the mountainous area of Northeastern Pennsylvania. A comparison is made of the synoptic weather data from the meteorological observing station at Wilkes College in Wilkes-Barre and the National Weather Service observing station at Avoca (approximately 11 miles north-east and 390 feet higher.) Results are discussed in terms of the known meso-scale influences of mountainous terrain on meteorological parameters.

INTRODUCTION

This study attempts to document the existence and magnitude of meso—scale synoptic weather variability in one area of the mountainous region of Northeastern Pennsylvania. It represents the first step in a continuing program of ascertaining the effects of local topography on local weather for the benefit of the area residents and scientists. Comparison is made of data collected at a college observing station and a nearby National Weather Service station. Experience indicates the existence of differences in the synoptic weather conditions at the two sites. Since the public weather data for the area is obtained from the N.W.S. site and most of the population of the area live or work near the college site, the result of this study can be of benefit to the citizens of this area in their interpretation and application of the abundant N.W.S. data.

DATA

Data covering a one year period (1974) were available for comparison. Figure 1 is a map of the study area. The college site is located near the middle of the Wyoming Valley on the valley floor at a height of 540 feet above sea level. The N.W.S. site is located 11 miles north-east on a small knoll on the eastern wall of the valley at a height of 930 feet. The college instruments are located approximately 40 feet above the ground (6 feet above a tar and gravel covered roof) on a building in an urban setting (Wilkes-Barre). The N.W.S. instruments are approximately 6 feet above grass covered earth near the landing areas of an airport in a rural setting (Avoca). All college instruments were purchased from a national scientific instrument manufacturer and are standard types meeting N.W.S. specifications. The valley floor has a slope of approximately 1 foot per mile with the N.W.S. site located upvalley from the college site. At both sites the eastern ridge has an average height of about 2100 feet and the western ridge about 1500 feet. The Susquehanna and Lackawanna Rivers flow through the valley. Comparisons are made with available college data (approximately 180 observations) and the nearest corresponding regular hourly N.W.S. observation. In general the college observations were taken once a day between 0800 and 1100 E.S.T. Data analyzed include temperature (maximum and minimum), wind (speed and direction), precipitation (amount and type), and relative humidity.

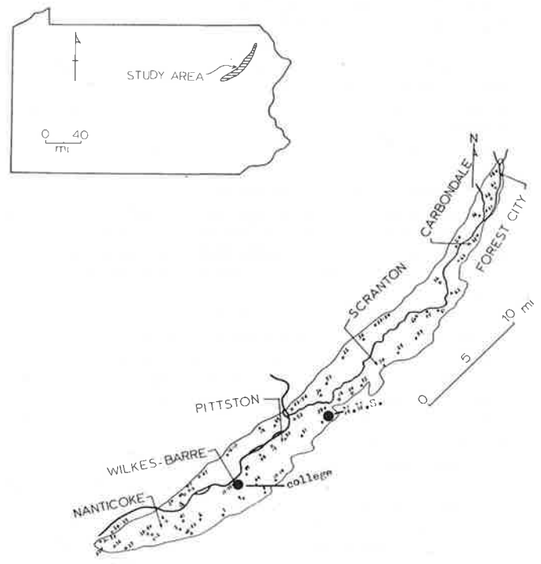

FIGURE 1. Generalized and detailed location maps of the study area.

TABLE 1
Seasonal averages of differences of synoptic weather data (college minus N.W.S.)

Data	Sp.	Su.	Fa.	Wi.	Ann.
T—max (F)	+5.1	+4.3	+2.3	+5.2	+4.2
T—min (F)	+2.9	+2.6	−0.1	+3.6	+2.2
R.H. (%)	−5.3	−12.0	−3.5	−3.7	−7.1
Precip. (in.)	+1.10	+1.12	+0.20	+0.48	+2.33

RESULTS AND DISCUSSION

Table 1 summarizes the temperature, relative humidity, and precipitation results. The college site averages nearly 4°F warmer than the N.W.S. site. The dry-adiabatic lapse rate implies an expected difference of 1.9-2.0°F between the two sites. In part the larger difference can be attributed to the roof-top location of the college site. Also, it may be associated with the general urban setting of the college site. With increasing frequency (1) researchers are identifying thermal domes or "heat islands" near urban areas. These result from the addition of waste heat into the urban environment by traffic, homes, and industries and from the greater solar heat absorbing qualities of concrete and asphalt in urban areas as compared with those of vegetation and earth in rural areas. The rivers flowing through the valley are assumed to exert no significant meso-scale thermal influences (2).

Ninety-five percent of all maximum temperature data show warmer college readings. The greatest differences in maximum temperatures occur in winter and spring when the college site is about 5°F warmer than the N.W.S. site. The minimum temperatures at the college site were also warmer than at the N.W.S. site (73% of the data). The largest average difference (3.6°F) occurs in winter. The fall difference is near zero and may reflect the influence of large-scale subsidence inversions frequently present in this part of the United States in the fall (3).

The relative humidity averages 7% less at the college site. Relative humidity is inversely related to temperature so in part the difference can be explained by the warmer air temperatures at the college site (it can also be noted that the largest relative humidity differences, −12%, occur during the warmest season). However, while the relative humidity was lower at the college site, the amount of water vapor present in the air was higher than it was at the N.W.S. site. For example, for the period 18 February through 12 March the college reported an average termperature of 45°F and an average relative humidity of 61%. During this same period the N.W.S. reported averages of 40°F and 66% at corresponding observation times. These data imply absolute humidities of 58 mb at the college site and 48 mb at the N.W.S. site (assuming a linear relationship between temperature and saturation vapor pressure over the small temperature range observed). Absolute humidity usually decreases with height since moisture is added to the atmosphere primarily from the surface, the precipitation processes remove moisture from the higher levels, the capacity of the air to hold moisture decreases upward due to the usually upward temperature decrease, and when air rises and expands the water vapor in the air also expands so that the moisture in a given volume becomes less (4). As regards this present study, there are additional influences related to the presence of the rivers, their tree lined banks, and automobiles (whose emission contain large quantities of water vapor). The higher levels of water vapor present at the college site also contribute to the higher temperatures by increasing absorption of long-wave radiation.

Total precipitation for the test period was 2.33 inches (or 6% of N.W.S. annual total) greater on the valley floor than on the valley wall. Nearly all of this increase occurs in the spring and summer. During these two seasons the college received the larger rainfall amount in 70% of those cases when precipitation occurred at both sites. In general, the area's greatest precipitation occurs with northeasterly, easterly, and southeasterly winds. This implies a partial rainshadow effect associated with the ridges on the immediate north, east, and south of the N.W.S. site.

Precipitation amounts associated with convective activity can be highly variable over relatively small regions. They depend upon a number of variables including cell pattern, longevity, and velocity; position of station with respect to passing cells; and time variations of rainfall rates (5). At the two stations studied it was not uncommon during the warm season for storm rainfall amounts to differ by a factor of 2 or more (approximately 20% of the cases) or for rainfall to be recorded at only one station (approximately 10% of the cases). In all cases of paired observations the two sites received the same type of precipitation. Apparently the difference in elevation between the two sites is not large enough to produce frequent instances of snow-melt during free-fall.

Table 2 contains the wind speed comparison data. When all data were averaged the college wind speeds were only half those at the N.W.S. site. There is a 7 times greater incidence of calms at the college. The last column of table 3 indicates that measurable winds at the college. site will not occur unless wind speeds at the N.W.S. site are in excess of 4-5 mph. On days that the college experienced wind, the speeds averaged 75% of those reported at the N.W.S. site Thus, for this variable, the difference in height between the two sites is very significant. The lower college site is greatly influenced by the sheltering and deflecting influences of the surrounding ridges.

TABLE 2
Average wind speeds at the college and N.W.S. sites

site	average wind speed (all data)	percentage of calms	average wind speed (calms excluded)	average wind speed (calms at college)
college	3.7 mph	43%	7.1 mph	0
N.W.S.	6.9 mph	6%	9.9 mph	4.5 mph

TABLE 3
Percent frequency of occurrence for wind directions at the college and N.W.S. sites.

	N.	N.E.	E.	S.E.	S.	S.W.	W.	N.W.
College	9	24	8	3	1	18	23	14
N.W.S.	16	5	0	4	7	31	15	22

The higher average wind speed readings at the airport are an additional influence to be considered when interpreting the temperature date. Strong daytime winds prevent high daytime temperature by increasing mixing and heat transport away from the surface. Strong winds also increase evaporation, thus also restricting the temperature rise. At night the effect of strong winds is to prevent low surface temperatures from being realized by mixing dowhward warmer air from above and thereby imparting some heat transfer to the ground by conduction (6). Thus windiness has a moderating influence on surface temperature and its diurnal variations. This is reflected in the 2°F larger annual average diurnal temperature range shown in the valley temperature data (Table 1). Although stronger winds increase evaporation, they also increase mixing, thereby preventing humidity accumulation and thus contributing to lower absolute humidity levels at the N.W.S. site (Table 1).

Table 3 presents the wind direction comparison data. Both sites show a preference for general westerly winds (S.W., W., N.W.) due to the mid-latitude location of the study area (7). The data include a disproportionately small number of winter season wind direction observations (due to equipment malfunction at the college). The most frequently observed wind direction at the college site is north-easterly. This is the result, in part, of the mid-valley location of the site and the channeling influence of the local ridges which are orientated N.E.S.W. The urbanization of the valley floor prevents detection of (and possibly formation of) an upslope-downslope valley breeze system (8). Easterly winds (N.E., E., S.E.) are distinctly lacking at the N.W.S. site. This reflects the sheltering influences of the east valley wall near the N.W.S. site.

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CLASSIFICATION OF ATMOSPHERIC HEIGHT CHARTS

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ABSTRACT

Analytical representation of height contours with Fourier-Bessel functions and linear correlation were used to classify upper-air wind patterns over the Northern Hemisphere. Data analyzed were the 640 days 1 January through 9 February of 1951 through 1966. It was found that a large percentage of days (nearly 80%) could be classified as resembling a relatively few types of circulation patterns (12). These recurring patterns were isolated, type charts constructed, and days labelled according to the basic upper-air circulation pattern present. Certain meteorological characteristics of the type charts were investigated.

INTRODUCTION

One practical reason for typing the earth's atmospheric general circulation is to gain knowledge of its complex behavior. Once some knowledge is obtained, its application to such studies as the development of long-range forecasting methods and energics investigations constitute a second and important reason for attempting the categorization.

The first map typing was probably done very soon after the first weather maps came into existence. This typing was the result of subjective mental averaging. This scheme allowed anyone familiar with weather charts to make a generalized forecast. This method has the advantage of being simple and crudely effective. It is probably practiced today by many who look at weather maps regularly. Today, when meteorologists have available to them increasing numbers of charts for larger areas at many levels in the atmosphere and are being asked to make increasingly more specific forecasts, it is essential that a more rigorous method of map classification be developed if the analague method of forecasting is to be seriously attempted or if map classification is to play a role in the study of atmospheric dynamics.

Researchers in various parts of the world have investigated serious chart typing techniques for over 80 years. Namias (1) presents a critical review of many of these investigations. Many of these studies suffer from lack of adequate time coverage, spatial coverage, or an objective scheme for selecting "typical" situations. Therefore, it is the purpose of this research to investigate the feasibility of chart typing with 16-years of daily Northern Hemispheric 700 mb height charts by objective statistical methods. This study will permit partial answering of the question posed by Kutzbach (2) ". . .if we double or triple the number of years of maps employed in the hemispheric map pattern classification study, will the number of identifiable map patterns double or triple?"

The technique makes extensive application of linear correlation of the Fourier-Bessel representation of height fields which was developed by Kutzbach and Wahl (3). It is a modification of the technique used by Kutzbach (2) and employs the method of Lund (4).

PROCEDURE

A basic data set for use in this investigation of large-scale hemispheric chart typing was obtained from the Extended Forecast Division of the National Weather Service. This basic data set consisted of a complete record of daily 1200 Z 700 mb Northern Hemispheric height charts for the period 1 January through 9 February of 1951 through 1966.

The Fourier-Bessel expansion described by Kutzbach and Wahl (3) was used to obtain an analytical description of the hemispheric height topography from 90N to 20N latitude for each of the charts. A set of 103 of the coefficients of the expansion was used to describe the charts. On the average this set explained over 99% of the total height variance of a chart. The expansion utilizes a 10^0 x 10^0 diamond grid and is represented by:

$$Z(r,\lambda) = \frac{1}{2}\sum_{k=1}^{\infty} A_{ok}J_0(h_{ok}r) + \sum_{n=1}^{\infty}\sum_{k=1}^{\infty}(A_{nk}\cos n\lambda + B_{nk}\sin n\lambda)J_n(h_{nk}r)$$

$$A_{nk} = \frac{1}{\pi d_{nk}}\int_{-\pi}^{\pi}\int_{0}^{1} Z(r,\lambda)J_n(h_{nk}r)\cos n\lambda\, r dr d\lambda$$

$$B_{nk} = \frac{1}{\pi d_{nk}}\int_{-\pi}^{\pi}\int_{0}^{1} Z(r,\lambda)J_n(h_{nk}r)\sin n\lambda\, r dr d\lambda$$

$$d_{nk} = \int_0^1 r[J_n(h_{nk}r)]^2 dr \text{ , the normalizing factor,}$$

where J_n is the Bessel function of order n and h_{nk} is the kth positive root of the equation Jn (r) = 0.

For two fields given in this expansion, the linear correlation between them is given by:

$$r_{1,2} = \sum_{n}{}' \sum_{k}{}' A_{nk}{}^*(1)A_{nk}{}^*(2)+B_{nk}{}^*(1)B_{nk}{}^*(2)$$

Where $A_{nk}{}^*$ and $B_{nk}{}^*$ are the normalized values of the Fourier-Bessel coefficients, each chart normalized to itself, primed summation denotes summation over selected values of n and k.

In this study, the correlations between the charts of the basic data set were obtained using the normalized set of coefficients representing the deviation of each map from a mean annual normal chart. This partially eliminates the general "polar vortex" feature from each map. With this feature removed, the distinctive features of the individual charts can be studied more precisely. For simplification, only 50 of the Fourier-Bessel coefficients were used when calculating the correlations between the deviation fields (i.e., for the A coefficient: k=1 n=0-9; k=2, n=0-8; k=3, n=0-3; k=4, n=0-2; k=5-6, n=0; and similarily for the B coefficients except there is no Bessel function of order zero for the B coefficients). These 50 coefficients consistently explain over 90% of a chart's variance.

The chart was found which had the greatest number of other charts in the basic data set highly correlated with it (+0.70 or more). When correlating, the 5-days on either side of a chart were excluded in order to obtain a reasonably independent set of correlations. Then the five daily charts were found which correlate highest with this day and which are not within three days of each other (to avoid biasing the set with the similarities that exist between successive daily charts). Next, a mean chart of this 6-chart set was formulated by averaging the respective coefficients. This composite chart is then used as a typing chart, representing the mean of the large-scale patterns of the 6 very

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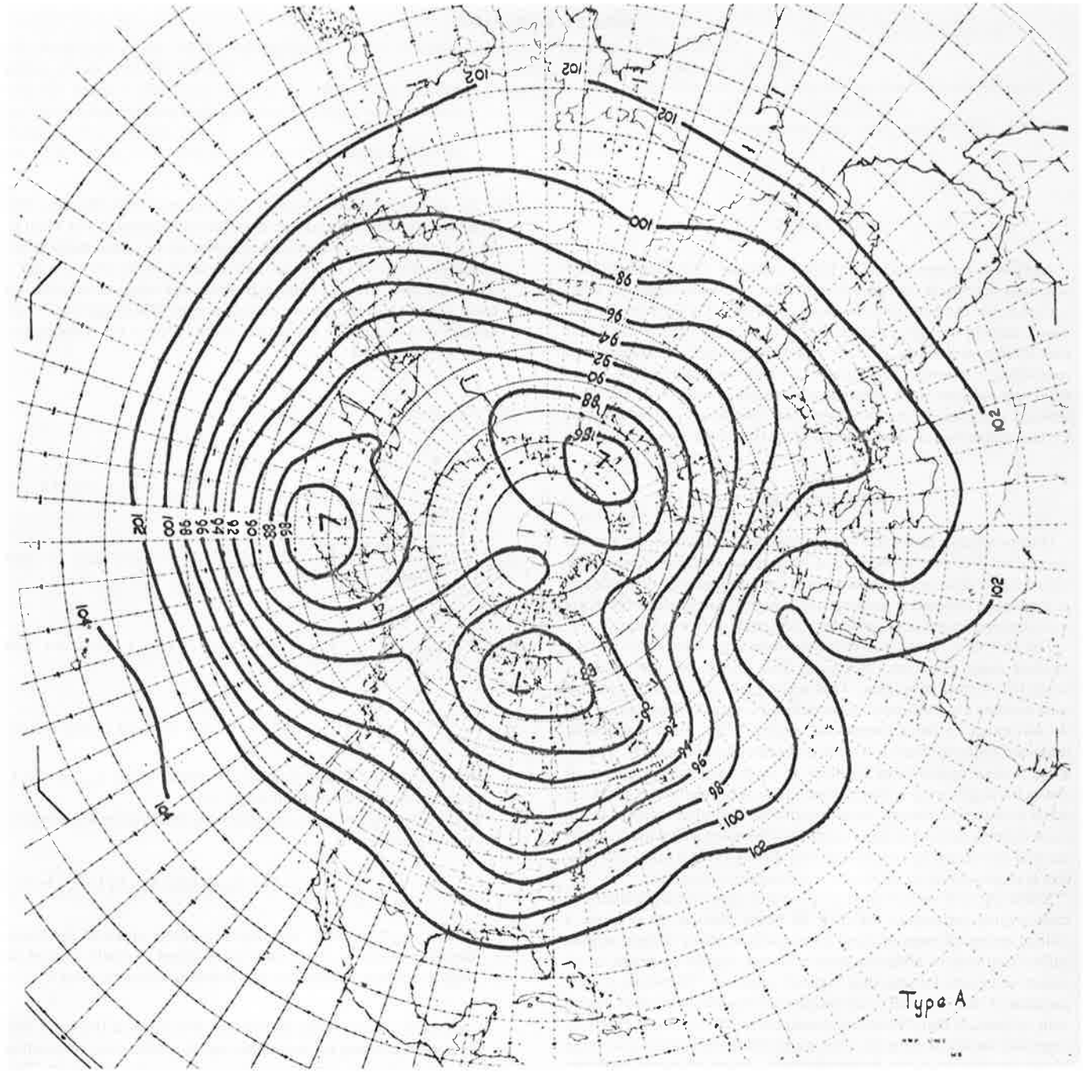

FIGURE 1. Example of the Northern Hemispheric 700 mb typing charts found in this study (Type A). Heights in 100's of feet.

similar charts from which it is composed. This characteristic of the typing chart is of advantage to a hemispheric-scale scheme where the large-scale features are of greatest interest. This mean field is then correlated with all the fields in the basic data set. All charts correlating highly with it (+0.75 or more) are called Type X charts and are considered to bear a close resemblance to the composite chart as far as the large-scale features are concerned. All those days correlating between +0.65 and +0.75 are called Type X' charts and are considered to bear an approximate resemblance to the typing chart. The significance of these latter charts is that they can be viewed as representing development periods of the types or as transition periods between typed charts.

The typing process is extended by removing from the set of height fields all those charts correlating highly (+0.75 or more) with a previously found typing chart, then again finding the date which has the largest number of days correlating highly (+0.70 or more) with it, and continuing the above approach. The typing process ends when there remains no field to which at least 5 other fields correlate highly (+0.70 or more).

It was found that the variance of a composite chart was only slightly smaller (3%) than the average variance of the individual charts from which it was composed (5). It is necessary to compare these variances since averaging smooths the contour patterns and thus may make direct comparison of mean charts with daily charts difficult (as to both wave amplitude and pattern in the case of a much smaller mean map variance).

TABLE 1

Dates labelled with appropriate type and approximate type designations.

1957	A	B	C	D	E	F	G	H	I	J	K	L	Date Type
Jan 1		B		D'	E'	F'	G'						B
2		B		D'	E'		G'						B
3		B		D'	E'		G	H'					B
4		B		D'	E'		G'	H'					B
5		B'					G'	H'					B'
6							G'	H'				L'	H'
7							G'	H'				L'	H'
8							G'	H				L'	H
9							G'	H				L'	H
10								H				L'	H
11	A'				E'			H'				L'	E'
12					E'			H'				L'	L'
13					E'			H'				L	L
14					E'								E'
15													—
16													—
17													—
18												L'	I'
19												L'	L'
20								H'					H'
21								H'					H'
22								H					H
23		B'					G'	H				L'	H
24		B		D'	E'		G	H				L	H
25		B					G	H				L'	G
26		B'					G	H					G
27		B'					G	H'					G
28							G	H'					G
29							G'	H'					G'
30							G	H'					G
31							G'	H'					H'
Feb 1								H'					H'
2								H'					H'
3													—
4													—
5													—
6													—
7													—
8													—
9													

The lower limits of +0.75 and +0.65 were used when selecting the charts that bear close or approximate resemblances to each other on the basis of a visual check of charts correlating in the +0.60 to +0.85 range. The choice of these limits is somewhat arbitrary. A higher limit was not used because of the small number of highly correlated (over +0.80) fields. A lower limit obviously produces loss of similarity since less variances is explained.

RESULTS

Two basic results were obtained with this procedure. Hemispheric type charts were obtained by adding to the deviation typing fields the annual normal chart originally subtracted from all charts and reconstructing the 700 mb height field from the resulting Fourier-Bessel coefficients. Also, a calendar of all days of the basic data set was constructed with each day labelled with the type chart to which it correlated. Certain meteorological and statistical characteristics of the charts and days were then investigated.

Figure 1 presents an example of one of the 12 composite typing charts found. The alphabetical identifiers refer to the order in which the typing charts were found. Table 1 is an example of the days labelled with all appropriate type designations. Table 2 is the calendar of each day of the basic data set labelled with the type chart to which it correlated highest within the prescribed limits. A blank indicates that the daily chart did not correlate greater than +0.65 with any of the typing charts. Two entries indicate the chart correlated equally with two of the typing charts.

Most of the flow patterns that occurred over the Northern Hemisphere during the 16 years used in this study are categorized by the 12 basic patterns found. In fact, of the 640 days used, 497 (or 78%) were typed; 296 as Type X and 201 as Type X'. The continuity of the results is striking. There are few isolated typed days. Rather, the types are seen to run in sequences. These sequences many time follow the pattern: X', X, X', and implies reasonble development of and transition from the typed periods.

The un-typed charts (22%, or 143 charts) may correspond to periods undergoing very rapid development and change. This impression

TABLE 2

Calendar of days, by type.

Year 19——

	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Jan 1	C'	H'	A	E	-	-	B	C'	-	G	F'	A	-	E'	-	H
2	H'	H'	A	E	-	-	B	A'	-	G	F	A	-	E	-	H
3	F'	H'	D'	A	-	-	B	D	-	G	F	L	-	E	-	C
4	F'	-	D'	A'	-	-	B	D	-	G	F	I	-	E	-	C
5	F'	-	J'	A'	-	-	B'	D	-	G'	F'	I	-	E	-	D
6	F	-	J'	A	C'	-	H'	B	-	E'	F'	I'	-	E	L'	D
7	F	-	J'	A	C'	-	H'	F	-	E'	F	B'	-	E	L'	D
8	F	-	J'	A	C'	-	H	F	-	E'	F	B'	K'	E	I'	D
9	F	H'	I	A	C	-	H	F	-	E'	F	B	K	E	I'	D
10	F	H'	I'	L'	C	-	H	F	-	-	D	B	K	E	B'	G'
11	F'	H	J'	L	C	-	E'	F	-	-	D	B	K'	E	B	D'
12	C'	H	D'	L	C	-	L'	F'	-	-	D	H	K	E	B	D'
13	C'I'	H	D'	H	C	-	L	F'	-	-	D	H	K	E'	B	-
14	C'	H	A'	H	C	-	E'	D'	I'	-	A	H	K	E'	B	-
15	C'	H	A'	H	C	-	-	A'	I	C'	D	H'	K	E	B	-
16	E'	H'	A'	H	C	-	-	-	I	-	D	H'	K	E	B	-
17	H'	H'	A	H	C	-	-	-	K'	C'	D	H'	K	E	B	-
18	I'	H'	A	H'	C	-	L'	-	K	C	D	H'	K	E	B	C'
19	I	-	A	L'	C	-	L'	-	K	C'	D	-	K	E'	B	C'
20	I	-	A	L'	C	C'	H'	J'	J	C'	D	-	K	E'	F	C'
21	I	-	A	L	C'	C'	H'	J'	J	-	D	H'	K	A'	F'	C
22	L	-	I	L'	A'	C	H	J	J	G'	D'	H'	K	A	G'	C
23	L	J'	I	L'	A	C'	H	J'	J	G'	G'	H'	K	A	G	C
24	L	J	L	-	A	C'	H	C'	A	G	J'	H'	K	A	G	C
25	L	J	L	L'	A	C'	G	-	A	G	G	L'	K	A	G'	C
26	L'	J	L'	-	B'	C'	G	-	D	J	G	I	K	A	-	J
27	G'	F	F'	-	G'	G'	G	-	D	J	G	I	K	A	-	C
28	G	F	J'	-	G'	G	G	D'	A	J	G	D	K'	F'	-	C'
29	G	F	-	-	G'	L	G'	E'	A	A	J	D	K'	F	-	C'
30	G	F	-	-	J'	L	G	E'	A	A	J	D	K'	F	-	C
31	G	F	F'	D'	F'	L	H'	E'	E	A'	I'	B	K'	F	K'	C'
Feb 1	G'	F	F'	-	-	D	H'	-	E	D	I	B	-	F	K	C
2	F'	F	-	D'	-	D'	H'	-	E	D	I	B	-	F'	K	G'
3	-	F	A'	A'	-	D'	-	-	E	D	I	B	-	-	K	G'
4	-	A	A'	A'	-	D'	-	-	E'	D	I'	B	J'	-	K'	G'
5	F'	A'	A'	D'	-	-	-	C'	E'	D	I'	B	-	-	A'	C'
6	G'	A	A'F'	B'	-	-	-	J'	E	A	B'	B	-	-	A'	C
7	D'	A	F	B	-	-	-	J'	E	A	I	L	-	-	-	C
8	J'	A	F'	B	-	-	-	J	E	A	I	L	-	-	-	C
9	-	A	B'	B	-	-	-	J	E	A'	I	L	-	-	-	C

is based upon the fact that the persistence (as measured by correlation) is lower for the un-typed periods than it is for typed periods. The un-typed charts may also represent similar recurring charts which did not occur frequently enough in the time period studied to be identified. The fact that 111 of the 143 un-typed days followed un-typed days appears to hint at a certain pattern in their occurrence which is not revealed by this typing technique. Also, it can be noted that there are no cases of direct transitions from Type X charts to the un-typed charts. This result implies that the un-typed charts are distinct from any of the typing charts which were identified.

Further study of the typed days was started by obtaining monthly values of the classically defined zonal and meridional index values (6). These values were placed into four classes depending on their magnitude relative to the mean values. The results indicate that the periods characterized by three of these classes (high zonal-high meridional, high zonal-low meridional, and low zonal-low meridional) are distinguished by stable flow patterns. These classes have large percentages (nearly 80%) of typed days. The low zonal-high meridional class shows a low percentage of typed days (nearly 60%) and thus these periods may be characterized by rapid development, transition, and movement of the contour patterns.

The existence of preferred groupings of the typed charts suggests application of these results to long-range forecasting problems. To study this, the correlations between the composite charts and those occurrences where a daily chart correlated highly (over +0.75) with more than one typing chart were investigated. These investigations indicated that within the set of charts A, B, D, I, and F there were frequent transitions between the types, high correlations between the composite charts (near +0.80) and numerous instances of daily charts correlating highly with more than one member of the set. On

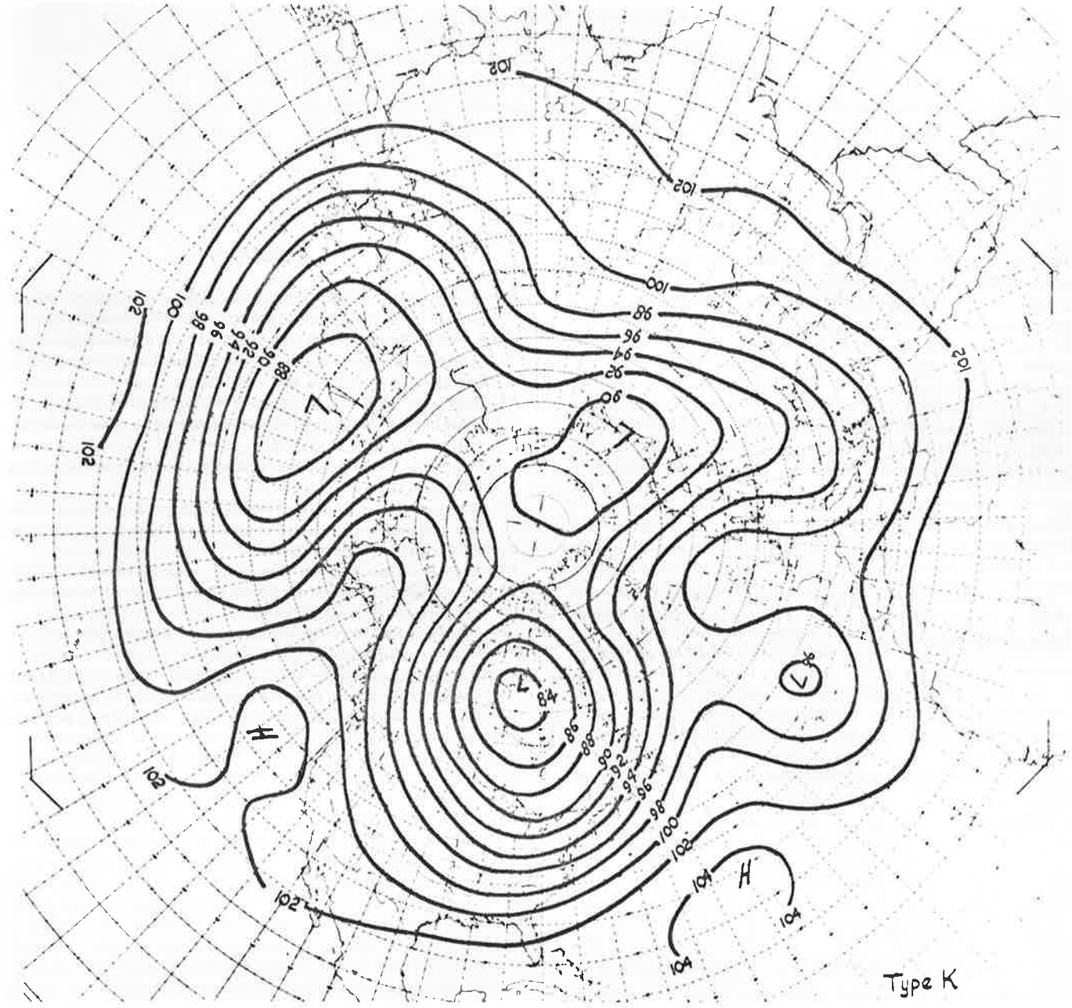

FIGURE 2. The Type K chart. Heights in 100's of feet.

the other hand, charts C, E, G, H, J, K, and L did not correlate highly (less than +0.70) with any of the other typing charts, did not develop into or from any of the other typing charts, and their labelled days did not correlate highly with other type charts.

It remains to point out some of the interesting characteristics of the Type K chart, characterized by an abnormally large amplitude three wave pattern (see figure 2). It is similar to the Pattern 3 mean map found by Kutzbach (2) at the 500 mb level and occurs during a similar calendar period. Table 2 shows that Type K charts occurred for the greater part of, and almost exclusively in, January 1963. O'Connor (7) has commented on the harshness and persistence of the cold weather that the Untied States experienced during that month. Lawson (8) studied this month and advanced the possibility that the pattern may be similar to at least some of those patterns thought to have occurred by some during the Neoboreal ("Little Ice Age") period (1200-1400's). Thus, it is implied that this present scheme may have some value in terms of the study and forecasting of long-term climatic trends. Further study of the Type K period lends physical significance to the typing charts constructed in this study. Gradient winds flow parallel to the contour patterns with higher values on the right of the wind. Thus, the Type K chart indicates northerly flow from Arctic regions into the North Platte (U.S.A.) and Munich (Germany) regions. The 1200Z (or 1230Z) surface temperatures for a number of stations were obtained for all years (except 1954 and 1955). The average temperatures for North Platte and Munich for each type situation is presented on table 3. Days on which Type K pattern was present averaged the lowest temperatures.

CONCLUSIONS

The basic purpose of this work was to apply the combined use of Fourier-Bessel analytical representation of height fields and the linear correlation of daily charts to 16 years of winter season 700 mb Northern Hemispheric circulation patterns in an investigation of the feasibility of chart typing. The orderly arrangements of the resulting

TABLE 3

Average temperature for each type situation.

Chart	A	B	C	D	E	F	G	H	I	J	K	L
North Platte (USA, F)	23	17	11	22	18	20	3	10	20	12	0	21
Munich (Ger., C)	−2	1	5	−2	−4	2	2	3	0	2	−8	−1

daily typed charts and the high percentage of days typed with relatively few typing charts implies that chart typing is feasible on a hemispheric-scale at mid-tropospheric levels. Also, the questions of the relationship between the number of identifiable map patterns and the length of record posed by Kutzbach (2) can be answered, at least partially. Considering the differences in method, the overall results appear to be that the number of types does not increase significantly as the period of available records increases. The physical implication of this study appears to be that the atmosphere is undergoing orderly change between relatively few large-scale circulation states in response to fixed geographic and limited varying thermal forcing functions.

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THE EFFECT OF SPATIAL FREQUENCY FILTERING ON OPTICAL IMAGE FORMATION

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ABSTRACT

The Abbey theory of image formation predicts that the spatial frequencies of the object will be displayed as a function of position in the image plane of the source. By use of apertures and beam stops, either high or low spatial frequencies or both may be blocked from reaching the image plane of the object. The effects of these filters on the images of some simple amplitude objects, are examined analytically and the design of an experiment to measure these effects using a microwave analog, is discussed.

INTRODUCTION

The empirical investigation of spatial frequency filtering can take many directions because of the combinations of the experimental variables available. Here, an attempt is made to establish, from an analytical approach, which effects of the filtering process are most conveniently adapted to experimental measurement, and of these, which ones are most conveniently predicted analytically. A microwave analog will be used for the experimental investigation. As was previously reported (1, 2) the experimental microwave apparatus was designed for studying diffraction effects; thus, the limitations of this equipment must be considered in this present analysis. For example, only intensities of the radiation can be measured and no phase measurements will be possible with this system. Relative intensities can be measured with an accuracy of about eight percent but measurements below 0.2 $\mu W/cm^2$ are unreliable. Further, absolute measurement of locations in the plane normal to the optical axis of the system are difficult to make, and situations in which small variations of one experimental parameter cause larger effects than large variations in the parameter being studied should be avoided.

The source available (1, 2) is composed of a VA-218 fixed frequency klystron radiating 2.85 cm microwaves through a pyramidal horn antenna. This results in a coherent cylindrical wave that is approximately plane over a large portion of its forward directed wave front. These facts indicate that cylindrical symmetry of the lens will be compatible with the source, and one dimensional translation of the detector (1, 2, 3) will be sufficient to map the wave fronts as is indicated in Figure 1.

THEORY

The Abbey (4, 5, 6) theory of optical image formation in one dimension predicts that for an object transparency illuminated by coherent radiation, the transmitted radiation in the object plane $f(x_0)$, and the radiation in the image plane of the source F(u), are related by a Fourier Transform (5, 7):

$$F(u) = \int_{-\infty}^{\infty} f(x_0)\ k(u, x_0)\ dx_0, \tag{1}$$

and the radiation in the image plane of the object f(x), is related to the radiation in the image plane of the source F(u), by another Fourier Transform (5, 7):

$$f(x) = \int_{-\infty}^{\infty} F(u)\, k(u,x)\, du \tag{2}$$

where

$$k(u,x_0) = e^{-i2\pi u x_0}\ , \tag{3}$$

$$k(u,x) = e^{-i2\pi u x}\ , \tag{4}$$

$$u = \frac{m\bar{x}}{\lambda\,(S' - D')} \tag{5}$$

and

$$x = m x_0 \tag{6}$$

Now the $\bar{x}$'s are coordinates in the image plane (Figure 1) of the source where the spatial frequency spectrum of the spatial signal, which is the object, is given by F(u). The u's are called the spatial frequencies and the x's are the locations of the spatial frequencies as shown in Figure 1. The $k(u, x_0)$ and k(u, x) are the Fourier kernels (8, 9) for the respective transformations (5, 6) in Equations (1) and (2). The remaining parameters λ, m, S', and D' are the wavelength of the illuminating radiation, the magnification of the lens, and the distances of the image plane of the object, and image plane of the source from the second principal plane (5, 3) of the lens respectively, as shown in Figure 2.

It is in the image plane of the source that filtering may be affected by inserting transparencies of desired type at the approximate position on the $\bar{x}$ axis as shown in Figure 2. Therefore, filtering concerns modifying F(u) and $f(x_0)$ the object phasor in Equation (1). An experimentally convenient $f(x_0)$ and one easily represented analytically is a rectangular aperture with edges perpendicular to both the optical axis and the x_0 coordinates as shown in Figure 1. The transmission function of this aperture is shown in Figure 3.c and is given by:

$$f(x_0) = h(x_0 - a) - h(x_0 - b), \tag{7}$$

where $h(x_0)$ is the Heaviside unit step function (7, 8, 9) defined by Equation (8) and shown in Figure 3.a.

$$h(x_0) \begin{cases} 0 & x_0 < 0 \\ \tfrac{1}{2} & x_0 = 0 \\ +1 & 0 < x_0 \end{cases} \tag{8}$$

F(u) then for this simple object is given by:

$$F(u) = \int_a^b k(u, x_0)\, dx_0 = (\pi u)^{-1}\ \mathrm{Sin}\,[2\pi u(b-a)]\ e^{-i\pi u(b+a)}\ . \tag{9}$$

The amplitude of F(u) an even function of u or $\tilde{x}$ is plotted in Figure 4.a. Also, it can be seen that the location of the object does not affect the location of the point of symmetry of the amplitude of F(u) but only the phase in Equation (9). If the object is symmetric with respect to the optical axis or a = −b, F(u) is real and the phase in Equation (9) is zero everywhere. If the object is not symmetrically placed about the optical axis, the phase of F(u) is an odd function of u or x. Since F(u) is the phasor describing the radiation passing through the image plane of the source, one can selectively affect the spatial frequency spectrum of $f(x_0)$ by putting opaque materials, like sheets of aluminum foil, at various locations in the image plane of the source and thus pass or block the radiation F(u) at any predetermined value of $\bar{x}$ or u (3, 4, 10, 11). This simple type of filter is called a binary filter (3, 4) with transmission functions similar to the $f(x_0)$ in Equation (7). A general expression for a binary filter is given by

$$T(u) = \sum_{i=1}^{N} h(u - c_i) - h(u - d_i);\ c_i < d_i\ , \tag{10}$$

where N is the number of apertures with unit transmission, and c_1 and d_1 are the locations of the edges on the u or $\bar{x}$ axis (Figure 4.b). Now a filtered image f'(x) of the object $f(x_0)$ is given from Equation (2) by:

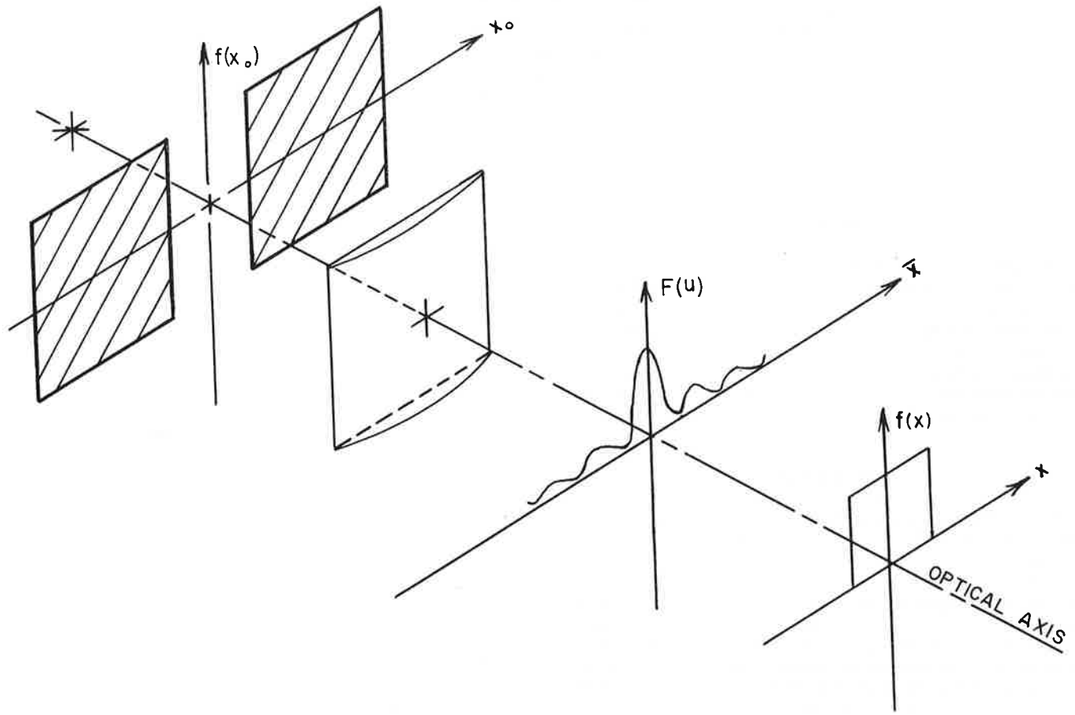

FIGURE 1. A schematic representation of an optical system identifying coordinates in the object plane (x_0), image plane of the source ($\bar{x}$) and the image plane of the object (x). The cylindrical lens forms the Fourier Transform F (u) of the object $f(x_0)$ in the image plane of the source.

$$f'(x) = \int_{-\infty}^{\infty} T(u)\, F(u)\, k(u,x)\, du. \tag{11}$$

For a binary filter like that in Equation (10):

$$f'(x) = \sum_{i=1}^{N} \int_{c_i}^{d_i} F(u)\, k(u,x)\, du \tag{12}$$

a form that is amenable to solution both analytically and numerically, if the c_1 and d_1 are chosen carefully.

An interesting and useful result of using the binary filters is gained by examining the complimentary filter of Equation (10):

$$\overline{T}(u) = 1 - T(u), \tag{13}$$

which is plotted in Figure 4.c. Now the image formed by this filter is given from Equation (2), Equation (12) and Equation (13) as:

$$f''(x) = \int_{-\infty}^{\infty} \overline{T}(u)\, F(u)\, k(u,x)\, du = f(x) - f'(x). \tag{14}$$

This means that the phasor that forms the image from the complimentary binary filter is simply equal to the difference of the unfiltered image phasor and the original binary filtered image phasor. In the following analysis integrals of the type in Equation (12) with N = 1 or 2 have been done and the images formed by the complimentary filters have been evaluated by Equation (14).

RESULTS

Calculations have been made using the above analysis. The integrals of the form of Equation (12) were done numerically on a Honeywell 430 computer. These integrals describe the image formed by using a band pass filter. The complimentary band reject filtered images were evaluated using Equation (14). The object used (Equation 7) was placed symmetrically about the optical axis and has unit width or a = −0.5 and b = +0.5. From Equation (9) it can be seen that the amplitude of the spatial frequency spectrum F (u) for this object will be an even function of u, and F (u) is real with a shape like that in Figure 4.a. The zeros of F(u) occur at $u = \pm n/2$, where n is a non-zero integer. If a single aperture binary filter, similar to the one in Figure 4.b with N = 1, is placed symmetrically about the optical axis as in Figure 2, with C = −0.5 and d = +0.5 the resulting image is as that shown in Figure 5.a. This corresponds to allowing spatial frequencies up to $u = 1/2(b-a) = \frac{1}{2}$ or frequencies corresponding to twice the fundamental spatial period of the object size to be passed by the filter but none higher. One can see from Figure 5 that the shape of the image or even the location of its edge is not clear until spatial frequencies of $u \approx 2.5$ are allowed to reach the image. However, if the image formed by the complimentary high pass or beam stop filter is evaluated, one can see from Figure 5 that the location of the edge of the object is well defined in the image. This implies in general that the location of edges or points of high contrast can be determined better in an image formed by a high pass filter than in one formed by its complimentary low pass filter. In the image formed by the high pass filters, there is a peak or resonance of intensity at the location of the edge. This peak becomes narrower and higher as the width of the beam stop is increased. In Figure8, both the quality factor and the intensity of their peaks are plotted against the width of the beam stop. The quality factor is a relative measure of the sharpness of a peak given by:

$$Q = \frac{X_{max}}{\Delta X_{\frac{1}{2}}}$$

Where $\Delta X_{\frac{1}{2}}$ is the width of the peak at one half maximum intensity and X_{max} is the location of the maximum of intensity. It is apparent that the quality factor increases as the width of the beam stop increases, but the maximum intensity decreases asymptotically to a value of 0.25.

A low pass filter with a beam stop inserted would correspond to a symmetric band pass filter, passing both positive and negative phasors of a given spatial frequency band. The form of this filter would be described by (Equation 10) with N = 2, $c_1 = -d_2$ and $d_1 = -c_2$. The effect of this type of filtering on the image of the object can be seen from some typical results given in Figure 6. The most significant object information contained in an image formed by this type of filter is a zero of intensity at the image location of the edge of the object. However, there are numerous other zeros also contained in this image. The image formed by the complimentary binary band reject filter does contain most of the important object information concerning both shape and edge location. As one might expect, a band reject filter would transmit more information than a comparable band pass filter

It is interesting for experimental design to determine what changes in the image due to a symmetric band pass filter are to be expected from positioning the edges improperly. Figure 7.a gives the results for an an asymmetric positioning of the edges of a filter like that in Figure 6.b. The difference is negligible and the result is typical of other calculations done to examine this possible source of experimental difficulty. If only the positive or negative phasors were passed by a band pass filter, the resulting image is found to be independent of whether the positive or negative phasors are chosen, and there seems to be only a slight change in the image for relatively large variations

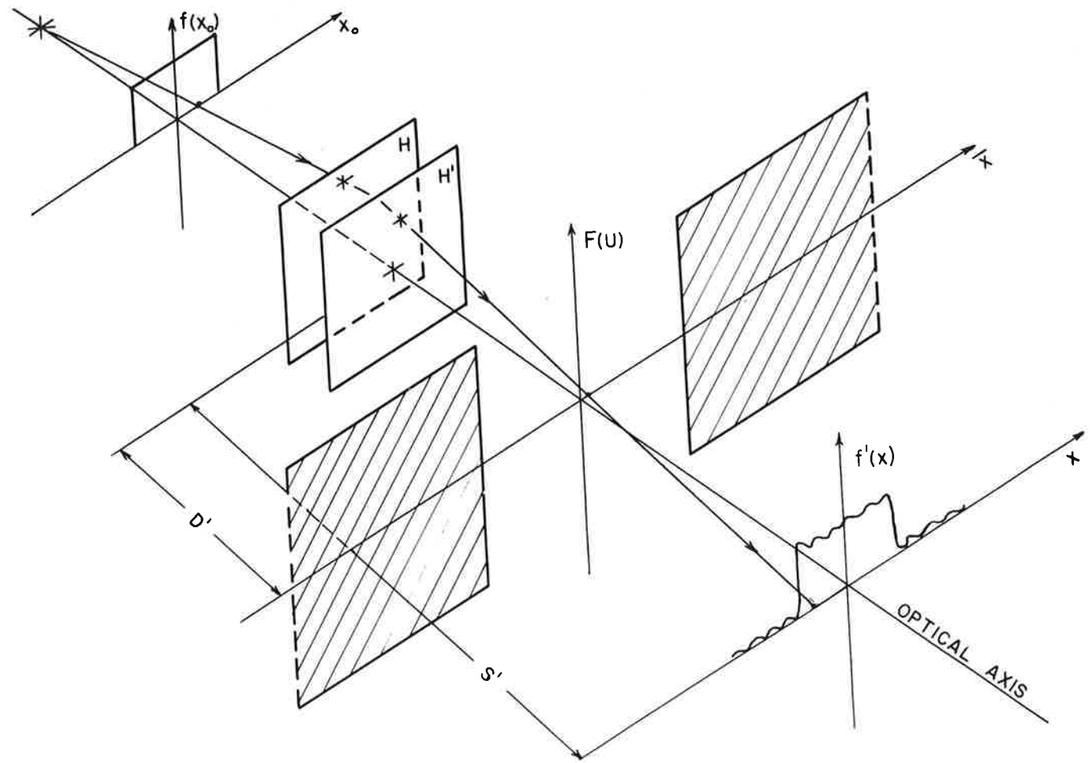

FIGURE 2. The distorted image f'(x) of the simple amplitude object $f(x_0)$ is formed at a distance S' from the second principal plane of the lens (H') by the spatial frequency spectrum, passed by the low pass filter placed in the image plane of the source, a distance D' from the second principal plane of the lens.

in the size or positioning of the asymmetric band pass filter. A typical result is given in Figure 7.b. The image formed by the complimentary asymmetric band reject filter is also shown and it typically shows about as much object information as the symmetric band reject case shown in Figure 6.b. The most striking effect of the asymmetric filters is the relative increase in the size of the imaginary parts of the image phasors, which become equal to the same order of magnitude as the real part, whereas the imaginary parts of the image phasors due to symmetric filters are 10^3 or 10^5 times smaller than the real parts and do not noticeably effect the intensity of the image.

The effect of a small asymmetry in the positioning of a low pass filter is shown in Figure 7.c. The result shows that small change in the position of a supposed symmetric low pass filter will not drastically change the resulting image, and the same is true in general of the placement of the complimentary high pass or beam stop filter.

Many other values of the filter parameters were studied and the results shown here were chosen to give an idea of typical results from the many cases examined. It must also be kept in mind that only one type of object has been examined.

The effect of placing the object asymmetrically on the optical axis has also been studied and the above results are in general valid for the resulting images, excepting that the location of the image is of course displaced an equivalent amount with respect to the optical axis.

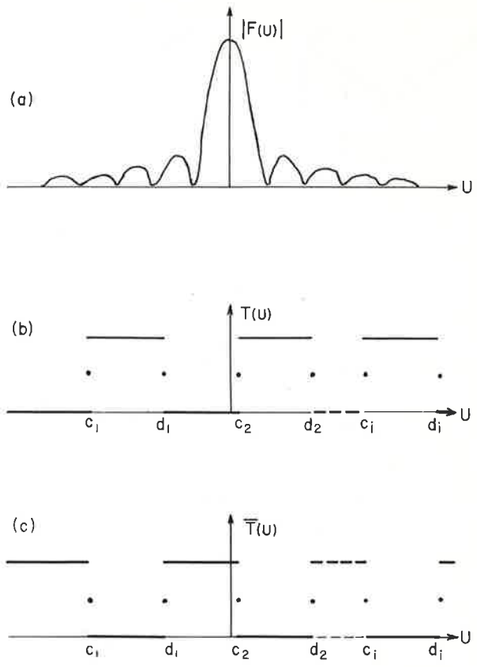

FIGURE 4. (a) An example of the amplitude of F (u) the spatial frequency spectrum of a single transmission object of unit width. (b) The transmission function T(u) of a multiple band pass binary filter and (c) its compliment $\overline{T}$(u).

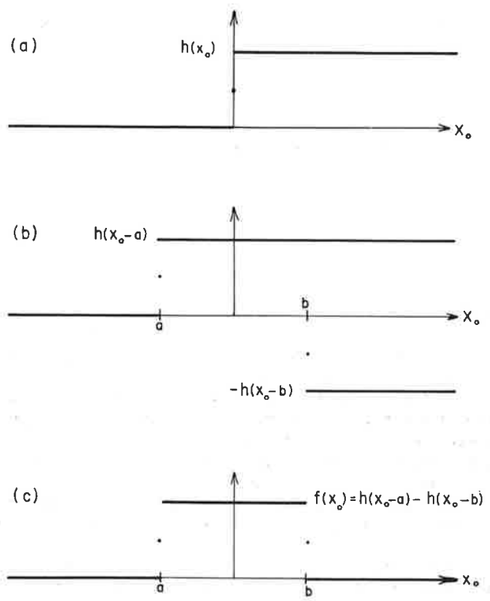

FIGURE 3. (a) An example of the Heavyside unit step function and (b) a combination of step functions forming (c) a square spatial pulse of unit height and with edges at $x_0 = a$ and $x_0 = b$.

CONCLUSIONS

From the foregoing study, several important experimental and analytical difficulties have been removed by the use of a simple transmission object and binary filters. One useful result is that the image due to the compliment of a binary filter is easily evaluated by use of Equation (14). Another usefl result apparent from this analysis occurs when studying the image formed by a narrow low pass filter where there is no information in the image giving the location of the edge of the object. By insertion of a beam stop, an image due to a symmetric band pass filter is formed and the location of the edge of the object in the image thus formed is quite clear. The beam stop can be inserted for identification of the edge position in the image and then removed for further study of the low pass filtered image without having moved the low pass filter at all. Another approach to this problem would be the use of the complimentary binary high pass filter to identify the edge position, but this technique would involve moving the low pass filter and thus introducing an uncertainty in the location of the filter when it is replaced.

A most fortunate discovery is the result that either small uncertainties in the location of the object in the object plane or the location of the filters in the filter plane do not cause large changes in the image information. This removes the difficulty of extraordinary effort of precise placement of the object or filter during the experimental investigation.

The overall conclusion is that if the intensity of the source is great enough and/or the transmission of the lens is close to unity, an empirical study of optical spatial frequency filtering can be done quantitatively by use of a microwave analog (1,2).

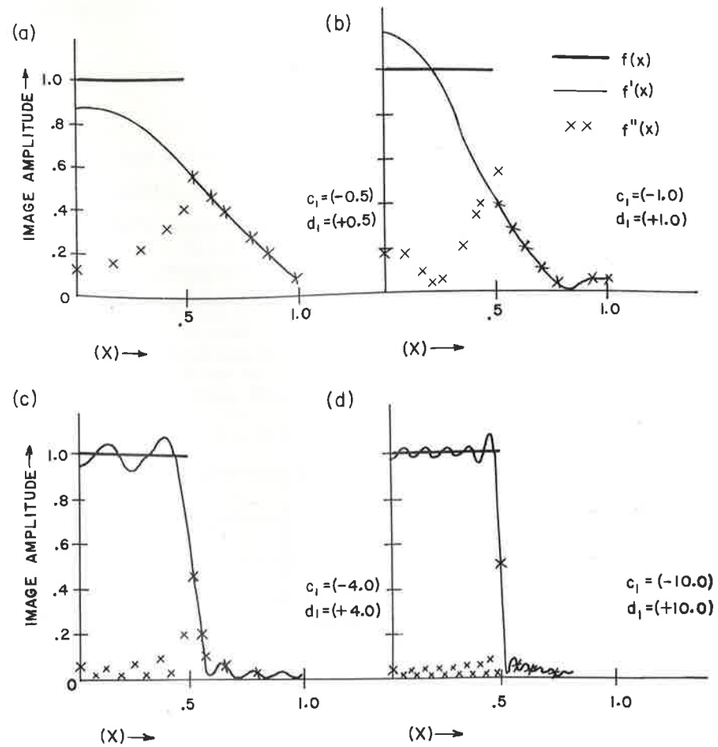

FIGURE 5. Images of a simple transmission object of unit width placed symmetrically about the optical axis. These images are formed by binary low pass filters with edges at the indicated locations u_i. Also indicated are the images formed by the respective complimentary high pass filter.

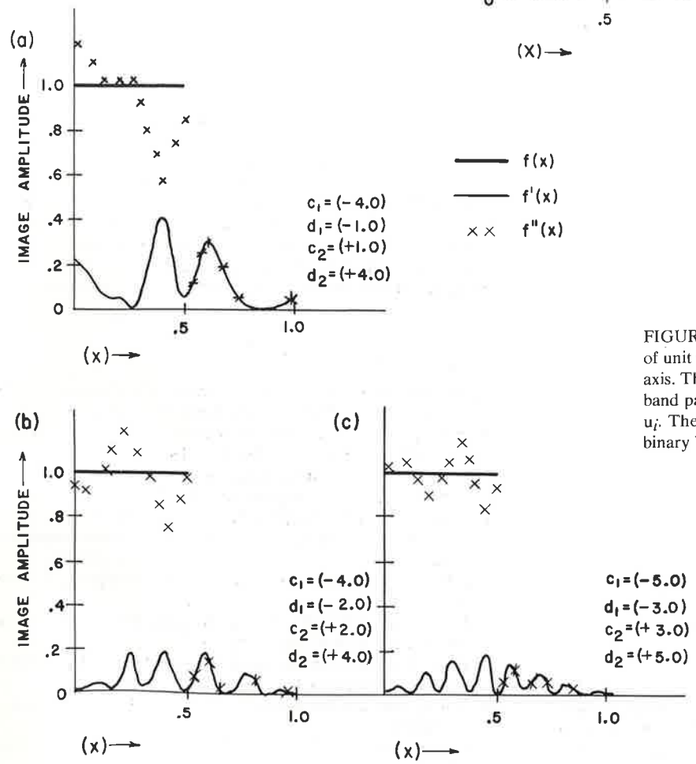

FIGURE 6. Images of a simple transmission object of unit width placed symmetrically about the optical axis. These images are formed by symmnetric binary band pass filters with edges at the indicated locations u_i. The images formed by the respective symmetric binary band reject filters are also indicated.

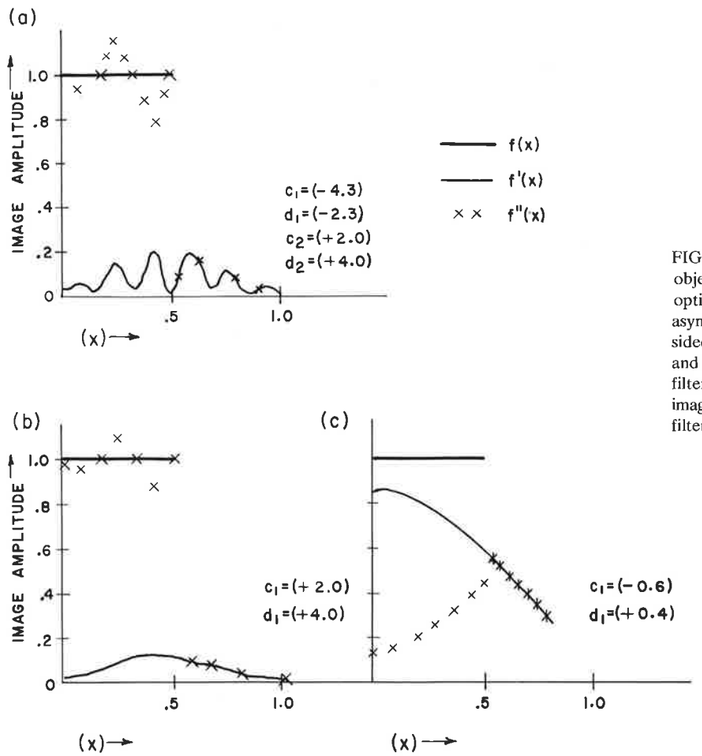

FIGURE 7. Some images of a simple transmission object of unit width symmetrically placed about the optical axis. These images are formed by (a) an asymmetrically placed band pass filter (b) a one sided band pass filter, passing only positive phasors, and (c) an asymmetrically placed low pass filter. All filters are placed with edges at the indicated u_i. The images formed by the respective complimentary binary filters are also indicated.

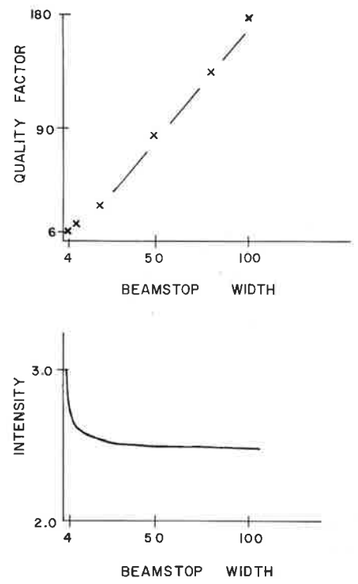

FIGURE 8. (a) The quality factor and (b) the maximum intensity of the amplitude of the peaks, which mark the edge of the object in images formed by high pass filters, are shown as they vary with respect to the width of the beam stop.

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