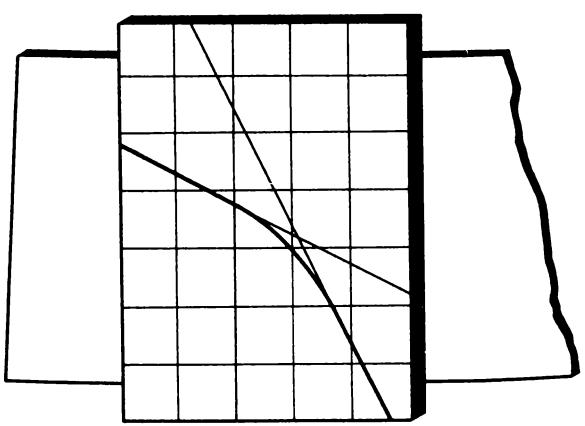
PROCEEDINGS

of the

NORTH DAKOTA ACADEMY OF SCIENCE

ABSTRACTS



64TH ANNUAL MEETING

MAY 5 AND 6, 1972

Dickinson State College
Dickinson, North Dakota

Volume 26 • Part I • May, 1972

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CHEMICAL STABILITY OF PRESERVED WATER SAMPLES. V. A. Adomaitis, J. A. Shoesmith and G. A. Swanson. Northern Prairie Wildlife Research Center, Jamestown, N. Dak.

Tests were conducted to determine changes in the chemical characteristics of oligotrophic waters collected at 15 sites in northeastern Minnesota and stored for different periods of time under chloroformed and nonchloroformed conditions. There was relatively little change in the pH of either series of samples for 8½ months. After about 13 months, both series were an average of 10 percent less acidic; however, the change was not statistically significant. Also, there was a slight drop, but no significant difference, in the specific conductivity at 25° C of either series after 13 months storage. Eleven other chemical characteristics of the paired samples showed no consistent differences after 13 months storage; however, the total dissolved solids content of the chloroformed samples was moderately higher. The other 10 chemical parameters (total alkalinity, bicarbonate and carbonate alkalinities, chloride, sulfate, silica, calcium, magnesium, sodium and potassium) had the same range. It was concluded that chloroform was unnecessary to preserve the chemical characteristics of these oligotrophic waters.

BEHAVIOR CHANGES AND ADAPTATION TO He-O_2 HYPERBARIC ENVIRONMENT. D. M. Allen, S. J. Brumleve, J. Carman, and C. B. Jensen. University of North Dakota, Grand Forks, North Dakota.

This series of experiments was designed to demonstrate some conditions in which rats adapt to a He-O_2 hyperbaric environment at 14.6 ATA. The conditioned anxiety paradigm of Estes and Skinner (1941) was used. The conditioned anxiety was obtained by repeated presentation of a warning stimulus (a light) upon a stable ongoing operant performance (lever pressing) maintained by food reinforcement. Termination of the warning stimulus was coincident with the delivery of a brief unavoidable foot shock. The behavioral consequence of this paradigm is one of reduced lever pressing during the warning stimulus. These experiments indicate that exposing the animal to He-O_2 and at the same time to 14.6 ATA retards the speed of acquisition of conditioned anxiety relative to that of rats under normal air. This can be prevented by increasing the ambient temperature to 90° F, or by changing only one environmental factor at two-day intervals. If the ambient gas is changed from air to He-O_2 for two days, then pressure increased for two days, the animal responds to shock as if in 1 ATA air. ONR Contract No. N00014-68-A-0499.

THE INFLUENCE OF TRYPTOPHAN ON GLUCONEOGENESIS AND PHOSPHOENOLPYRUVATE CARBOXYKINASE(PEPCK) IN DIABETES. F.L. Alvares and P.D. Ray. Dept. of Biochem., Sch. of Med., Univ. N. Dak., Grand Forks, N. Dak., 58201.

Gluconeogenesis in intact normal rats or perfused normal livers can be regulated by L-tryptophan(Try) via its metabolite quinolinic acid(QA) which, acting at the level of PEPCK inhibits the conversion of oxalacetate to PEP even though the assayable specific activity(Sp.Act.) of PEPCK is, paradoxically increased. We have previously reported that Try and 3-hydroxyanthranilic acid(3-HAA) have no effect on gluconeogenesis or on the Sp.Act. of PEPCK in intact alloxan-diabetic rats; however, Try, 3-HAA, and QA can inhibit gluconeogenesis in perfused alloxan-diabetic livers even though the Sp.Act. of PEPCK remains unaltered. We now report qualitatively similar patterns of inhibition of gluconeogenesis by Try, 3-HAA and QA in perfused streptozotocin-diabetic rat livers, and again(except for QA) the concomitant absence of any effect on the Sp.Act. of PEPCK. QA increases the Sp.Act. of PEPCK in a manner quantitatively similar in both normal and streptozotocin-diabetic livers. Thus Try and its metabolites can regulate hepatic gluconeogenesis in both normal and diabetic isolated perfused livers; the inability of whole diabetic rats to respond to Try remains unanswered but may relate to the loss of control of gluconeogenesis in diabetes. (Support: NIH AM 12705)

ARYL ISOXAZOLE CARBOXYLIC ACID ESTERS. Jill Armbrust, Karen Vosburg, and Franz H. Rathmann. Dept. of Chem. NDSU, Fargo, N. Dak.

Following the procedure used by Quilico and Fusco (Gazzetta Chimica Italiana, 67: 589-603, 1937) for the preparation of 5-methyl-3-phenyl-isoxazole-4-carboxylic acid, we prepared the corresponding 3-biphenylyl-5-methyl-isoxazole-4-carboxylic acid ethyl ester. 5.47 grams of 4-biphenylcarboxaldehyde are reacted with 2.19 grams hydroxylamine hydrochloride in the presence of 1.46 grams sodium hydroxide to yield 5.47 grams of 4-biphenylcarboxaldoxime, m.p., 150°C. When reacted with chlorine gas this oxime gave 5.66 grams of the 4-biphenylcarboxaldchloroxime. By reacting the chloroxime with sodioacetoacetic ethyl ester, the 3-biphenylyl-5-methyl-isoxazole-4-carboxylic acid ethyl ester was prepared. The 3(2',4'-dichlorophenyl)-5-methyl-isoxazole-4-carboxylic acid ethyl ester has been prepared using 2,4-dichlorobenzaldehyde. Work on the preparation of the 3-pyrenyl-isoxazole and the tetrachlorophenylene-bis-isoxazole is in progress.

SHEYENNE RIVER WATER STUDY. L. Becker, M. Graven, M. Lacy and D. Nix. Dept. of Chem. and Biol., V.C.S.C., Valley City, N. Dak.

The water from 16 strategic sites along the Sheyenne River was collected 3 different times during June and July of 1971 and tested for the following factors to determine its quality: pH, biological oxygen demand (B.O.D.), dissolved oxygen, EDTA hardness, nitrate, nitrite, phosphates, dissolved solids, acidity, alkalinity, bacterial and algal counts and classifications. The above mentioned chemical and biological tests were run according to Standard Methods for the Examination of Water and Waste Waters, APHA, 1965. Rainfall data was obtained from the U.S. Dept. of Commerce and recorded. The purpose of this study was to obtain base-line data on the conditions of the Sheyenne against which future data may be compared. Several trends were noticed during the study. The acidity, nitrates, nitrites, phosphates, dissolved solids, dissolved oxygen, and B.O.D. generally increased as the summer progressed. The alkalinity and pH decreased, as would be expected, with an increase in acidity. A large majority of the sites tested had positive presumptive and confirmatory Coliform tests. The bacteria count varied, showing no obvious trend throughout the summer, but at most sites the algae counts declined during the summer. This work was supported in part by a grant from the N.S.F., GY-5309.

LATE CRETACEOUS AND PALEOCENE TROCHIFORM SPECIES OF VIVIPARUS (GASTROPODA: VIVIPARIDAE) OF THE GREAT PLAINS AND ROCKY MOUNTAIN REGIONS. David Bickel. Dept. of Physical Sci., Minot State Coll., Minot, N. Dak.

Trochiform gastropod shells are characterized by conical spires and flattened whorls with lightly impressed sutures. Late Cretaceous and Paleocene rocks of the Great Plains and Rocky Mountains contain fossils of several species in the freshwater genera, Viviparus and Tulotoma, that possess these characters. In some species this shell form tends to persist throughout ontogeny, while in others it occurs on immature shells only to grade into convex penultimate or body whorls on adult specimens. Species groups can be characterized by the representative forms, V. conradi, V. trochiformis, and V. tasgina. Some aspects of viviparid evolution are discussed and the Late Cretaceous and Paleocene trochiform species of North Dakota are reviewed.

REGULATION OF ENZYME ACTIVITIES DURING HEPATIC GLUCONEOGENESIS IN RABBITS. R.A. Brunsvold and P.D. Ray. Dept. of Biochem., Sch. of Med., Univ. of N. Dak., Grand Forks, N. Dak., 58201.

Phosphoenolpyruvate carboxykinase(PEPCK), first reported as a mitochondrial(m_w) enzyme in rabbit liver, has recently been reported in cytosol(c_s) too. The importance of its cellular location to pathways of gluconeogenesis in rabbits has led to our current study which, by using m_w marker enzymes cytochrome oxidase and citrate synthetase, verifies both locations for the enzyme. Total activity of c_s PEPCK has been shown to increase over a relatively long period of time with fasting, alloxan diabetes and hydrocortisone(HC). We now report more rapid increases in this enzyme's total c_s activity by treatment with mannoheptulose(MH) (4 hrs) or HC(8-12 hrs). These data support the idea that gluconeogenesis in rabbits and rats can at least in part occur by similar processes. Since pyruvate carboxylase(PC), fructose 1,6-diphosphatase(FDPase) and glucose 6-phosphatase(G-6-Pase) are also considered as key enzymes in gluconeogenesis, effects of MH, HC, fasting and diabetes on total activities of these enzymes were also studied in rabbit liver. PC shows no significant responses. Alloxan diabetes, MH and HC all increase the total activity of G-6-Pase; only alloxan diabetes and MH increase the total activity of FDPase. Our data with rabbits are in general consistent with data from other species. (Support by NDEA and NIH AM 12705)

VITAMIN B_{12} PRODUCTION AND UTILIZATION IN A SEWAGE OXIDATION LAGOON. F. Choy, G. M. Fillipi and J. W. Vennes, Dept. of Microbiol., Sch. of Med., Univ. N. Dak., Grand Forks, N. Dak.

The role of vitamin B_{12} in an overloaded sewage oxidation lagoon was investigated. Early production of B_{12} (2650 ng/l) was observed during the June anaerobic phase of the lagoon. Emergence of the purple sulfur phase in June resulted in better than 90% removal of the vitamin. Disappearance of the purple sulfur organisms early in July resulted in an increase in B_{12} (1000 ng/l) in the lagoon. Subsequent emergence of the algal phase late in July resulted in about 90% removal of the vitamin. Pure culture studies with the predominant purple sulfur bacteria in the lagoon, Chromatium vinosum and Thiocapsa floridana and the predominant lagoon algae, Chlorella spp. and Scenedesmus spp., showed these photosynthetic species were capable of utilization of the vitamin. Lagoon heterotrophs, isolated in pure cultures, were grown in vitamin B_{12} assay medium. Only two organisms, Escherichia spp. and Flavobacterium spp. were capable of B_{12} production in this medium. Undoubtedly other lagoon organisms are capable of production and utilization of B_{12}, however it is concluded that since the photosynthetic species were the predominant organisms during B_{12} utilization they are likely candidates for the removal of the vitamin.

THE EARLY DETECTION OF DIFFICULTIES IN RATS DURING DECOMPRESSION FROM 13 ATMOSPHERES HELIUM-OXYGEN. J. A. Cromer, S. J. Brumleve, J. Carman, and E. S. Halas. University of North Dakota, Grand Forks, North Dakota.

The object of these experiments was early detection of difficulties due to rapid decompression so adjustments could be made to prevent trauma. Fasted rats trained to lever press for food were decompressed from 13 ATA He-O_2 compression and the rate of lever pressing recorded. A comparison of lever pressing rates during various constant decompression rates (0.08, 0.13, 0.27 ATA/min) suggested that a decreased rate of lever pressing was indicative of difficulties in the animal. At 0.27 ATA/min decompression the change in lever pressing rates indicates a partial paralysis early enough for change of the decompression to allow recovery of the animal. A 50% fatality rate occurred with animals decompressed at 0.27 ATA/min without any corrective procedure. Recompression to 5 ATA followed by constant decompression at 0.08 ATA/min prevented injury to the animal. At 0.13 ATA/min irregular lever pressing patterns were seen when the animals were decompressed to 0 ATA. Recompression followed by slow decompression prevented injury. ONR Contract No. N00014-68-A-0499.

LAND SNAILS OF NORTH DAKOTA: A PRELIMINARY REPORT. *A. M. Cvancara, J. B. Van Alstine*, and *W. E. Fenner*.
Dept. of Geology, Univ. N. Dak., Grand Forks, N. Dak. 58201

In July and August, 1969, two east-west transects (along 46° 30' and 48° 30' N.) and two north-south transects (along 98° 00' and 103° 00' W.) were made across North Dakota for land snails. General localities (26) along parallels were at the intersections with whole meridians; those along meridians were at the intersections with half-parallels. An open (no woody vegetation except wolfberry) and covered (with woody vegetation) habitat, selected randomly, was sampled adjacent to each meridian-parallel intersection. Twenty-one species were taken alive from the transects–1 cionellid, 1 valloniid, 6 pupillids, 3 succineids, 3 endodontids, 1 limacid, and 6 zonitids. The five most frequently occurring species were *Vallonia gracilicosta* Reinhardt, *Catinella avara* (Say), *Deroceras laeve* (Müller), *Euconulus fulvus* (Müller), and *Discus cronkhitei* (Newcomb). Three species, *Gastrocopta pentodon* (Say), *Vertigo binneyana* Sterki, and *Punctum minutissimum* (Lea), are newly reported for the state. Generally 1-3 species (up to 5) occupied an open habitat and 1-11 were in a covered habitat. More species, and generally more individuals, occurred in covered habitats. More species and presumably more individuals occurred in the eastern one-fourth of the state, east of about 99° 00' W. Supported in part by N. Dak. WRRI.

DIGESTION OF ANIMAL TISSUES FOR MERCURY DETERMINATION USING VANADIUM PENTOXIDE AS A CATALYST. F. D. Deitz, J. L. Sell and D. Bristol. An. Sci. and Biochem. Dept., NDSU, Fargo, ND.

A simple digestion method using sulfuric and nitric acid with vanadium pentoxide as a catalyst has been developed for the determination of total mercury (Hg) in milk and meat at the 5 to 15 nanogram level. Digestion of 1 to 3 gm samples was accomplished in open 250 ml volumetric flasks within approximately 30 minutes. The temperature necessary for complete recovery of Hg from methyl mercury chloride in standard solutions was at least 140°C. After oxidation of organic matter, Hg is determined by atomic absorption spectrophotometry using the cold vapor technique. Recovery of 10 ng of Hg added to milk and meat in the form of mercuric chloride was 92 and 93%, respectively, while recovery of 10 ng of added Hg as methyl mercuric chloride was 110 and 112% from milk and meat, respectively. The coefficient of variation for determination of standards with 10 ng of Hg from mercuric chloride was 8.1%, while that for 10 ng of Hg from methyl mercuric chloride was 6.1%. The standard curve obtained from either mercuric chloride or methyl mercuric chloride was linear from 0-150 ng. Although more study is needed, this method of preparing samples for Hg determination appears to have considerable merit, particularly for biological materials which contain less than 20 ppb of Hg.

SEASONAL VARIATION IN SWINE SEMEN CHARACTERISTICS. W. Eide, J.E. Tilton and D.T. Jensen. Dept. of Anim. Sci., NDSU, Fargo, N. Dak.

Semen was collected from eight mature boars of two breeds with certain semen characteristics recorded from the samples as well as ambient temperatures at the time of collection in an attempt to determine the effects of low temperature on semen production and quality. The data indicated there were no differences in semen characteristics between boars as well as between breeds. Within individual variation as well as within breed variation was too large to observe significant mean differences. Semen samples were grouped according to temperature at time of collection and semen characteristics correlated with the low daily recorded temperature. Statistically significant correlation coefficients ($P < .05$) indicated as ambient temperature decreases, very little loss of semen quality occurs. The percent abnormalities increased whereas the motility and concentration tended to decrease as ambient temperature increased. Although these values were statistically significant, they were small and accounted for five to six percent of the total variation in swine semen quality. Also concentration per ml. and total sperm tend to increase as temperatures decline, thus if the semen characteristics examined in this study are true criteria of a boars' fertility capacity than a cold environment should have no effect on conception rate and litter size potential

DIGESTION OF BROME AND ALFALFA AS DETERMINED BY CHEMICAL CONSTITUENTS. D.O. Erickson, Dale B. Ferebee, C.N. Haugse and K. L. Larson. Dept. of Animal Sci., NDSU, Fargo, N. D.

Alfalfa (medicago sativa) and brome (Bromus inermis) plots (4 replicates) were sampled 15 times a season for 3 years. Estimated digestion coefficients were determined using the in vitro "summative equation" derived by Van Soest in 1967. The equation is based on the negative relationship of acid detergent fiber, lignin, cell wall and silica to digestibility. The digestibility of alfalfa (25% bloom) for cattle was 61.8% $\pm$ 5.3 and ranged 70.9% to 37.8% from early growth to the over ripe stage. Brome (near heading) was 54.6% $\pm$ 4.9 and during the season ranged from 67.2% in May to 34.4% in late summer and fall. Most of the simple and multiple regression equations using chemical constituents to estimate digestibility were significant. Protein was positively correlated (r=0.84 and 0.77)to the digestibility of alfalfa and brome. Phosphorus followed the same pattern as protein in relation to digestibility. There is a considerable decrease in the digestibility of alfalfa and brome with advancing maturity. Many of the chemical fractions or combinations of these fractions are useful to estimate forage digestibility.

CHEMICAL COMPOSITION OF BROME GRASS AND ALFALFA AS AFFECTED BY PHYSIOLOGICAL GROWTH STAGE. D.B. Ferebee, D.O. Erickson, C.N. Haugse, and K.L. Larson. Dept. of Anim. Sci., NDSU, Fargo, N.D.

North Dakota harvested approximately 1,328,000 acres of alfalfa (Medicago sativa) and approximately 802,000 acres of brome grass (Bromus inermis) a year during a 3-year period 1967-69. Plots (4 replicates) of brome and alfalfa were sampled 15 times a season during 3 growing seasons. Alfalfa and brome were divided into 12 and 8 physiological growth stages respectively. Samples were freeze-dried and analyzed for dry matter, ash, acid detergent fiber, acid detergent lignin, cell wall, protein, silica and phosphorus. Fiber, lignin and cell wall increased with growth stage (r=0.82, 0.72 and 0.84 for brome and 0.43, 0.37 and 0.38 for alfalfa). Fiber increased from 16.3% to 46.9% in alfalfa and 20.9% to 39.4% in brome respectively with growth stage. Lignin, fiber and cell wall were highly correlated in both alfalfa and brome. Protein and phosphorus decreased with growth stage in both alfalfa and brome (r=-0.44, -0.39 for alfalfa and -0.92, -0.82 for brome). Total silica is much higher in brome than alfalfa and in brome increases with maturity. From August on, protein and phosphorus in brome are below recommended requirements for ruminants at any stage of the reproductive cycle or growth. Chemical constituents vary with growth stage.

OSTRACODA FROM THE TONGUE RIVER FORMATION (PALEOCENE), WARD COUNTY, NORTH DAKOTA. Diane Leedy Gruber and David Bickel. Dept. of Physical Sci., Minot State Coll., Minot, N. Dak.

Five freshwater ostracode forms were tentatively identified from the Tongue River Formation (Paleocene) in southern Ward County, North Dakota. The material was recovered from calcareous, lacustrine sediments in association with a large molluscan assemblage. There are few published reports of freshwater Ostracoda in the Tongue River-Sentinel Butte interval. This apparent rarity may be due to the small percentage of near-shore lacustrine sediments in these strata and limited examination of this facies for microfossils in the past.

MAGNESIUM AVAILABILITY STUDIES UTILIZING A COMBINATION OF COMPARATIVE-BALANCE AND ISOTOPE-DILUTION TECHNIQUES. W. Guenter and J. L. Sell. Dept. of An. Sci., NDSU, Fargo, ND.

Five experiments were conducted with mature male chickens to establish a valid and reliable technique to determine Mg availability from foodstuffs. Chromic oxide was included as an unabsorbable marker in all diets, to facilitate collection of comparative-balance data. ^{28}Mg was administered intramuscularly to label the endogenous Mg pool so that the use of the isotope-dilution method could also be exploited. Studies on the effect of time after ^{28}Mg injection and influence of dietary Mg level on ^{28}Mg equiliberation indicated that with dietary Mg levels not exceeding 400 ppm, ^{28}Mg equilibrated among plasma, urine, bile, pancreas, heart, liver and intestinal mucosa within 48 hrs as shown by their relative specific activities. On this basis it was concluded that the specific activity of plasma Mg and of Mg in digesta could be used to partition dietary and endogenous Mg in the excreta. A formula was derived into which comparative-balance and isotope-dilution data could be applied to calculate Mg availability. Subsequent experiments with $MgSO_4$ as reference material (100% available) resulted in relative availabilities of this mineral from grains as follows: wheat, 95%; corn, 89%; barley, 95%; oats, 182%; soybean meal, 105% and polished rice, 75%. Supported in part by USPS, NIH (Grant 5R01 AM12512).

CHYMOTRYPSIN-CATALYZED HYDROLYSIS OF N-CARBOBENZOXY-L-AMINO ACID P-NITROPHENYL ESTERS. L.M. Gutnik and J.A. Stewart, Dept. of Chem., Univ. of N. Dak., Grand Forks, North Dakota 58201.

Because of the large extinction coefficient of the nitrophenolate ion released as one of the products, nitrophenyl esters are among the most suitable of the wide variety of substrates available for the study of chymotrypsin. These substrates allow both the transient phase and steady state kinetics to be determined. However, only limited work has been reported using the nitrophenyl esters of amino acids. N-carbobenzoxy-L-phenylalanine (CPNE), N-carbobenzoxy-L-tyrosine (CTNE) and N-carbobenzoxy-L-alanine (CANE) p-nitrophenyl esters were investigated in 50% (V/V) isopropanol-aqueous solutions. The initial release of phenolate ion was recorded and the data analyzed using the Guggenheim method. From the results with excess substrate the rate constants associated with complex formation were determined to be $\sim 10^5$ l m^{-1} s^{-1}. Because of the low solubility of CPNE and CTNE, it was not possible to isolate the rate constant for acylation with these substrates. However, tentative studies with CANE using stopped-flow techniques (excess enzyme) gave an acylation constant of 3 s^{-1}; indications are that this stage is rate-controlling. This work was supported by research grants USPHS (GM-16167) and NSF (GY-8979).

TWO FORMS OF 3,4,5-TRIMETHOXYBENZALDOXIME. John W. Hamilton and Franz H. Rathmann. Dept. of Chemistry, NDSU, Fargo, N. Dak.

3,4,5-Trimethoxybenzaldoxime was reported by Semmler and Mauthner to melt at 83-84°, and by Heffter and Cappelmann to melt at 91° (Beilstein, Vol. H VIII, 391). We found it to exist in two forms. When first prepared by the action of hydroxylamine on 3,4,5-trimethoxybenzaldehyde and purified by (re)crystallization from ethyl alcohol, benzene, methanol, isopropyl alcohol, or n-butyl alcohol, the oxime melts at 83-84°C. When left standing the melting point slowly rises to 104°C after about 4 days. When recrystallized the resulting 84° melting point of the product again gradually rises to 104°C after 4 days. DTA (differential thermal analysis) graphs for the 84°C crystals exhibited two equal endothermic peaks at 84°C and 91°C respectively; the 104°C crystals yielded a sharp endotherm at 104°C. DTA on intermediately aged crystals showed a gradual disappearance of the 91°C peak, a shift and broadening of the sharp 84°C peak toward higher values, narrowing again to a sharp peak at 104°C.

VASCULAR FLORA OF DIVIDE, WILLIAMS, BURKE, AND MOUNTRAIL COUNTIES, NORTH DAKOTA. Glen D. Hegstad and W. T. Barker, Dept. of Botany, N. D. St. Univ., Fargo, N. D.

This study is based upon fieldwork completed during the 1969, 1970, and 1971 growing seasons and on a study of the specimens previously collected from the area and deposited in the NDSU herbarium. The area's topographical features typify the southern glaciation limits of the last ice age. This northwestern area of the state includes a portion of the Drift Prairie, the Coteau Slope, and the Missouri Coteau. The latter dominates the central area. Badland features are found along the Missouri River, its tributaries, and locally elsewhere. Aspen bordered sloughs, alkali lakes, seepage areas, and eroded clay buttes offer rather unique habitats for the area. As a result of this study 695 species are known for the area. Thirty nine, previously collected plants, were not found in this present study. Two hundred twenty one new NDSU herbarium records for the area were recorded. Four new state records were recorded. They are: Carex rossii, Thelesperma marginata, Penstemon procera, and Gnaphalium uligonosum.

FREQUENCY INDEPENDENT PHASE SHIFTING AND LEVEL SHIFTING USING INEXPENSIVE ANALOG MULTIPLIERS, D. C. Hicks Electrical and Electronics Engr. Dept., North Dakota State University, Fargo, North Dakota 58102.

The major disadvantages of conventional phase shifting networks, frequency dependence, can be totally eliminated with the use of an inexpensive multiplier. Also converting a square wave into sine wave calls for some kind of level control for the generated sine wave, which can again be accomplished by an analog multiplier. Phase shifting and automatic level control are vital functions when trying to obtain sine waves from square waves. The author developed a system where a ramp voltage obtained from sq:wave is converted into a DC reference voltage, whose relative value can be controlled by an analog multiplier and an associated control voltage. Variation of a control voltage results in a variable phase shift introduced into the original wave train, which is independent of frequency. The filtered output of the system, a sine wave, is again processed through a multiplier used as a divider for obtaining a constant amplitude sine wave, independent of frequency.

SOME EFFECTS OF ETHANOL ON AMINO ACID TRANSPORT. F.A. Jacobs, J.C. Crandall and C.B. Fabel. Dept. of Biochem., Sch. of Med., Univ. N. Dak., Grand Forks, N. Dak. 58201

These investigations were undertaken to study the influence of ethyl alcohol upon the absorption of amino acids, especially methionine, from the intestinal lumen of the rat in vivo. Amino acids were perfused through cannulated segments of the upper small intestine of animals under sodium pentobarbital anesthesia. Feeding liquid diets with or without ethanol from 2 to 3 weeks before perfusion had no significant effect on amino acid (5 x plasma concentration mixture) absorption under the conditions of these experiments. However, ethanol (2.5% by vol) did inhibit the absorption of 3 mM L-methionine (^{14}C-methyl). When 2.5 ml of ethanol (20% by vol), were injected into the intestinal lumen just distal to that perfused, an inhibition in methionine absorption was also observed. Not all animals responded to ethanol to the same extent. Experiments were performed to determine physiological concentrations of amino acids in the intestinal lumen under post-absorptive and under feeding conditions in order to establish lumenal base-line levels for amino acids. Perfusion experiments are continuing with varied concentrations of methionine and ethanol. (Supported in part by NIH Research Grant No. MH 19235-02 ALC).

REMOTE MULTISPECTRAL SENSING: AN ALTERNATIVE VIEW OF THE EARTH. Gary E. Johnson. Dept. of Geography, Col. of Arts and Sciences, Univ. N. Dak., Grand Forks, N. Dak.

In order to evaluate the performance characteristics of remote multispectral sensing techniques a cover type identification capability study was conducted at the Laboratory for Applications of Remote Sensing (LARS) at Purdue University using data collected in five flights over Tippecanoe County, Indiana by an optical-mechanical scanner equipped aircraft. Using techniques and procedures developed by LARS a computer classification analysis of the multispectral data was conducted on nine classes of cover type. It was found that identification performance ranged from an average of over 99 percent correct recognition for the water class to 57 percent correct recognition for pasture under the conditions present at the time of data collection. The additional cover types considered were wheat, oats, corn, soybeans, hay, trees, and bare soil. The study indicates that remote multispectral sensing techniques offer an alternative to presently recognized means of viewing characteristics of the earth environment to a degree of accuracy which can be quantitatively determined. This research was supported in part by LARS and Indiana State University.

IBR VIRUS-INDUCED BOVINE ABORTION: DETECTION BY VIRUS ISOLATION AND IMMUNOFLUORESCENCE. C. L. Kelling, I. A. Schipper and J. E. Tilton. Dept. of Vet. Sci., Col. of Agric., N. Dak. State Univ., Fargo, N. Dak.

Thirty-four cases of infectious bovine rhinotracheitis (IBR) virus-induced abortion were detected by testing composite tissue inoculums obtained from 425 aborted bovine fetuses utilizing virus isolation techniques. IBR isolates were identified by cytopathogenic effect (CPE) and neutralization of the virus with IBR specific antiserum. Virus isolation attempts with individual tissues indicated small intestine, lung and placental tissues were superior to kidney and spleen tissues for isolation tests. The relative competence of immunofluorescent techniques for detection of IBR virus-induced bovine abortion was determined. The specificity of two fluorescein isothiocyanate (FITC) conjugated anti-IBR-serum preparations was tested utilizing cryostat-sectioned fetal tissues and bovine endocardial tissue culture cells growing on Leighton coverslips. The fluorescence that was produced was demonstrated to be nonspecific. Absorption of IBR virus-infected cells with hyperimmune anti-IBR-serum did not inhibit fluorescence. Nonspecific fluorescence was observed in heterologous lysed cells. Nonspecific fluorescence was observed when FITC-conjugated anti-IBR-serum was applied to bovine virus diarrhea virus-infected cells.

USE OF REMOTE SENSING TECHNIQUES IN DELINEATION AND MEASUREMENT OF PLANT COMMUNITIES. A. T. Klett and H. K. Nelson. Northern Prairie Wildlife Research Center, B.S.F.W., Jamestown, N. Dak.

Personnel of the U.S.D.I., the Univ. of Michigan, and S. Dak. St. Univ. have been investigating the practicality of using multispectral scanner data and photography to detect and measure wetlands and associated plant communities. Between 1968 and 1970 five remote sensing missions were flown over a study area near Woodworth, N. Dak. at altitudes varying from 610 to 18,350 meters. Similar flights were made over a forested area near Bemidji, Minnesota in 1970 and 1971. Data were collected with a multispectral scanner and a variety of cameras using different film/filter combinations. Computer programs were used with the scanner data to automatically recognize wetlands and broad vegetative types, to measure area and perimeter of individual wetlands, and to generate analog and digital maps. Some of the photographic products were used to delineate vegetative characteristics that were not discernible on standard aerial photographs.

NORTH DAKOTA FLEAS. IV. COLD TOLERANCE IN THE BIRD FLEA, CERATOPHYLLUS IDIUS. Omer R. Larson. Dept. of Biol., Univ. N. D., Grand Forks, N. Dak.

In eastern North Dakota, fleas of a migratory bird such as the purple martin spend 7-7½ months of each year without their hosts. In order to investigate winter survival of these ectoparasites, adult C. idius were collected in nest material from a purple martin birdhouse in Grand Forks during late October 1971. On November 1st active adults were isolated at random and placed in groups of 10 in 22 small, cotton-stoppered test tubes. Half of these were placed in a freezer at -8°C, the others in a refrigerator at +5°C. During the next 4 months, tubes of fleas were removed at 2-week intervals and allowed to reach room temperature. Survivorship counts indicate that specimens held at -8° live longer than those at +5° (83.8% versus 47.5%). At either temperature more females survive than males (87.0% versus 79.4% at -8°, and 53.2% versus 30.2% at +5°). Occasional samples from the martin house throughout the fall and winter failed to yield living adult fleas after mid-January. Whether some adults can survive the winter to reinfest the martins each spring is problematic. Perhaps winter survival occurs in some other stage of the flea's life cycle.

LYMPHOCYTOSIS STIMULATING HORMONE: A THYMUS HORMONE. F. E. McCoy, Jr., Williston High School, Williston, ND 58801

The purpose of this project is to answer these questions: 1a) Is lymphocytosis stimulating hormone (LSH) present only in the thymi of neonates? or 1b) Is LSH also present in the thymi after maturation? 2a) Is LSH an agent that aids in the maturation of the developing lympho-immune system of the newborn? or 2b) Does LSH cause an acute lymphocytosis at any age? To investigate 1a and 1b, I gave intraperitoneal injections of extract of thymi of 4-month-old Wistar rats to 32 rats 24 hr. after birth. I injected another group of 31 neonates with extract of thymi of 8-day-old rats. To answer 2a and 2b, I gave subcutaneous injections of material from thymi of 4-month-old rats to 4-month-old male rats. Another group of 4-month-old males received extract of thymi from 8-day-old rats. Differential and total white blood cell counts were done on all groups. Eight days after injections, neonates receiving extract of thymi of 4-month-olds had 2900 lymphocytes per mm^3 blood. Newborn given extract of thymi from 8-day-olds had 5200. No significant differences were found between the two groups of 4-month-old males. These data support the conclusion that LSH is present only in prepubertal thymi and aids in the maturation of the lymphatics.

NONTOXIC COATINGS, APPLICATIONS AND SYNTHESIS. J. R. McDermott, Dept. of Polymers and Coatings, NDSU, Fargo, North Dakota.

A nontoxic coating in which the organism Bacillus popilliae could survive on exposure to water was developed through a study of various natural oils and driers. Conventional heavy metal driers could not be employed due to their toxic effect on Bacillus popilliae. Raw tung oil and tri-n-octylaluminum in various combinations were found to be nontoxic. Linseed, safflower and soya oils did not present desirable characteristics when reacted with tri-n-octylaluminum. Tung oil with tri-n-octylaluminum did provide a degree of moisture resistance but further improvements in the impermeability of the coatings are needed. The exclusion of water was necessary to insure the viability of the test organism since on contact with water Bacillus popilliae is activated and soon dies from lack of food. Moisture tests were facilitated by use of phenolthalein indicator and a slightly basic solution. The tests were qualitative but gave an indication of water permeability. The study was partially supported by the Agricultural Experiment Station and by an undergraduate institutional grant.

SUPPLEMENTING SWINE RATIONS WITH LYSINE AND METHIONINE. J. L. Nelson and W. E. Dinusson, Dickinson Experiment Station, Branch of N.D.S.U., Fargo, N. Dak.

This study compares swine rations composed of barley and oats supplemented with either soybean oilmeal or L-lysine and DL-methionine. In Phase 1, 78 Yorkshire barrows and gilts (16kg) were fed either a 12% protein ration of barley and oats (base) or the base ration plus SBOM to make a 16% protein feed or the base ration plus 0.3% L-lysine and 0.15% DL-methionine. The pigs were fed both in dry lot and on pasture. A 14% protein ration of the base plus SBOM, 0.15% L-lysine and 0.125% DL-methionine was added in Phase 2, where 140 pigs (18kg) were fed in dry lot and on pasture. In Phase 1, the 16% protein ration produced the best gains, followed by the L-lysine - DL-methionine supplemented ration. In Phase 2, the 16% protein ration gained 17.1% faster and was 9.82% more efficient; the 14% protein ration gained 19.15% faster on 7.77% less feed; and the base ration plus L-lysine and DL-methionine gained 18.8% faster and was 9.37% more efficient than the base ration.

ZOOPLANKTON-PHYTOPLANKTON INTERRELATIONSHIPS IN HOOKER LAKE, ROLETTE COUNTY, N. DAK. R.N. Nordin and J.B. Owen, Dept. of Biology, Univ. N. Dak., Grand Forks, N. Dak.

Population levels of zoo- and phytoplankton in Hooker Lake, were investigated from June 1970 to May 1971. The organisms were identified in a Sedgwick-Rafter counting chamber. An inverse relationship was found between populations of zoo- and phytoplankton, when one was high the other was low. Blue-green algae (Aphanizomenon flos-aquae and Oscillatoria spp.) increased during the summer to maxima of 5000 cells/ml in July and 1100 cells/ml on 21 August, respectively. Keratella cochlearis increased to a maximum of 5300 organisms/l on November; there was a concomittant decline in phytoplankton. The zooplankters Brachionus angularis attained a population of 2200 organisms/l on 11 May and K. cochlearis, 1700 organisms/l on 27 May; the phytoplankton population at this time was minimal. Supported by the North Dakota Game and Fish Department and the U.S. Bureau of Sport Fisheries and Wildlife (Dingell - Johnson Act).

ISOLATION AND CHARACTERIZATION OF SURFACE-ACTIVE EXTRACTS OF RAT LUNG. R.E. Olson and R.H. Wilson. Dept. of Physiol. and Pharmacol., UND Sch. of Med., Grand Forks, N.D.

It is generally accepted that pulmonary surfactant is composed chiefly of dipalmitoyl lecithin. However, there is considerable debate as to the protein content of surfactant. This study was made to determine the presence of protein in surfactant. Female Sprague-Dawley rats were used. By perfusing isolated heart-lung preparations while alternately compressing and expanding the lungs in a closed chamber, foamy extracts were obtained. These extracts contain pulmonary surfactant and exhibit surface-activity. Each sample was separately dialyzed to remove the NaCl, lyophilized for purposes of concentration and storage, redissolved in a small amount of saline and subjected to disc electrophoresis using polyacrylamide gel. Serum samples were taken from each rat to serve as controls. A comparison of the extract samples to the serum controls indicated that there was no protein in the extracts except that from serum contamination.

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THE VERTICAL AND LATERAL DISTRIBUTION OF GOLDEYE, HIODON ALOSOIDES, AND YELLOW PERCH, PERCA FLAVESCENS, IN THE LITTLE MISSOURI ARM, LAKE SAKAKAWEA. J. B. Owen and C. H. Wahtola Jr. Dept. of Biol., Univ. of N. D., Grand Forks, North Dakota.

Goldeye, Hiodon alosoides, and yellow perch, Perca flavescens, are the two most abundant fish in Lake Sakakawea (Garrison Reservoir). Both species have similar food habits, but fill separate niches. Goldeye were found most often in the upper strata of the water column and were most abundant in the more turbid upstream portion of the arm. Yellow perch were most commonly encountered in the lower strata of the water column but were equally abundant along the entire arm. Water temperature and turbidity were considered to be influential in the distribution of both species. Supported by North Dakota Game and Fish Department and the U.S. Bureau of Commercial Fisheries (N.M.F.S.) under P.L. 88-309.

ISOPROPYL CARBANILATE [PHENYL-^{14}C(U)] METABOLISM IN THE RAT AND GOAT: RATES AND ROUTES OF ELIMINATION AND TISSUE RESIDUES. G. D. Paulson and A. M. Jacobsen. USDA, ARS, Metabolism and Radiation Research Laboratory, Fargo, North Dakota 58102

Rats and a milking goat were given a single dose (100 mg per kg of body weight) of isopropyl carbanilate [phenyl-^{14}C(U)]. Urine, feces, and milk were collected for 48 hr. after dosing. The animals were then sacrificed, and tissues were removed for analysis. The rats excreted 95.8% of the administered carbon-14 in the urine, and the goat excreted 90.2% of the activity given in that fraction. The elimination of carbon-14 in the feces of the rat and goat accounted for 2.3 and 3.3% of the dose, respectively. Goat milk collected during the 48-hr. period contained 0.45% of the carbon-14 given. The liver in both the rat and goat was the tissue with the highest specific activity 48 hr. after dosing; total carbon-14 residues in the rat and goat accounted for 0.56 and 1.05%, respectively, of the activity given.

EFFECT OF CARBOXIN ON IN VITRO AND IN VIVO GROWTH OF USTILAGO NUDA. V. D. Pederson and Mei Mei Yang. Dept. of Pl. Path., NDSU, Fargo, N. Dak.

The systemic seed treatment fungicide 5,6-Dihydro-2-methyl-1,4-oxathiin-3-carboxanilide (Vitavax 75[1] = carboxin) was applied to Larker barley seed at the rate of 4 oz/cwt. The chemical was absorbed during imbibition by the germinating seed. After germination of carboxin-treated seed, hyphae of Ustilago nuda (Jens.) Rostr., were limited to the scutellum, scutellar node, and cells of the ground meristem. Hyphae were never found in the head primordium. In infected seedlings from untreated seed, U. nuda hyphae were found in the growing points within 5 days, and in head primordia 16-21 days after seeding. U. nuda cultured 8 days on PDA amended with 3 ppm carboxin grew slightly, but was inhibited by 5 ppm or higher concentrations. These mycelia grew normally after transferring to PDA. In other experiments, U. nuda mycelium was treated for various lengths of time in up to 100 ppm carboxin-amended liquid media. After removing and washing the mycelia in sterile water, they resumed growth on PDA. These results indicate carboxin is fungistatic rather than fungicidal.

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INTESTINAL CONJUGATION AND TRANSPORT OF ^{14}C-NAPHTHOL IN VITRO. Jerome C. Pekas. USDA, ARS, Metabolism and Radiation Research Laboratory, Fargo, N. Dak. 58102

Intestinal synthesis of naphthol glucuronide has been reported (Am. J. Physiol. 220: 2008, 1971). The effect of increasing the concentration of ^{14}C-naphthol in the mucosal and serosal media was investigated using everted sacs of rat small intestine. As the concentration was increased from 0.01 to 0.5 mM, the following changes were observed: The tissue ^{14}C concentration increased linearly to values 10 times the mucosal fluid concentration; cranial tissue was lowest, and caudal tissue the highest. Serosal fluid contained 98% conjugated ^{14}C-naphthol at 0.01 mM, but this decreased to 35% in 0.5 mM medium. The quantity of conjugate synthesized and released into mucosal and serosal fluids rose sharply and approached an apparent maximum in 0.05 mM medium; the cranial sac was most active and caudal least active at each concentration tested. Net transfer of ^{14}C-label to the serosal fluid also approached an apparent maximum in 0.05 mM but decreased as the conc was increased to 0.5 mM. The caudal sac was most active in 0.01 mM medium but dropped sharply to the least active in 0.25 and 0.5 mM media. There was some evidence that 0.5 mM naphthol was toxic.

CAUSES AND CONTROL OF ALGAL BLOOMS IN SPIRITWOOD LAKE, NORTH DAKOTA. John J. Peterka and John W. Held. Dept. of Zoology, NDSU, Fargo, N. Dak.

Sources and possible means of control of algal blooms in Spiritwood Lake, a popular recreational lake, were investigated during the summer of 1970. The objective was to provide a preliminary estimate of sources of nitrogen and phosphorus and to recommend necessary research approaches toward solving eutrophication problems of the lake. Of the estimated nitrogen and phosphorus entering the lake during spring runoff, 62% of the nitrogen and 95% of the phosphorus appeared to come from diffuse land surface sources, such as drainage from fields and grasslands. Nitrogen, and to a lesser degree, phosphorus in livestock excrement are potentially important contributors to nutrient enrichment. Field experiments indicated that additions of inorganic nitrogen increased algal production whereas phosphorus inhibited it.

MASS SPECTROSCOPY OF SOME LONG-CHAIN ALKYL AND ALKENYL MONO- AND DI-ETHERS OF ETHYLENE GLYCOL AND PROPANEDIOLS. Franz H. Rathmann, NDSU, Fargo, N. Dak., and Ralph T. Holman, Hormel Institute, Univ. of Minn., Austin, Minn.

Alkyl and alkenyl mono and diethers of ethylene glycol, of propylene glycol, and of trimethylene glycol were subjected to 7-100 eV electron bombardment in a Hitachi-Perkin Elmer RMU 6D Mass Spectrometer. The parent peak $ROCH_2CH_2OR$ (R = hexadecyl) is strong, as are those for the fragments R, RO, and $ROCH_2CH_2O$. The $ROCH_2$ and $ROCH_2CH_2$ peaks are relatively weak. Peaks intermediate between P and $ROCH_2CH_2O$ are very weak. For $ROCH_2CH_2CH_2OR$ (R = octadecyl) the $ROCH_2$ peak is very strong, $ROCH_2CH_2CH_2$ strong, R and $ROCH_2CH_2CH_2O$ prominent, while RO and $ROCH_2CH_2$ are very weak. 1-Octadecyloxy-3-cis-9-octadecenyloxypropane shows somewhat similar fragmentation. 3-Octadecyloxypropanol, $R\text{-}OCH_2CH_2CH_2OH$ yields a weak P pattern, a strong $ROCH_2$, but no $ROCH_2CH_2$, and all other C-O and C-C fission fragments. Primary fission in all cases occurs mainly at the C-O bonds. Fragments smaller than R are mainly in the C_4, C_5, C_6 range.

THE EFFECT OF SUCCINATE ON BRAIN GAMMA-AMINOBUTYRIC ACID LEVELS IN MICE. R. L. Reinke and R. H. Wilson. Dept. of Physiol. and Pharmacol., UND Sch. of Med., Grand Forks, N. D.

The purpose of this experiment is to determine the effect of intraperitoneal (ip) injections of succinate on gamma-aminobutyric acid levels in the brains of male Swiss-Webster mice and the resulting tolerance to high pressure oxygen. The weight of the mice ranged between 20-30 gms. Control mice were injected (ip) with isotonic saline and were decapitated after either 15, 30, 60, 120, or 180 min. Experimental mice were injected ip with 10 mM/kg of isotonic sodium succinate (0.4 M; pH adjusted to 7.4). These mice were decapitated after either 15, 30, 60, 120, or 180 min. GABA was determined on an ACTA II spectrophotometer. A significant increase in GABA was found in the succinate injected mice at every time interval when compared to the corresponding control group.

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SELECTIVE PREDATION BY MINK ON WATERFOWL. A. B. Sargeant, G. A. Swanson and H. A. Doty. Northern Prairie Wildlife Research Center, Jamestown, N. Dak.

Predation by mink (Mustela vison) on captive, pen-reared-released, and wild ducks was documented in two studies at the Northern Prairie Wildlife Research Center. In the first study 36 of 60 flightless adult and juvenile ducks held on eight 0.1-acre experimental ponds disappeared between July 10 and August 4, 1969. All were believed to have been killed by a large adult mink. The mink selected recently released incubator-hatched ducklings, females in the process of incubating, and adults and juveniles on a marginal food supply.

In a second study 152 wood duck ducklings (Aix sponsa) were released on a 76-acre marsh during 1971. Half of the birds were released from four floating pens when 28 to 31 days old and the remainder flew from a shoreline pen when approximately 60 days old. The shoreline of the marsh was periodically searched, and the remains of 21 (28 percent) of the floating-pen birds were identified in food remains found at 16 mink dens. Coots (Fulica americana) were also taken commonly. Wild ducklings appeared to have been almost totally consumed, but the legs and feet of the wood ducks were often left uneaten because of bands and tags.

A MORE ACCURATE URANYL-OXALATE ACTINOMETER. R. Skodje, Bismarck High School, Bismarck, ND 58501

The uranyl-oxalate actinometer has ben used widely in the past to measure light intensities through the monitoring of the photochemical breakdown of oxalic acid sensitized by the uranyl ion. My method of obtaining intensities differs from the classical procedure as I monitor the production of CO, carbon monoxide, a product of the oxalic breakdown, rather than by titration of the decrease of the oxalic acid. To determine CO concentration, I passed an inert gas over the uranyl-oxalate solution in the reaction cell, this will encompass the CO in the gas flow. The gas flow, with an oxygen impurity added, is passed over a heat source where the CO is burned with the oxygen. The gas flow now is routed into an electrolytic oxygen detecting cell. The intensity of the light can, therefore, be expressed as a function of the difference between the currents before and after combustion. My technique has proven to be accurate to approximately 4×10^{14} quantas/sec. With CO detection it is also possible to receive an almost instantaneous reading on changes in intensities. This method is, therefore, of more value than the standard procedure.

A GENETIC ANALYSIS OF LEAF RUST RESISTANCE IN LEEDS DURUM WHEAT. G. D. Statler. Dept. of Pl. Path., NDSU, Fargo, N. Dak.

The inheritance of resistance to wheat leaf rust incited by Puccinia recondita Rob. ex Desm. f. sp. tritici, was investigated in Leeds durum wheat. Leeds is moderately resistant to leaf rust in the field and is resistant to 70-1, a culture of race 1 which is widely avirulent. Leeds was crossed to the rust susceptible line D6618 for the genetic analysis. All F_1 plants of reciprocal crosses were susceptible to 70-1 indicating one or more recessive genes conditioning resistance. The F_2 plants inoculated with culture 70-1 segregated approximately 15 susceptible to 1 resistant indicating that two recessive genes condition resistance in Leeds. The 281 F_3 families tested satisfactorily fit a 2 factor ratio of 7:4:4:1. One hundred fifteen families were homozygous susceptible, 82 segregated 3 susceptible : 1 resistant, 73 segregated 15 susceptible : 1 resistant and 11 families were homozygous resistant.

A TEST FOR THERMISTOR RELIABILITY AND ACCURACY AT HIGH PRESSURE. L. C. Stetzner and B. DeBoer. Dept. of Physiol. and Pharm., Sch. of Medicine, University of North Dakota, Grand Forks, North Dakota.

In order to measure physiological changes at high pressure, it is important that the sensor or instrumentation be an accurate index to a given response. Thermistor probes (Models YSI 402--small animal, and YSI 405--air) were tested by placing them, one at a time, in a U-shaped housing of reinforced pipe. The temperature from the thermistor was read on a tele-thermometer via a high pressure electrical throughput. The housing was submerged in a constant-temperature-controlled water bath. Readings were taken at 1 ATA, after compression to 41 ATA with helium, and after decompression. This procedure was repeated 10 times for each thermistor probe. The results indicated a variation in temperature of less than $\pm$ 0.1° C for all trials.

(Supported by ONR Contract No. N00014-68-A-0499)

SELECTION OF THERMODURIC ESTEROLYTIC BACTERIA FROM RUMINAL DIGESTA. R. L. Stolzenberg and P. P. Williams. USDA, ARS, Metabolism and Radiation Research Laboratory, Fargo, N. Dak.

Certain esterolytic ruminal bacterial strains are capable of degrading a carbamate insecticide (Mobam). Additional esterolytic strains have been isolated on the basis of their heat tolerance and ability to hydrolyze tributyrin in a differential medium maintained under CO_2. Serial dilutions of bovine ruminal digesta were heated at 80 C for 20 min along with unheated (control) samples and inoculated into the medium. The medium was solidified in roll tubes and incubated at 39 C for 14 days. The control samples showed colonies with zones of hydrolysis up to 10^{-8} dilution. These colonies at 10^{-7} dilution contained gram negative straight and curved bacilli. The heat-treated samples contained thermoduric esterolytics up to 10^{-6} dilution. On the basis of morphology and gram reaction, the thermoduric bacteria were categorized into five groups. Groups 1 and 2 were gram positive bacilli (1.7 X 1.8 μ or 3.0 X 8.0 μ); group 3, gram variable bacilli (1.3 X 6.5 μ); and groups 4 and 5, gram negative curved or straight bacilli (1.1 X 8.7 μ or 1.4 X 4.6 μ).

SODIUM IN NORTH DAKOTA LIGNITE. F. T. C. Ting, Dept. of Geology, Univ. N. Dak., Grand Forks, N. Dak. 58201

Sodium in the ash of lignite has long been considered one of the major elements that causes fouling of boilers. Deposits of ash on boiler tubes cause severe heat transfer problems and may even damage the boiler itself.

Analyses of the ash of ten lignite samples from the upper Tongue River Formation (Paleocene) indicate that the sodium content varies with the petrographic composition and geographic distribution of the lignite. Vitrain (anthraxylon) tends to have a higher sodium content than durain and fusain. The average Na_2O contents of the ash of vitrain, durain, and fusain at the Glenharold Mine, Stanton, N. Dak., are 12.5%, 7.4%, and 6.3%, respectively. The Na_2O contents of the ash of vitrain and durain at the Baukol-Noonan Mine, Center, N. Dak., are 3.9% and 1.5%.

Preliminary X-ray analysis of low-temperature ash of a high-sodium lignite (Beulah Mine, Beulah, N. Dak., ashed with a Tracerlab low-temperature ashing apparatus, Model 500A) failed to show any sodium minerals in the lignite. This strongly suggests that sodium occurs as organic salts attached to the lignite. Partly supported by University of North Dakota Faculty Research grant. Ash analyzed by U. S. Bureau of Mines, Grand Forks, N. Dak.

POPULATION ESTIMATES OF CHANNEL CATFISH IN THE LITTLE MISSOURI ARM, LAKE SAKAKAWEA, 1969-1971. C. H. Wahtola Jr. Biol. Dept., Univ. N. Dak., Grand Forks, N. Dak.

A population of channel catfish (Ictalurus punctatus) in the Little Missouri Arm of Lake Sakakawea (Garrison Reservoir) was studied during the summers of 1969-1971 to determine population size. Of 3334 catfish captured, 2460 were tagged and released. Population estimates were made using the Schnabel and Schumacher-Eschmeyer methods. A population of 25,000 to 40,000 catfish was postulated on the basis of 71 recaptures. Supported by the U.S. Bureau of Commercial Fisheries (N.M.F.S.) and the North Dakota Game and Fish Department under P.L. 88-309.

EFFECT OF SOME ENVIRONMENTAL FACTORS ON THE DISTRIBUTION AND PRODUCTIVITY OF THE PRODUCERS IN AQUATIC ECOSYSTEMS.

M. K. Wali and *D. W. Blinn*, Dept. of Biol., Univ. of N. D., Grand Forks, North Dakota.

The chemical characteristics of water, sediments and plants from 39 aquatic habitats in Nelson and Ramsey counties, northeastern North Dakota, were studied. Habitats were selected randomly and on the basis of size and depth (>2 meters). These systems showed a specific conductivity range from a low of 0.08 to a high of 75.9 millimhos/cm. The dominant ion in solution was calcium or sodium. The distribution of 47 species of aquatic macrophytes along a salinity gradient was demonstrated.

Intra-seasonal variation in nutrient composition and biomass production were investigated at six sites, sampled bi-weekly; 42 species of algae were identified. Changes in biomass production and nutrient composition (major ions-Ca, Mg, K, Na; trace metals-Al, B, Cu, Fe, Li, Mn, Ni, Pb, Si, Sr, Zn) were investigated for *Typha latifolia, Phragmites communis, Phalaris arundinacea, Carex atherodes, Sagittaria cuneata, Scirpus acutus, Scolochloa festucacea* and *Ruppia maritima.*

Supported by OWRR Grant No. A-031-NDAK.

ANALYSIS OF A SALINE TALL GRASS PRAIRIE ECOSYSTEM. V. FURTHER OBSERVATIONS ON SOIL-PLANT-ANT INTERACTIONS.

M. K. Wali and *P. B. Kannowski*, Dept. of Biol., Univ. of N. D., Grand Forks, North Dakota.

Soil-plant-ant interaction studies (*Proc. N. D. Acad. Sci.*, 25 (1): 30, 1971) were continued in 1971. The sampling was done in NW¼ of section 9 of Oakville Prairie, 13 miles west of Grand Forks, northeastern North Dakota. Of the 25 ant mounds studied, located in lowland prairie vegetation, 21 were under the influence of a fluctuating water table. These mounds were inhabited by *Formica montana, F. pergandei, Lasius umbratus, Myrmica brevinodis and Polyergus breviceps.* Relative frequency and density were determined for 32 plant species; *Distichlis stricta, Spartina gracilis, Muhlenbergia asperifolia and M. richardsonis* were the dominants. In contrast to the mounds in SW¼ of section 16, in upland prairie, these (NW¼, Section 9) were smaller in size, 6.0-35.2 cu. dm., have higher bulk densities, 0.9-1.3 gm/cm^3, greater specific conductivity, 3.0-12.5 millimhos/cm, and higher replaceable Na values, always exceeding 2 me/100 gm. Other replaceable major cations, and complexed/and or chelated trace metals, also show interesting differences.

ENERGY BUDGETS OF GRAZED AND UNGRAZED GRASSLAND. Warren C. Whitman. Dept. of Botany, N. Dak. State Univ., Fargo, N. Dak.

Energy budgets were determined on grazed and ungrazed native mixed grass prairie at the Dickinson Experiment Station during the 1970 summer season. The observation period began on May 15 and ended on Oct. 17, a period of 155 days. On the ungrazed site the following percentage utilization of measured net radiation occurred during this period: Plant production - 1.04%; evapotranspiration - 50.77%; soil heat flux - 0.42%; and sensible heat - 47.77%. On the grazed treatment equivalent values were: Plant production - 0.79%; evapotranspiration - 57.57%; soil heat flux - 0.14%; and sensible heat - 41.50%. Total net radiation was less on the grazed treatment than on the ungrazed treatment. Apparently reradiation from the grazed site was appreciably greater than from the ungrazed site. The greater reradiation from the grazed area was related to higher soil temperatures and lower bulk of mulch on the soil surface on this area. Energy losses in evapotranspiration in the early part of the season on the grazed site were greater than total net radiation on this site during this period, and advected heat from the ambient air was necessary to provide the required heat. Total net radiation measured on the ungrazed site was always greater than the energy utilized in evapotranspiration.

INTESTINAL CONCENTRATION GRADIENTS VS. TIME DURING ^{14}C-GLUCOSE TRANSPORT IN VITRO (RAT). Jean Wilk and J. C. Pekas. USDA, ARS, Metabolism and Radiation Research Laboratory, Fargo, N. D. 58102

The effect of length of incubation on concentration gradients between mucosal fluid, mucosal and nonmucosal tissue, and serosal fluid during transfer of substrates across everted sacs of intestine was investigated using glucose as a model substrate. The cranial one-third of rat small intestine (capable of rapid glucose transport) and a caudal section (slow glucose transport) were incubated for 1, 5, 10, 30, and 120 min in 50 ml of Ringer medium containing 1 X 10^{-2}M ^{14}C-glucose. The ^{14}C glucose concentration of the mucosal water space increased rapidly during the first 5 min and reached an apparent maximum at 30 min (21 and 13 mM for cranial and caudal sacs, respectively); that of the nonmucosal tissue increased at a slower rate but continued to rise through 120 min (35 and 25 mM, respectively) to exceed that in the mucosa. The low mucosa concentration relative to that of nonmucosal tissue may be partially explained by mucosal fluid retention on the mucosa. Although the serosal fluid concentration increased more slowly initially than that of the nonmucosal tissue which enveloped it, it continued to increase through 120 min (35 and 15 mM, respectively).

RARE VASCULAR PLANTS OF SOUTHWESTERN NORTH DAKOTA, N. K. Zaczkowski and W. T. Barker. Dept. of Botany, N. D. St. Univ., Fargo, N. D.

Of the 600 native or naturalized species of vascular plants in southwestern North Dakota about 75 are restricted to a single station or to a very few small areas locally, rarely found throughout the four counties of Billings, Bowman, Golden Valley and Slope. The specific factor limiting a plant to "rare" is seldom apparent but falls within the broad categories of climatic, edaphic, or biotic. Some species, reaching the limits of their ranges in the Badlands, exhibit the general pattern of isolated stands at the periphery of distribution (Chaenactis douglasii, Gilia congesta, Stephanomeria tenuifolia, Pinus flexilis). Others are found only in greatly restrictive habitats such as sandy blowouts or shaded moist ravines, situations that are much restricted in the area (Astragalus ceramicus, Astragalus tegetarius, Betula papyrifera, Pyrola secunda). A few may be only temporarily established along roadsides or railroad right-of-ways (Verbascum thapsus, Chorispora tenella, Tamarix gallica).

PALEOENVIRONMENT OF THE UPPER ESTEVAN COAL SEAM, SASKATCHEWAN. Paul L. Broughton, Department of Mineral Resources, Regina, Saskatchewan.

The Upper Estevan coal seam (Estevan No. 3) is the main economic horizon of the Estevan coalfield in southeastern Saskatchewan. The seam has the following maceral percentages: 62 per cent structured vitrinite, 6 per cent structureless vitrinite, 11 per cent groundmass vitrinite, 8 per cent fusinite, 7 per cent exinite, 1 per cent micrinite and 5 per cent inorganics. The microlithotypes present are 8 per cent carbargillite, 55 per cent vitrite, 10 per cent duroclarite, 4 per cent clarodurite, 11 per cent clarite, 4 per cent fusite, 7 per cent vitrinertinite and 1 per cent shale parting. Three environmental facies are recognized in the original peat deposition from the distribution of macerals in the 12 foot thick lignite seam. The seam is predominantly of forest-moor origin, with strong influences of reed-moor in the upper one-third and open-water in the lower one-third of the seam.

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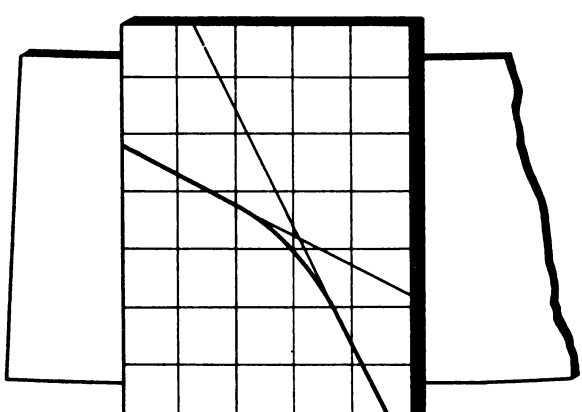
PAPERS



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DEADLINES

Abstracts.—Both student competition and professional paper abstracts are due February 15 at the Office of the Secretary. Abstracts must be submitted on the prescribed form (available from the Secretary's Office) so that they can be published in time for the annual meeting of the Academy during the last week in April.

Papers.—Complete papers for student competition are due at the Office of the Secretary on March 22 so that time is available for judging. Complete professional papers are due at the time of oral presentation during the last week in April.

PRESENTATION OF MANUSCRIPT

General.—The general style for papers of the Proceedings will be that of the *Council of Biology Editors Committee on Form and Style* (CBE Style Manual Third Edition, 1972). Available from: American Institute of Biological Sciences, 3900 Wisconsin Avenue NW, Washington, D.C. 20016. Manuscripts that do not conform to the *Style Manual* or to the specific instructions given below will be returned to the authors for correction before consideration.

Authors are to write with clarity and conciseness so that the result is professional and consistent in style. The manuscript should be in completed, final form when submitted; changes after the galley proof is set can be made only with the approval of the Editor, and costs for these changes will be assessed to the author.

All parts of the manuscript must be typed double spaced with wide margins on 8½ inch x 11 inch white paper. Each original manuscript must be accompanied by two copies (Xerox or similar copy), including illustrations.

A separate title page, numbered one, should include the authors names, their affiliation and complete addresses (including zip code). Subsequent pages should be numbered consecutively and the principal authors name should precede each page number.

A carefully organized paper should consist of the following parts introduced by major headings. ABSTRACT, INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION, ACKNOWLEDGMENTS, and LITERATURE CITED; RESULTS and DISCUSSION may be treated together.

Headings.—Major headings are centered and capitalized. Subheadings are indented, underlined for italics, and followed by a period and dash (two hyphens on the typewriter) as used in these instructions.

Figures.—Maps, drawings, graphs, structural formulas and complex tables cannot be set in type and must be drafted and reproduced as line cuts. These illustrations must be drafted in India ink so they

reproduce well, and should be submitted on separate sheets ordinarily not exceeding the size of the manuscript page. Therefore larger drawings should be reduced photographically; if so, lines, lettering and symbols must be bold enough to stand the appropriate reduction.

Photographs must be unblurred and clearly show what is intended.

Each figure (drawing or photograph) must be proportioned to fit precisely on the printed page of the *Proceedings*. A full page figure should be 4⅛ x 6¾ inch to allow *adequate space* for a caption at the base of the figure. To reduce publishing costs, consider carefully if a full page figure is necessary, or whether a carefully cropped photograph or smaller line cut would convey the visual impression as well.

Each figure must be identified on the back with the figure number, author's name, and the "Top of figure" should be indicated.

Figure captions are to be typed on a separate page and included with the manuscript. An example of a figure caption is as follows:

Figure 1. Frequency occurrence of vegetation for each sampling station.

Tables.—Complex tables (those with vertical rules, characters on fraction of successive lines or unusually extensive characters or words) should be drafted as mentioned under Figures. Tables are to be double spaced on separate sheets, numbered (Arabic numbers) consecutively and given a short title. An example of a table caption is as follows:

Table 1. Effect of pH on reactivity of chymotropsin.

The same material should not be repeated in tables and figures.

References.—References are to be listed at the end of the paper alphabetically and in the format of the *CBE Style Manual.* Abbreviations of journals are also those suggested by the *Manual.* Examples of listing a book and journal are as follows:

Council of Biology Editors, Committee on Form and Style. 1972. *CBE Style Manual.* Third edition. Amer. Inst. Biol. Sci., Washington, D.C.

Groenewold, G. H. ,and F. R. Karner, 1970. Preliminary classification of concretions and nodules in the Cretaceous Hell Creek Formation, North Dakota. N. Dak. Acad. Sci., Proc. 23 (II): 64-73.

Citations.—Citation of references in the text is by the name and year system. It may appear as Smith (1970, p. 21) or (Smith, 1970, p. 21). Figures and tables are also to be cited in the text. For example: In the second and later years females grew faster than males (Table 1, Figures 2-4).

Footnotes.—Footnotes are costly and are to be avoided. Footnote material can usually be incorporated in the text or included under the major heading Acknowledgments.

Acknowledgments.—Grants and other aid are to be acknowledged under the major heading Acknowledgments.

Full Papers.—Manuscripts of full papers consist of the following parts arranged in the indicated order (each page, beginning with the title page, is to be given a consecutive page number):

1. Title page (separate sheet)
2. Manuscript text
3. Tables (separate sheets)
4. Figure captions (separate sheet)
5. Figures

Other.—Words underlined in the text are placed in italics when set in type. Authors are to use the metric system for all measurements; equivalent values of the English system may be placed in parentheses.

CHARGES, GALLEY PROOFS, AND REPRINTS

For papers in excess of five printed pages, authors will be charged $10.00 per page for each page in excess of five. Exceptions may be granted in unusual cases. Authors are encouraged to include page charges in grant or other budget requests.

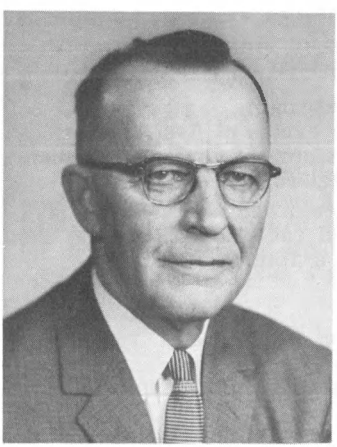
Galley proofs are to be corrected and returned within three days, to the Editor. Reprints are to be ordered (at prices shown on the order form) at the time the galley proof is returned.

IN MEMORIAM

DR. R. B. WITMER
1900-1972

Dr. R. B. Witmer, long-time dean of the College of Science, Literature, and Arts and professor of physics at the University of North Dakota, died in a Grand Forks hospital on February 21, 1972 after an illness of several months.

Dr. Witmer was born on January 30, 1900 at Bathgate, North Dakota. He earned a bachelor's and a master's degree in electrical engineering from the University of North Dakota. Serving as a graduate assistant in physics for two years, he was subsequently appointed to the physics faculty in 1924. Encouraged by colleagues, he pursued graduate studies at the University of Washington and at the University of Michigan where he received the Ph.D. degree in physics and mathematics in 1935. His specialization was in the field of X-ray. Additional graduate work was done later at Columbia University. At UND, Dr. Witmer advanced through the academic ranks to professor in 1938.



Dr. R. B. Witmer

The University of North Dakota and its friends have been the benefactors of the many talents of Dr. Witmer for nearly half a century. He will probably be remembered best as Dean of the College of Science, Literature, and Arts (now the College of Arts and Sciences), a position he held from 1949 to 1965. Prior to this, he was freshman scholarship advisor from 1936 to 1939, dean of freshmen from 1939 to 1946, and dean of the junior division from 1946 to 1948. After one year as acting dean, he assumed the position as dean of S.L.A. During World War II he spent one year, 1942-43, as head of the mathematics-physics department of the Navy pre-flight school at Iowa City, Iowa.

During Dr. Witmer's deanship, the University as a whole, but his college in particular, experienced considerable growth and up-grading of staff. Appreciable changes took place in the offerings and organization of the entire college. Among these were increased options in the natural and physical sciences. Several new departments were stablished and completely accredited degree programs were developed in these areas along with medical technology and a program in fisheries and wildlife management.

Dr. Witmer was on sabbatical leave in 1964-65, spending one semester at the University of Michigan and one semester at the University of California at Riverside. He retired as dean in 1965 and received the title of University Professor, an honor reserved for UND's most distinguished faculty members, and Dean Emeritus, College of Arts and Sciences. He returned to teaching duties on the physics faculty, an activity which he obviously enjoyed immensely. Students and colleagues remember him as an excellent teacher and lecturer. He was enthusiastic, energetic, and diligent in the classroom and expected the same dedication from his students.

Throughout his years at the University, Dr. Witmer devoted much of his time to student and university organizations, and to university committees. He was a member of Phi Beta Kappa, scholarship honorary; Sigma Xi, scientific honorary; Sigma Tau, engineering honorary; Phi Delta Kappa, education honorary; Blue Key, men's service honorary; and, the American Association of University Professors. He was included in Who's Who in America and in American Men of Science. He had been a member of the North Dakota Academy of Science since 1924. He maintained a strong interest in the aims and activities of the academy and served as president in 1948. In recent years he served the academy as a member of the Editorial Advisory Committee and as a member of the Necrology Committee.

Dr. Witmer retired from the University faculty in 1970. On June 7, 1969 he was honored at ceremonies formally dedicating the new physics-mathematics building on the campus. The building was named Witmer Hall, marking the first time a UND building was named in honor of an individual while he was still a faculty member.

PATRIC K. McILWAIN
1938-1971

Patric K. McIlwain died at the age of 33 on December 2, 1971. He earned his bachelors in 1960 and his master's degree in 1962 from North Dakota State University.

He served as Chief Laboratory Bacteriologist, U.S. Army Medical Command Japan 1962 to 1964 and Laboratory Director, Duluth Clinic, Duluth, Minnesota from July to December 1964. He was appointed Assistant Professor of Veterinary Science, North Dakota State University in 1965 and served in that position until his untimely death in December 1971. Mr. McIlwain was a member of Section 2, 311th General Hospital, U.S. Army Reserve, Fargo, ND. since December 1964 and served as commanding officer from his appointment October 1, 1971 until his death.

He was the author of several publications on infections and toxins of *Listeria monocytogenes*, nitrate poisoning in cattle, endemic rabies, and on laboratory diagnosis of various disease entities. He was a member of the North Dakota Academy of Science since 1965.

CHEMICAL STABILITY OF PRESERVED OLIGOTROPHIC WATER SAMPLES

V. A. Adomaitis, J. A. Shoesmith and G. A. Swanson
U.S. Bureau of Sport Fisheries and Wildlife
Northern Prairie Wildlife Research Center
Jamestown, North Dakota 58401

ABSTRACT

Tests were conducted to determine whether changes that may occur in the chemical characteristics of stored oligotrophic waters collected on 15 sites in northeastern Minnesota were affected by chloroforming. Chloroform was added on site to one of each pair of samples to stabilize the organic content of the water by preventing biological decomposition. The samples were subsequently stored at 25°C, and pH and specific conductivity were measured at intervals for a period of 13 months at which time nine additional chemical parameters (total dissolved solids, total alkalinity, chloride, sulfate, silica, calcium, magnesium, sodium and potassium) were measured.

pH increased and specific conductivity decreased. Average changes occurring in time from the original levels were not influenced by treatment, and first differed signficantly ($P<0.05$, paired t test) at 14 days for pH and 8.5 months for specific conductivity. Total dissolved solids and sulfate were significantly ($P<0.01$ and <0.05, respectively) larger in treated than untreated samples after 13 months storage while the reverse was true for calcium ($P<0.05$). Total alkalinity, chloride, silica, and magnesium, however, did not differ significantly ($P>0.05$). Sodium and potassium levels were too low to provide meaningful comparisons. It was concluded that chloroform may be advantageous in preserving oligothrophic waters with respect to total dissolved solids, sulfate and calcium.

INTRODUCTION

Chemical analysis of water samples provides a means of evaluating the quality of wetlands utilized by breeding and migratory waterfowl and other wildlife. Since samples sometimes have to be procured some distance from laboratory facilities, and, in addition, stored for considerable periods in the laboratory, chloroform is commonly introduced as a preservative to stabilize the organic content of that water. Since changes in chemical characteristics may take place during storage, tests were conducted to determine any difference existing between preserved and unpreserved samples.

STUDY AREA AND METHODS

The surficial deposits of northeastern Minnesota as described by Eddy (1963) consist of Precambrian rocks covered by a thin layer of red drift. A scarcity of soluable salts in the surface materials and bedrock, and precipitation rates that exceed evaporation rates pro-

vide surface waters low in dissolved materials. The lakes of this area are typically oligotrophic: deep, low in dissolved solids, with a small littoral area and a hypolimnion with sufficient dissolved oxygen to support fish during the summer months (Moyle, 1956; Eddy, 1963; Bright, 1968). Amphipods, gastropods and aquatic insects are present in most of the waters but are usually not abundant.

Water samples were collected September 24 and 25, 1968, at 15 sites in the Superior National Forest northeast of Minnesota State Highway #1 between the South Kawishiwi River and the Temperance River. Six sample sites were located along the highway between Ely and Finland, and nine along Forest Routes #153 and #165 between the Gunflint and the Sawbill Trails.

Temperature, pH and specific conductivity were determined from samples taken on site at a depth of six inches . Paired water samples were obtained at each collected station and placed into two half-gallon, amber, polyethylene bottles (Nalge Co., Rochester, N.Y.)* The bottles had been cleaned with concentrated hydrochloric acid, thoroughly soaked with Barnstead distilled water for a minimum of three days, and finally rinsed thrice with Barnstead-Corning, double-distilled water. The pH was monitored with a wide range (4-10) colormetric field kit (Model 17-N, Hach Chemical Co., Ames, Iowa), and the specific conductivity was determined with a battery-operated conductivity meter (20-100 micromhos/cm; Model RA-2A, Beckman Instruments, Inc., Cedar Grove, N.J.). After analysis, chloroform (Baker A.C.S. Analyzed Reagent) was added to the test samples at the rate of 12 ml/1. Both sets of samples were stored at 25° C.

The pH and specific conductivity were determined periodically thereafter in the laboratory with the same equipment that had been used on the original samples. In addition, pH was spot-checked electrometrically with a laboratory instrument (Model 76A Expandomatic, Beckman Instruments, Inc., Fullerton, Calif.); the specific conductivity was spot-checked with a line-operated instrument (Model RC 16B2, Industrial Instruments, Inc., Cedar Grove, N.J.).

Nine other chemical characteristics were analyzed at approximately 13 months past collection. These included total dissolved solids, total alkalinity, chloride, sulfate, silica, calcium, magnesium, sodium, and potassium. The wet chemical analysis of the anions was conducted by recommended analytical procedures (A.P.H.A., 1965). The cations were determined by atomic absorption spectrophotometry (Perkin-Elmer Corp., 1968).

For both pH and specific conductivity, paired *t* tests (Steel and Torrie, 1960) were used to determine whether or not chloroforming affected the average change occuring between initial and later measurements. Similar tests were carried out to compare average changes in the concentrations of nine other chemical parameters after

*Use of brand names does not imply endorsement of commercial products by the Federal Government.

13 months of storage. This test is meaningful, however, only when changes are consistent in sign because positive and negative changes would have a cancelling effect, possibly resulting an an average change near zero and an erroneous conclusion that no changes occurred.

To circumvent this difficulty, certain comparisons were made by considering the squared changes occurring between measurements taken at different times. The sum of squared changes in n_1 untreated samples divided by the sum of squared changes in n_2 treated samples provides an F statistic with n_1 and n_2 degrees of freedom for testing the composite hypothesis of equal means and equal variances for the treated and untreated samples. For pH, the actual changes were squared and used because the magnitude of changes was independent of the value of pH; for specific conductivity, the squares of the relative changes were used to insure homogenity of the variances because changes appeared to increase with increasing specific conductivity.

RESULTS AND DISCUSSION

The history of the stored oligotrophic waters was especially interesting in regard to pH and specific conductivity (Figure 1). Average changes occurring in time from the original levels were not influenced by treatment, and first differed significantly ($P<0.05$, paired t test) at 14 days for pH and 8.5 months for specific conductivity. In general, the trend in most of the stored samples was a shift from the acidic to the more neutral. Some samples became slightly alkaline with time. A statistical analysis of the data by the F test failed to demonstrate any significant ($P>0.10$) treatment effect in the changes in pH that occurred during storage for 13 months. Although the specific conductivity decreased slightly during the 13-month period, the F test failed to demonstrate a significant ($P>0.10$) treatment effect in the paired water samples.

Values of seven chemical characteristics of the paired samples after storage for 13 months are given in Table 1. At the end of the 13 months, no significant difference in total alkalinity, chloride, silica, and magnesium could be demonstrated by the use of the paired t test; however, significantly greater readings in the treated than in the untreated samples were demonstrated for total dissolved solids ($P<0.01$) and sulfate ($P<0.05$), while the reverse was true for calcium ($P<0.05$). Sodium and potassium levels were very low, and most were not detectable at the sensitivity of the analytical procedure used (0.05 mg/ml). For this reason, comparison of these cations between treated and untreated samples were not made.

The significant difference in total dissolved solids may have been influenced by the breakdown of organic matter in the untreated samples and may also reflect the combined influence of individual differences in sulfates, calcium, and possibly some of the other dissolved inorganics. Past experience in this laboratory has indicated

that stored waters usually become more alkaline with time. No explanation can be given for the shift in specific conductivity other than the possibility of changes on the surface of the storage bottles.

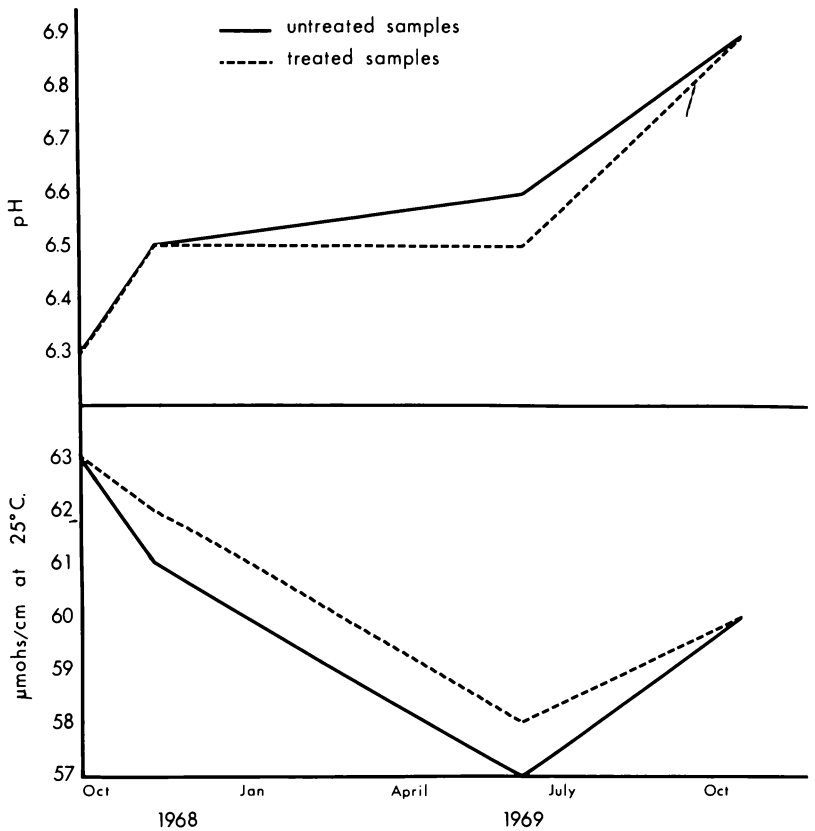


Figure 1. Changes in the mean pH and specific conductivity of treated and untreated water samples in relation to storage time.

Although the differences in some of the chemical characteristics were statistically significant, they were not of sufficient magnitude to influence the general classification of these oligotrophic waters. One exception was the change in mean pH from 6.3 to 6.9 after 13 months storage.

Table 1. Chemical characteristics of 15 paired water samples from small wetlands, lakes, and rivers of northeastern Minnesota after 13 months of storage. Data expressed as the means (mg/l) and the standard deviations (in parentheses) of chemical parameters in treated (chloroform added) and untreated samples.

Chemical Parameter	Treated	Untreated
Total dissolved solids[a]	59 (40.2)	48 (32.8)
Total alkalinity	34 (34.6)	36 (37.4)
Chloride	9.0 (3.4)	8.3 (2.4)
Sulfate[b]	5.2 (6.8)	4.2 (5.5)
Silica	7.4 (3.2)	7.5 (3.8)
Calcium[b]	4.6 (3.1)	4.7 (3.2)
Magnesium	3.6 (3.3)	3.6 (3.4)

[a] Paired samples differed significantly ($P<0.01$, paired **t** test)
[b] Paired samples differed significantly ($P<0.05$, paired **t** test)

CONCLUSIONS

1. Significant changes in pH and specific conductivity were detected after 14 days and 8½ months, respectively. Conductivity decreased and pH increased with time; the trends were independent of treatment.
2. Chloroform may be advantageous in preserving stored oligotrophic waters with respect to total dissolved solids, sulfates and calcium.

ACKNOWLEDGMENTS

We wish to thank D. H. Johnson and M. I. Meyer for aid in statistical analysis. Also appreciation is expressed to L. M. Cowardin for assistance in the field and to P. F. Springer for critical review of the manuscript.

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α-CHYMOTRYPSIN CATALYZED HYDROLYSIS OF N-CARBOBENZOXY-L-AMINO ACID P-NITROPHENYL ESTERS

L. M. Gutnik and J. A. Stewart
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University of North Dakota, Grand Forks, North Dakota 58201

ABSTRACT

N-carbobenzoxy-L-phenylalanine (CPNE), N-carbobenzoxy-L-tyrosine (CTNE) and N-carbobenzoxy-L-alanine (CANE) p-nitrophenyl esters were investigated in 50% (v/v) isopropanol-aqueous solutions. The initial release of phenolate ion was recorded and the data analyzed using the Guggenheim method. Rate constants associated with complex formation were determined to be $\approx 10^5$ $lm^{-1}s^{-1}$. Because of the low solubility of CPNE and CTNE, it was not possible to isolate the rate constant for acylation with these substrates. Tentative studies with CANE, using stopped-flow techniques (excess enzyme) gave an acylation constant of $3s^{-1}$; indications are that this stage is rate-controlling.

INTRODUCTION

Because of the large extinction coefficient of nitrophenolate ion released as one of the products, nitrophenyl esters are among the most suitable of the wide variety of substrates available for the study of chymotrypsin. These substrates allow both transient phase and steady state kinetics to be determined. However, only limited work has been reported using the nitrophenyl esters of amino acids.

Kassera and Laidler[1] reported that N-carbobenzoxy-L-alanine-p-nitrophenyl ester (CANE) is hydrolyzed by the enzyme trypsin. Since α-chymotrypsin shows aromatic specificity[2], this substrate as well as N-carbobenzoxy-L-phenylalanine-p-nitrophenyl ester (CPNE) and N-carbobenzoxy-L-tyrosine-p-nitrophenyl ester (CTNE) should be particularily reactive with α-chymotrypsin.

The kinetics of α-chymotrypsin is consistent with the following three step mechanism as illustrated using p-nitrophenyl acetate[3]:

(1) This first stage is extremely rapid, and deals with the enzyme-substrate complex.

$$E + S \underset{k_{-1}}{\overset{k_1}{\leftrightarrows}} ES$$

(2) This stage is relatively slow and involves liberation of a phenol group plus the acylation of the enzyme.

$$ES \xrightarrow{k_2} ES' + \text{phenol group}$$

(3) This third stage, deacylation, is slow and liberates acetate and regenerates enzyme.

$$ES' \xrightarrow{k_3} E + \text{products}$$

The rate of reaction is followed spectrophotometrically since the phenol release is related to acylation.

EXPERIMENTAL

A master solution of chymotrysin was prepared by dissolving a known amount in cold de-ionized water. This stock solution was diluted by a factor of 10-100 depending on the various concentrations required. Equal amounts of phosphate buffer, at pH 6.9, were added to each solution to give a final concentration of 0.025M after enzyme and substrate were mixed.

In the determination of k_1, the final enzyme concentrations were 1.0875×10^{-7} and 7.25×10^{-8}M for the phenylalanine ester (CPNE) 1.0875×10^{-7} and 5.44×10^{-8}M for the tyrosine ester (CTNE), and $4.025 \times 10 \times {}^{-7}$ and 2.0125×10^{-7} M for the alanine ester (CANE).

Substrate solutions were prepared by dissolving various substrates in isopropyl alcohol (redistilled, reagent grade). After mixing with α-chymostrysin the final concentration of isopropyl alcohol was 50% (v/v), and the final substrate solution concentrations were 5.5, 11.02, and 22.05×10^{-5} for the phenylalanine ester, 3.79, 7.575, and 15.15×10^{-5} M for the tyrosine ester and 9.46×10^{-4} M for the alanine ester.

All rates were recorded on the Cary Model 14 spectrophotometer and the results were obtained by manually mixing enzyme and substrate solutions using split compartment cells (Pyrocell, Westwood, N.J.).

THEORETICAL TREATMENT OF THE DATA

According to Michaelis-Menten kinetics[4]

(1) $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES$

$$\text{(2)}\quad ES \xrightarrow{k_2} \text{products}$$

where [E] = the concentration of enzyme at any time t
[S] = the concentration of substrate at any time t
ES = the concentration of complex at any time t
E_o = the initial concentration of enzyme
S_o = the initial concentration of substrate

Since

$$\text{(A)}\quad dp/dt = k_2\,ES$$

and according to steady state theory

$$\text{(B)}\quad dES/dt = k_1[E][S] - k_{-1}ES - k_2ES = 0$$

therefore, one can now solve for ES.

If $$\text{(C)}\quad K_m = \frac{k_{-1} + k_2}{k_1}$$

then $$\text{(D)}\quad ES = \frac{[E][S]}{K_m}$$

and $$\text{(E)}\quad dp/dt = k_2ES = \frac{k_2[E][S]}{k_m}.$$

Now $$\text{(F)}\quad E_o = [E] + ES = [E](1 + [S]/K_m);\quad [E] = \frac{E_o}{1 + [S]/K_m}$$

Substitute for [E] back into the preceeding rate equation and

$$\text{(G)}\quad dp/dt = \frac{k_2E_o[S]}{K_m(1 + K_m/[S])} = \frac{k_2E_o[S]}{K_m + [S]}$$

Usually $[S] >> E_o$ and it is assumed that $[S] = S_o$ and

$$\text{(H)}\quad dp/dt = \frac{k_2E_oS_o}{K_m + S_o}$$

If $S_o < K_m$,

$$\text{(I)}\quad dp/dt = \frac{k_2E_oS_o}{K_m}$$

This equation is first order with respect to substrate concentration
If $S_o > K_m$:

$$\text{(J)}\quad dp/dt = k_2E_o,$$

which is zero order with respect to substrate concentration.

When fast reactions are studied one should not substitute S_o for [S], and a different interpretation of steady state theory for Michaelis-Menten kinetics should be developed as follows:

From the preceeding page

$$(G)\quad dp/dt = \frac{k_2 E_o [S]}{K_m + [S]}$$

Under steady state conditions

$$(K)\quad -ds/dt = \frac{k_2 E_o [S]}{K_m + [S]}$$

is also true.

Rearrange and integrate (K),

$$(L)\quad \frac{K_m}{k_2 E_o}\int_{S_o}^{[S]} -dS/[S] - \int_{S_o}^{[S]} \frac{d[S]}{k_2 E_o} = \int_o^t dt$$

$$(M)\quad \frac{K_m}{k_2 E_o} \ln \frac{S_o}{[S]} + \frac{1}{k_2 E_o}(S_o - [S]) = t$$

$$(N)\quad \ln [S]/S_o = \frac{-k_2 E_o}{K_m} t - \frac{1}{k_2 E_o}(S_o - [S])$$

$$(O)\quad [S] = S_o e^{\frac{-k_2 E_o t}{K_m}} + \frac{(S_o - [S])}{K_m}$$

$$(P)\quad [S] e^{[S]/K_m} = S_o e^{\frac{-k_2 E_o t}{K_m}} e^{S_o/K_m}$$

$$(Q)\quad [S] e^{[S]/K_m} = S_o e^{-ft} e^{S_o/K_m}$$

where $(R)\quad f = k_2 E_o / K_m$

Since $e^x = 1 + x/1 + \frac{x^2}{2}$ etc.,

then $(S)\quad e^{[S]/K_m} = 1 + [S]/K_m + \frac{[S]^2}{2K_m^2}$ etc.

Now substitution of the value for $e^{[S]/K_m}$ in equation (P) leads to:

$$(T)\ [S] + \frac{[S]^2}{K_m} + \frac{[S]^3}{2K_m^2} = S_o e^{S_o/K_m} e^{-ft}$$

When the concentration of [S] is small compared to the value of K_m where $f = \frac{k_2 E_o}{K_m}$ then

$$(U)\ [S] = S_o e^{S_o/K_m} e^{-ft}$$

Accordingly, when the above equation is evaluated via the Guggenheim method for f, and -f is plotted versus S_o, the results should be independent of S_o.

The Guggenheim method yields a final form of

(V) $\ln (r_2 - r_1) = \ln \text{constant} - ft$

so that Figure 1 is the general plot observed.

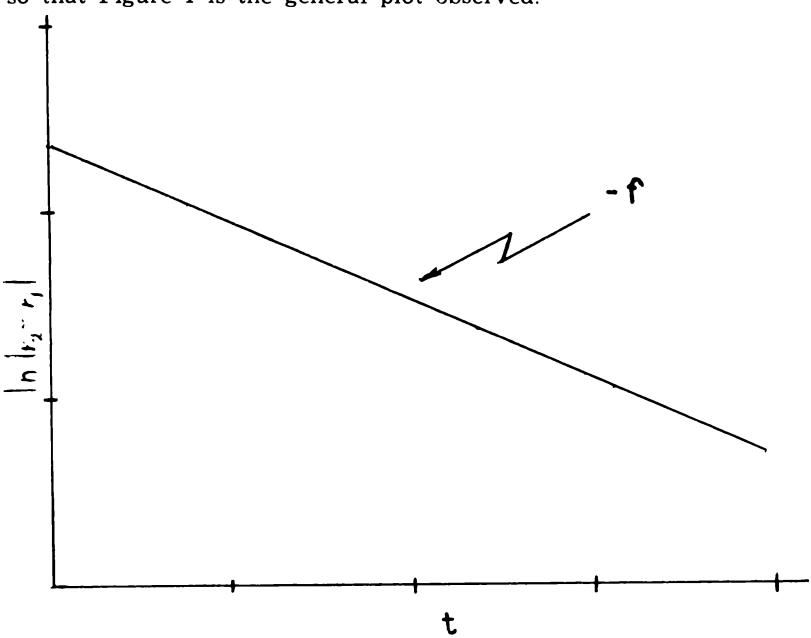

Figure 1: Plot of the general Guggenheim method. Where ln $|r_2-r_1|$ is plotted against t and the slope obtained is -f.

S_o = Substrate Concentration

From transient phase kinetics[4], when $S_o > E_o$ then

$$(W) \quad f = \frac{k_2 + S_o}{K_m + S_o}$$

Thus f is dependent on S_o and independent of E_o.

When $E_o < S_o$

$$(X) \quad f = \frac{k_2 + E_o}{K_m + E_o}$$

f is independent of S_o and dependent of E_o.

For steady state kinetics when $S_o > E_o$ and $S_o = [S]$

$$(R) \quad f = k_2 E_o / K_m$$

In the theoretical equation (R) the value of f would be independent of S_o and dependent on E_o.

This interpretation will be shown to check with the experimental data involved. A typical example of equation (R) would be shown in graphical form as in Figure 2.

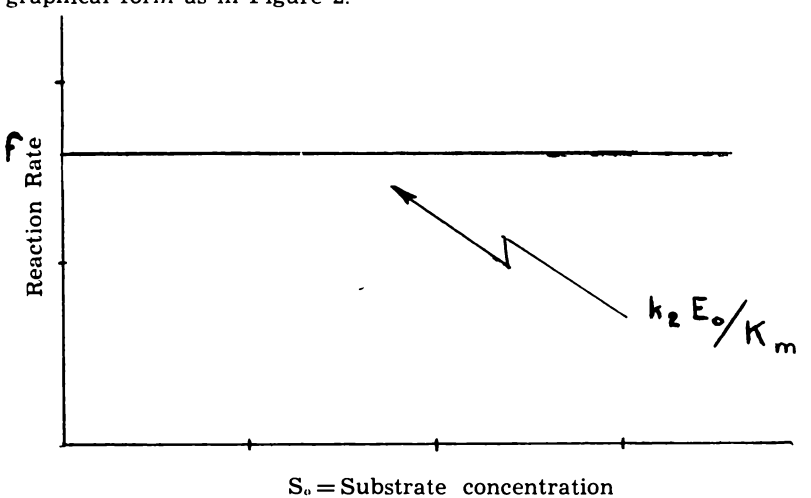

Figure 2: Plot f is substrate independent. The intercept equals k_2E_o/K_m thus is enzyme dependent. The intercept divided by E_o gives k_2/K_m. The result, k_2/K_m eqauls k_1.

X 10^{-5} S_o = Substrate

RESULTS AND DISCUSSIONS

Water and dioxane associate with the enzymic cavity, the possibility exists that isopropanol, a solvent for the substrates, also associates with the cavity; in high concentration isopropanol inhibits catalysis so that complex formation can be conveniently measured.

The reaction was of the first order with respect to the production of phenol. However, the results did not conform to conventional transient phase kinetics as expressed in equation W for excess S_o, since the slopes of the f versus S_o plots were dependent upon the concentration of enzyme, and not upon the concentration of substrate as shown in Figures 3, 4, and 5. This is opposite to that indicated by an equation W under the condition of $S_o > E_o$.

At first the results were confusing, until it was realized that the error was due to the approximation $[S] = S_o$ in equation (H) as is commonly done. However, this approximation is not valid due to the unusual rate of product formation.

Equations (T) and (R) validate the graphical variation of the f versus S_o plots, as shown in Figures 2, 3, 4, and 5.

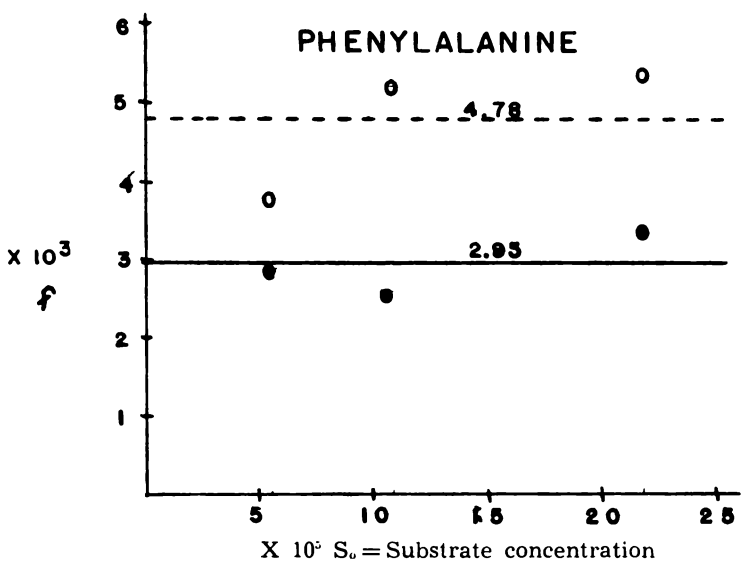

Figure 3: Plot of f vs. S_o (Substrate CPNE) used to obtain the intercept of $\frac{k_2 E_o}{K_m}$ equation (R), and in Figure 2. The O's represent an E_o of 1.0875 x 10^{-5}M and the ●'s an E_o of 0.725 x 10^{-5}M. [refer to Table 1 for a value for $\frac{k_2}{K_m} = k_1$ for this substrate.]

X 10^5 S_o = Substrate Concentration

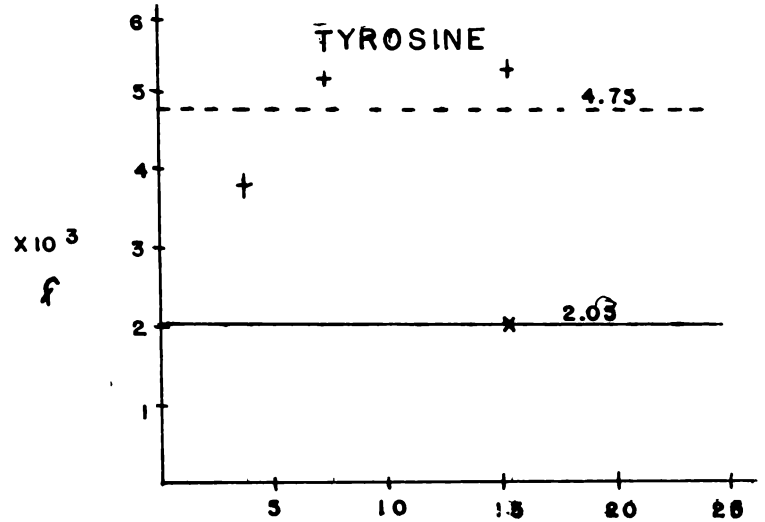

Figure 4: Plot of f vs. S_o (Substrate CTNE), used to obtain the intercept of $\frac{k_2E_o}{K_m}$ in equation (R), and in Figure 2. + represents an E_o of 1.0875×10^{-7}M, x represents an E_o of E_o of 0.544×10^{-7}M. [Refer to Table 1 for a value for $\frac{k_2}{K_m} = k_1$ for this substrate]

X 10^6 S_o = Substrate Concentration

In excess substrate equation, (W) shows enzyme independence (substrate dependence). However enzyme dependence as shown in equation (R), Table 1 and Figures 2, 3, 4, and 5 correlate the data and the theory.

Table 1 summarizes Figures 3, 4, 5, and expresses in tabular form their graphical approach. It takes the intercepts from the graphs respectively, which are equal to f. f in turn is equal to k_2E_o/K_m from equation (R). Thus when the intercept is divided by E_o the result is k_2/K_m which is an approximation for k_1.

CONCLUSION

The mechanism postulated in the introduction can even be reduced more generally to the following

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES$$

$$ES \xrightarrow{k_2} \text{products}$$

This approach facilitates the mathematical steps involved in letters (A) through (J).

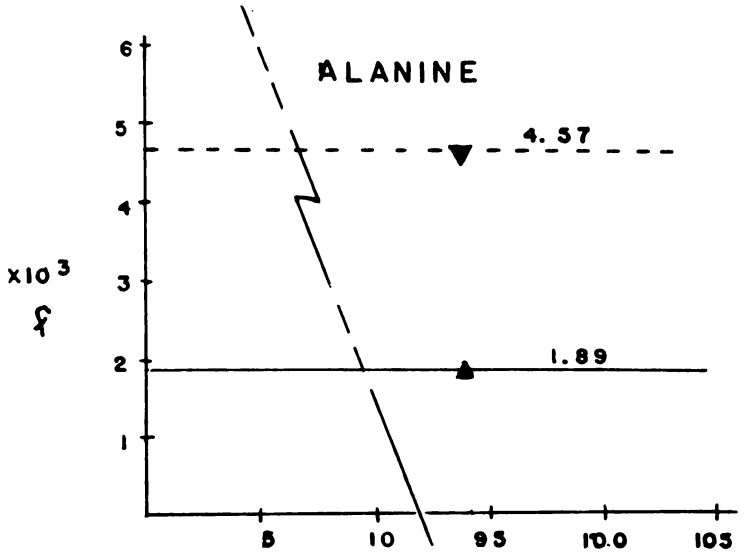

Figure 5: Plot of f vs S_o (Substrate CANE), used to obtain the intercept of $\frac{k_2E_o}{k_m}$ in equation (R) and in Figure 2. ▲'s represent an E_o of 2.0125 x 10^{-5}M and the ▲'s represent an E_o of 4.025 x 10^{-5}M. [Refer to Table 1 for a value for $k_2/K_m = k_1$ for this substrate.]

Table 1. Characteristic values of various substrate reactions with a chymotrypsin.

	SUBSTRATES	
CPNE	CTNE	CANE
$f = 4.48 \times 10^{-3}$ $E_o = 1.0875 \times 10^{-5}$ $k_1 = \frac{f}{E_o} = 4.12 \times 10^{4} s^{-1} lm^{-1}$	$f = 4.75 \times 10^{-4}$ $E_o = 1.0875 \times 10^{-5} M$ $k_1 = \frac{f}{E_o} = 4.36 \times 10^{4} s^{-1} lm^{-1}$	$f = 3.57 \times 10^{-4}$ $E_o = 4.005 \times 10^{-5} M$ $k_1 = \frac{f}{E_o} = 8.85 \times 10^{3} s^{-1} lm^{-1}$
$f = 2.95 \times 10^{-4}$ $E_o = .725 \times 10^{-5} M$ $k_1 = \frac{f}{E_o} = 4.07 \times 10^{4} s^{-1} lm^{-1}$	$f = 2.03 \times 10^{-4}$ $E_o = 0.544 \times 10^{-5}$ $k_1 = \frac{f}{E_o} = 3.77 \times 10^{4} s^{-1} lm^{-1}$	$f = 1.73 \times 10^{-4}$ $E_o = 2.125 \times 10^{-5} M$ $k_1 = 8.58 \times 10^{3} s^{-1} lm^{-1}$

It would appear that the constant for enzyme complex formation can be determined without expensive equipment. It seems very probable because the reaction was conducted under the condition of 50% isopropyl alcohol.

The values of f normally vary with substrate and not with enzyme under the condition of excess substrate. In the case of the reactions studied the theoretical values of f were shown to be independent of substrate and vary with the enzyme concentration.

ACKNOWLEDGEMENT

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LATE CRETACEOUS AND PALEOCENE TROCHIFORM SPECIES OF *VIVIPARUS* (GASTROPODA: VIVIPARIDAE) OF THE GREAT PLAINS AND ROCKY MOUNTAINS

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INTRODUCTION

Fossil fresh-water snails of the genus *Viviparus* in North America were first studied extensively by F. B. Meek from 1856 to 1885. Later work by C. A. White and others continued to be descriptive with occasional comments about systematic relationships within the genus. Prashad (1928) discussed the North American fauna in a re-

view of living and fossil Viviparidae but not in the detail that characterizes his work with European and Asiatic forms.

Viviparid snails are important elements of molluscan faunules in Upper Cretaceous and Paleocene continental rocks of western North America. Upper Cretaceous strata have 24 forms and Paleocene rocks 18, but younger Tertiary beds have yielded relatively few species. The living North American fauna by comparison includes 21 to 27 Viviparidae, depending upon the number of species placed in synonymy. Most living species in North America are concentrated in the Eastern United States.

The present review is concerned with Late Cretaceous and Paleocene species of *Viviparus* from western North America that possess a trochiform shell during some stage of development. *Viviparus* includes medium to large-sized snails with globose to turbinate shells. Trochiform shells are characterized by having conical spires, flattened whorls, and lightly impressed sutures.

MATERIALS AND METHODS

Types from the U.S. National Museum of Natural History, the Geological Survey of Canada, and the University of Alberta were examined and photographed. Material in the University of North Dakota Paleontological Collection collected by Charles I. Frye and specimens collected by the author supplement published data on the Norh Dakota fauna. The types studied are as follows: *Viviparus trochiformis* (USNM 2158), *Tulotoma thompsoni* (USNM 9026), *V. leidyi formosus* (USNM 2159), *V. panguitchensis* (USNM 8145), *V. wyomingensis* (USNM 8295), *V. conradi* (USNM 2156), *V. peculiaris* (USNM 2157), *V. planolatere* (U. Alberta Pa 11), *V. westoni* (GSC 10122), *V. mokowanensis* (GSC 10298), *V. tasgina* (GSC 6676), *V. nidaga* (GSC 6675), and *V. crickmayi* (GSC 6674).

SYSTEMATIC PALEONTOLOGY

Three groups of trochiform *Viviparus* can be recognized from the Late Cretaceous and Paleocene of western North America.

Phylum Mollusca
Class Gastropoda
Subclass Prosobranchia
Order Mesogastropoda
Family Viviparidae
Genus *Viviparus* Montfort, 1810

Viviparus (Viviparus) Montfort, 1810
Viviparus Montfort, 1810, Conchyliologie systématique . . . 2, p. 247.

Medium to large for genus; shell ovate-conic to globose-conic; whorls convex to slightly convex, often carinate surface smooth or sculptured with fine spiral ridges or striations; aperture ovate to sub-ovate; imperforate or slightly perforate.

This subgenus includes most living and fossil species of *Viviparus*. Several shells with a trochiform aspect from the Late Cretaceous and Paleocene belong in this group. These include species with all whorls somewhat flattened, such as *V. conradi*, and species in which juvenile whorls tend to be flattened and later whorls convex as in *V. westoni*:

V. conradi (Meek and Hayden) [*Paludina conradi* Meek and Hayden, 1856, Proc. Acad. Nat. Sci. Philadelphia 8:122.] Upper Cretaceous. Foremost and Milk River Formations, Alberta; Judith River Formation, Montana (Russell, 1964); Hell Creek Formation, North Dakota (Frye, 1967).

V. peculiaris (Meek and Hayden) [*Paludina peculiaris* Meek and Hayden, 1856, Proc. Acad. Nat. Sci. Philadelphia 8:122.] Paleocene. Tongue River Formation, North Dakota. Possibly a synonym of *V. conradi*.

V. planolatere Russell, 1926, Trans. Roy. Soc. Canada 20:209. Paleocene. Paskapoo and Upper Willow Creek Formations, Alberta (Tozer, 1956). Possibly a synonym of *V. conradi*.

V. westoni Tozer, 1956, Mem. Geol. Surv. Canada 280:58. Upper Cretaceous. Edmonton, Brazeau, Lower Willow Creek, and St. Mary River Formations, Alberta (Tozer, 1956); Hell Creek Formation, North Dakota (Frye, 1967).

V. tasgina Dyer, 1930, Bull. Natl. Mus. Canada 63:10. Upper Cretaceous. Lower Edmonton Formation, Alberta.

V. mokowanensis Tozer, 1956, Mem. Geol. Surv. Canada 280:52. Upper Cretaceous. St. Mary River Formation, Alberta.

V. retusus (Meek and Hayden) [*Paludina retusa* Meek and Hayden, 1856, Proc. Acad. Nat. Sci. Philadelphia 8:122.] Paleocene. Fort Union Group, North Dakota, Montana, and Wyoming; Ravenscrag Formation, Saskatchewan (Henderson, 1935).

Viviparus (Paludotrochus) Cossman, 1921

Viviparus (Paludotrochus) Cossman, 1921, Rev. Crit. Paléozool. 25, p. 79.

Viviparus (Tulotomops) Wenz, 1938-1944, Handbuch der Palaozoologie 6. Gastropoda I, p. 491.

Medium to large for genus; shell ovate-conic; whorls with two prominent, sub-carinate, revolving ridges; surfaces between ridges flat; adult whorls with two ridges, or revolving series of elongate nodes, or unornamented and convex; shell imperforate.

Late Cretaceous and Paleocene species from the Rocky Mountains and Great Plains include:

V. trochiformis (Meek and Hayden) [*Paludina trochiformis* Meek and Hayden, 1860, Proc. Acad. Nat. Sci. Philadelphia 12:185.] Synonyms: *V. leidyi* (Meek and Hayden, 1856) and *V. leidyi formosus* Meek, 1876. Paleocene and Upper Cretaceous. Paleocene strata of North Dakota, Montana, Wyoming, Utah, Colorado, New Mexico, Saskatchewan, and Alberta (Henderson, 1935). Occa-

sionally reported from Lance and Hell Creek Formations, Wyoming and North Dakota. One possible earliest Eocene record (Hickey, 1967).

V. thompsoni (White) [*Tulotoma thompsoni* White, 1876, Geol. Uinta Mountains (Powell Surv.), p. 104.] Late Cretaceous. Lance Formation, Wyoming; Mesaverde Group, Colorado and Utah; Fruitland Formation, New Mexico (Henderson, 1935).

V. laevibasalis (Yen) [*Tulotomops laevibasalis* Yen, 1954, U.S. Geol. Surv. Prof. Pap. 254-B, p. 62.] Upper Cretaceous. Nelsen Formation, Utah; a Mount Garfield and Hunter Canyon Formations. Colorado.

Viviparus leidyi and *V. leidyi formosus* possess the proportions and ornamentation of *V. trochiformis* on early whorls, but adult whorls tend to be more or less convex and smooth. The types of these speces are much larger than typical specimens of *V. trochiformis.* White (1886, p. 31) placed *V. leidyi formosus* in the synonymy of *V. trochiformis.* The close relationship between *V. trochiformis* and *V. thompsoni* was noted by White (1883) and Prashad (1928). The early whorls of these species have the same pattern of ornamentation and it is only the elongate nodes on penultimate and body whorls of large specimens of *V. thompsoni* that separate them. *Viviparus laevibasalis* may be closely related to *V. thompsoni.* Wenz (1928) proposed the subgenus, *Tulotomops,* for *T. thompsoni,* but Cossman's subgenus, *Paludotrochus,* with *V. trochiformis* as its type, is the oldest name for this group. Prashad (1928) rejected *Paludotrochus* and presented a classification of the Viviparidae that kept generic and subgeneric catagories to a minimum.

? Group of *Viviparus panguitchensis* White, 1876

Viviparus panguitchensis White, 1876, Geol. Uinta Mountains (Powell Surv.), p.101. Upper Cretaceous. Straight Cliffs, Kaiparowits, and possibly Blackhawk or Price River Formations, Utah (Gregory, 1950; Henderson, 1935).

Medium-sized *Viviparus;* shell ovate-conic whorls with wide, sharp shoulders, flattened sides; bases of whorls angular at suture line; whorls ornamented with many prominent, spiral ridges; umbilicus possibly perforate.

Viviparus panguitchensis seems to differ enough from other species of *Viviparus* to merit separate ranking; however, the poorly preserved type material needs to be supplemented with more complete topotypes and other specimens to satisfactorily establish the relationship of this species to other Viviparidae. White (1886) considered *V. panguitchensis* a form of *V. trochiformis. Viviparus plicapressus* White, 1876, a shouldered and relatively high-spired form from the uppermost Cretaceous of Montana, Wyoming, and Colorado may belong in this group. Illustrations of this species in White (1883) resemble Paleocene species of *Campeloma.* The U.S. National Mu-

seum staff has been unable to locate the types of *V. plicapressus*, and specimens of this species were not examined during this study.

North Dakota trochiform species. — The following records are from Meek (1876), Henderson (1935), Frye (1967) and Frye's specimens, Delimata (1969), Russell (1964), Hickey (1967), and the author's collections. Eastern Montana and southern Saskatchewan records that indicate to me the species probably occurs in North Dakota are included.

Upper Cretaceous
- Hell Creek Formation
  - *Viviparus westoni*
  - *Viviparus conradi*
  - *Viviparus trochiformis*
  - *Viviparus thompsoni*
  - *Viviparus tasgina* ?
  - *Viviparus mokowanensis* ?
  - *Viviparus retusus* ?

The last three records are based on poorly preserved specimens collected by Frye (1967) that compare favorably with these species but do not allow positive identification. Tozer (1956) stated that *V. mokowanensis* and *V. tasgina* in Alberta are restricted to strata that are apparent time equivalents of the Montana Group. Most records of *V. retusus* are from the Fort Union Group.

Paleocene
- Tullock Formation
  - *Viviparus tasgina* ? (A tentative comparison made by Frye, 1967)
- Tongue River Formation
  - *Viviparus trochiformis*
  - *Viviparus retusus*
  - *Viviparus peculiaris*
- Sentinel Butte Formation
  - *Viviparus trochiformis*

Paleocene—Eocene
- Golden Valley Formation, Hebron Member (Paleocene)
  - *Viviparus retusus* ?
- Golden Valley Formation, Dickinson Member (Eocene)
  - *Viviparus trochiformis*

DISCUSSION

Characters most useful in determining the relationship between trochiform species of *Viviparus* seem to be 1) convexity of whorls and development of shoulders, 2) type and pattern of ornamentation, and 3) expression of these features during ontogeny. Size and relative spire height seem to be less reliable characters for this purpose.

Prashad (1928) concluded that the Viviparidae is a polyphyletic family that had independent origins in North America, western Europe, India, and possibly Australia. The internal anatomy of living viviparids suggests that the family arose from the marine families Trochidae and Turbinidae, whose geologic ranges extend to the early Paleozoic. The geologic range of North American viviparids extends from the Late Jurassic (Morrison Formation), and the North American fauna possibly originated earlier in the Jurassic. Yen (1952) found similarities between viviparids from the Morrison Formation and those of the British Purbeck beds but concluded that the North American and European species were distinct.

Jurassic and Early Cretaceous *Viviparus* and related forms tend to have convex whorls and smooth or lightly sculptured shells. The common occurrence of trochoid shells, shouldered whorls, and prominent ornamentation is more characteristic of Late Cretaceous and Paleocene species. Variation in the cross-sectional shape of whorls and development of prominent ornamentation were the primary modes of diversification during this time interval.

A common ontogenetic pattern among the trochiform species is for flattened whorls and spiral ornamentation to be distinct on early whorls, and less distinct or absent on later whorls. However, *V. conradi, V. trochiformis, V. thompsoni,* and *V. panguitchensis* tend to retain the features of juvenile whorls on adult whorls. In a few cases, surface ornamentation becomes more pronounced on adult whorls. The shoulders and ornamentation on *V. panguitchensis* seem to become more prominent on mature whorls and the development of nodes is a characteristic of adult whorls in *V. thompsoni.*

The geologic range of *V. thompsoni* is restricted to the Upper Cretaceous (Campanian to Maestrichtian), whereas *V. trochiformis* commonly ranges throughout the Paleocene with occasional records from the uppermost Cretaceous (Maestrichtian). The morphological similarity and geologic ranges of these species suggest that *V. trochiformis* evolved from *V. thompsoni* stock near the end of the Cretaceous.

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OSTRACODA FROM THE TONGUE RIVER FORMATION (PALEOCENE), WARD COUNTY, NORTH DAKOTA

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INTRODUCTION

A highly fossiliferous outcrop of the Tongue River Formation (Paleocene) was brought to our attention in January, 1971, by Mr. Fred Ballentyne of Sawyer, North Dakota. The outcrop has yielded an unusual faunule of fresh-water mollusks (Bickel, 1973). The site is an isolated, man-made cut on the wall of a shallow coulee at about 1800 ft. above sea level in the NW¼ sec. 19, T. 152 N., R. 81 W., Ward County, North Dakota. A measured section is described below:

Unit		Thickness (feet)
	Paleocene: Tongue River Formation	
4	Buff to light gray limestone, friable, blocky; lower half coarse grained near base, finer textured toward top; mollusks abundant in limonitic band near middle.	11.3
3	Dark gray calcareous shale, soft, strongly fissile.	1.0

2	Light gray to buff limestone, blocky; moderately indurated at base; moderately friable above.	6.2
1	Brown to gray lignitic clay, weakly calcareous; base not exposed	0.3
	Total thickness	18.8 ft.

Both sides of the outcrop are covered and more typical Tongue River clastic sediments are exposed at the same elevation about 100 yd. to the west. An outcrop of carbonate sediments with similar stratigraphy occurs at the same elevation about 1 mi. to the east, but its transition into the clastic facies exposed nearby is also concealed. Abrupt facies changes and the limited number of outcrops have prevented accurate determination of the stratigraphic position of these beds. Lemke (1960, p. 30, 33) placed the Tongue River-Cannonball Formation contact at an elevation of 1540 ft. along the Souris River 8-9 mi. to the northeast. Allowing for a gradual northeast dip in this area, the sampled units are probably 200-250 ft. above the base of the Tongue River Formation.

Swain (1949) recorded non-marine Ostracoda from four localities in the Tongue River Formation in Montana and North Dakota. An exhaustive search of Williston Basin literature for records of non-marine ostracode fossils in Fort Union strata was not made, but ostracodes seem to be uncommon in the section. This may be due to the small percentage of near-shore lacustrine sediments in these strata and to limited examination of this and other facies for microfossils. Occasionally a few specimens are found uring examination of sediments for small aquatic mollusks, but so far no locality has provided the number of specimens found in this study. We wish to thank Dr. Frederick M. Swain for confirming the identification of *Cypridea (Bisulcocypridea) bisulcata* and noting the presence of *Herpetocypris*, but we assume responsiblity for all identifications.

MATERIALS AND METHODS

Samples from all four units in the section were soaked in water until disaggregated and then passed through a No. 60 sieve. Sieve residues were dried, and ostracode valves were removed and mounted. Specimens were recovered only from Unit 4; however, ostracodes were abundant on bedding planes of the soft shale in Unit 3. These specimens were poorly preserved and attempts at separating them from their matrix were unsuccessful. A total of 125 nearly complete fragments and whole valves were examined.

SYSTEMATIC PALEONTOLOGY
Subclass Ostracoda
Order Podocopida
Family Ilyocyprididae
Subfamily Cyprideinae
Genus *Cypridea* Bosquet, 1852
Subgenus *Bisulcocypridea* Sohn, 1969
Cypridea (Bisulcocypridea) bisulcata Swain, 1949

1

2

Figure 1. *Cypridea (Bisulcocypridea) bisulcata* Swain, right valve of large specimen wth shallow sulci, X25.

Figure 2. *C. bisulcata* Swain, right valve of specimen with prominent sulci and lobe, X25.

Cypridea bisulcata Swain, 1949, J. Paeontol. 32(2), p. 180, pl. 33, fig. 13-19.

Description.—Valve subquadrate-oviform in side view, greatest height about one-fourth from anterior end; hinge margin nearly straight to slightly curving, covering about two-thirds of shell length and defined by blunt hinge angles; anterior margin broadly rounded, truncate above, extended into a beak below; posterior margin rounded, narrow; ventral margin almost straight, medially somewhat depressed as edges of valves turn slightly inward; anterodorsal surface of valve with two, narrow, sub-ventral sulci separated by small lobe; surfaces near anterior and posterior margins with several papillate nodes varying from rounded to spine-like; left valve larger than right valve.

Remarks.—Valve surfaces anterior and posterior to the sulcate area are raised into large, round nodes on some specimens. The sulci and lobe show varying degrees of development, from deep and conspicuous, to shallow and grading into the general contour of the valve. Surface punctations vary from almost unnoticeable at 70X to uniform and obvious structures at 10X. We recovered 115 valves of *C. bisulcata* from this locality.

Peck (1951, p. 314) considered *C. bisulcata* a synonym of *Ilyocypris arvadensis* Swain, 1949. He concluded that *I. arvadensis* is a single species that reacts to slightly different environments by subtle changes in its morphology and that these are not two isomorphic species as suggested by Swain (1949, p. 180).

Family Cyprididae
Subfamily Candoninae
Genus *Candona* Baird, 1845
Candona sp.

3

Figure 3. *Candona* Baird (= *Herpetocypris* Sars), right valve, X20.

Erpetocypris Brady and Norman, 1889, Trans. Roy. Soc. Dublin 4, p. 84.

Herpetocypris Sars, 1890, Vid. Selsk. Forhandlinger 1, p. 61.

Candona Baird, 1845, Swain, 1961, Treatise Invertebrate Paleontol. Q(3), p. 233.

Description. — Valve sub-elliptical in lateral view; dorsal margin convex, somewhat flattened along hinge line; ventral margin slightly concave near mid-length; anterior margin broadly rounded, posterior margin slightly pointed; surface finely pitted.

Remarks. — Swain (personal communication, 1972) noted one specimen of *"Herpetocypris"* in our material, and a total of two valves are assigned to this form. *Herpetocypris* Sars was proposed as an emendation of *Erpetocypris* Brady and Norman. Under rules of the International Code of Zoological Nomenclature, this change is invalid and *Erpetocypris*, although an apparent typographical error, has priority. *Erpetocypris* and *Candona* have the same type species, so *Erpetrocypris* is an objective junior synonym of *Candona* (Swain, 1961, p. 234).

Candona sp.

6

Figure 6. *Candona* Baird, left valve, X20.

Description. — Valve elongate-subreniform in lateral view; dorsal margin moderately arched; ventral margin slightly convex; anterior

margin widely rounded, slightly extended below; posterior margin more tightly rounded than anterior margin, slightly extended below; surface smooth.

Remarks. — Four specimens show the characteristics of *Candona*, including muscle scars, and are distinct from the form described above. Swain (1949, p. 175) found *Candona* in one Tongue River Formation sample from Mercer County, North Dakota.

Family Darwinulidae
Genus *Darwinula* Brady and Robertson, 1885
Darwinula cf. *D. stevensoni* (Brady and Robertson, 1870)

4

Figure 4. *Darwinula* cf. *D. stevensoni* (Brady and Robertson), left valve with portion of antrodorsal margin missing, X30.

Polycheles stevensoni Brady and Robertson, 1870, Ann. Mag. Nat. Hist. 6, p. 25.

Description. — Valve elongate-ovate in lateral view; ventral and dorsal margins almost straight; anterior and posterior margins rounded, posterior margin more widely rounded than anterior; greatest inflation of valve about one-third distance from posterior margin; surface smooth.

Remarks. — Two slightly damaged specimens were found. Swain (1949) reported forms that compare favorably to the living species, *D. stevensoni*, from the Tongue River Formation in Powder River County, Montana. The genus *Darwinula* has a fossil record extending back to the Pennsylvanian and possibly to the Ordovician, with little morphological variation.

Family Notodromadidae
? Genus *Cyprois* Zenker, 1854

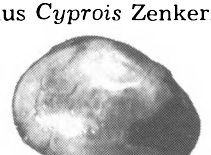

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Figure 5. ? *Cyprois* Zenker, right valve, X20.

Description. — Valve subovate in lateral view, greatest height near mid-length; dorsal margin strongly arched; anterior margin

broadly rounded. posterior margin apparently narrowly rounded; ventral margin almost straight; surface smooth.

Remarks. — The two valves on hand are poorly preserved fragments and can only be tentatively identified as *Cyprois*. These specimens may belong to *Procypris* Swain, 1964.

DISCUSSION

Very little is known about the Paleocene non-marine ostracodes of the Fort Union Group and our findings cannot contribute useful information on the age and correlation of the section sampled. We hope this report will stimulate collection and publication of data on Paleocene non-marine Ostracoda in North Dakota, because it may be possible to zone and correlate Fort Union Group rocks in North Dakota and adjacent areas with these micro-fossils. Swain (1964) zoned early Tertiary rocks in the Uinta and Piceance Creek Basins with non-marine ostracodes.

Swain (1964) found *Cypridea bisulcata* to be a stratigraphically useful fossil in the Uinta and Piceance Creek basins where it defines a zone that includes the Eocene Wasatch-Green River transition beds and lower Green River Formation. Swain (1949) also reported it from the lower Flagstaff Formation (Paleocene) of Utah. Any zone for the Williston Basin defined on the range or relative abundance of this species will probably include rocks of late-middle or early-late Paleocene age along with younger beds. Swain (1964, p. 261, 267) did not find *C. bisulcata* abundant in any samples but recovered it from a variety of lacustrine facies. *Cypridea bisulcata* apparently preferred well circulated and calcium-rich lacustrine habitats.

No generalizations about water temperature, depth, current, or vegetation preferences can be made on the generic level with living non-marine ostracodes. Species with thick, calcareous shells are indicative of alkaline water (Staplin, 1963), but this adds little to the obvious interpretation of the enclosing sediments of our material. Many species of egg-laying ostracodes are mainly inhabitants of temporary bodies of fresh water, and have short life cycles in which only the eggs survive dry periods. Other egg-laying species inhabit both temporary and permanent water bodies seemingly without preference. The eggs and young of *D. stevensoni* are retained within the shell of adults through early development and the species does not occur in ephemeral lakes or streams (Staplin, 1963, p. 761). If early Tertiary species of *Darwinula* had similar life cycles, the presence of *Darwinula* is an indication of a permanent water body. The freshwater Mollusca recovered from the same units sampled for ostracodes in this study indicate a permanent water body.

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SEASONAL VARIATION IN SWINE SEMEN QUALITY

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INTRODUCTION

Numerous studies concerning the effect of climatological conditions on the reproductive performance of the bull and ram have been done. However, there has been little research to evaluate fertility patterns in boars. Simpson and Rice (1) reported rams exposed to elevated environmental temperatures suffered decreases in sperm motility and increases in numbers of abnormal spermatozoa but semen volume was unchanged. Male sheep maintained at 45-48 degrees F. had significantly greater deterioration in semen quality as measured by percent motile and percent abnormal spermatozoa in comparison with rams subjected to enviromnental temperatures of 88.7 degrees F. (Dutt and Bush, 2). Cooled rams had improved mating performances based on services per conception (1.9 vs 5.3). Swierstra (3) observed the effect of low temperatures on reproduction by housing one group of 14 boars in a temperature controlled chamber (17 degrees C.) and a similar group at temperatures from

-30 degrees to zero degrees C. These extremely low temperatures were found not to interfere with testicular development, sperm production or seman quality.

The purpose of this study was to examine the variation in the quality of swine semen and to determine if seasonal lows in semen quality existed. The results were used to determine the effects of extreme low temperatures on the sperm production and potential fertility in boars.

EXPERIMENTAL

Semen samples were collected from eight fertile boars maintained at the North Dakota State University Animal Science Department. These boars ranged from one to four years of age and were four Durocs and four Chester Whites. They were housed individually with outside runways with feed and water located opposite the shelter for exercise purposes. A 15% protein ration consisting of ground barley and soybean oil meal were fed. Minimum and maximum temperature recordings on the day of collection were made.

Semen was collected at ten day intervals in a heated barn. Each boar was permitted to mount a restrained female for purposes of collection of the ejaculate in 500 ml beakers covered with cheesecloth that served to strain out foreign particles and the gelatinous portion of the seminal plasma. The semen was examined and the strained volume recorded. A droplet of semen was placed on a warmed slide to a categorize motility. Sperm motility and semen color were rated on separate scales of zero to five, with zero indicating a clear and nonmotile sample and five, a thick, creamy sample with strong, swirling wave motion.

Semen was diluted with 3% NaCl solution in a red blood cell pipette. A portion of this dilution was used to flood a Bright-Line hemacytometer and concentration per ml established by multiplying the average count by 10,000. The number of sperm per ejaculate was determined for each total ejaculate. The percentage of abnormal sperm were determined by staining with 5% eosin and ascertaining the number abnormal per 100 sperm. Abnormal sperm were those that deviated from the normal appearance of the acrosome cap, head, midpiece or tail.

The relationship of low ambient temperatures to the quantity and quality of swine semen were tested by correlation analysis following a classification into three groups based on temperature at the time of collection. The first group included temperatures greater than or equal to 32 degrees F., the second group from 0 to 32 degrees F. and the third group less than 0 degrees F.

RESULTS

During the study 247 collections were taken from eight boars. Using the procedures previously discussed, observations as to quality and quantity were analyzed and are presented in Table 1. Slight differences within the observed semen characteristics occurred be-

Table 1. Mean semen characteristics of eight boars maintained at ambient temperatures for two years.

Boar	Observations	Color	Volume (ml)	Motility	Conc/ml (x 10^7)	Percent Abnormal	Total sperm/ ejaculate (x 10^{10})
Chester Whites							
Gee	43	3.2 ± 1.0	388.0 ± 82.0	4.0 ± 1.1	23.3 ± 10.7	6.9 ± 6.1	8.7 ± 3.6
Holtie	41	4.6 ± 0.5	222.2 ± 31.7	4.3 ± 0.8	37.9 ± 13.9	5.7 ± 2.6	8.5 ± 3.4
Abbe	31	3.8 ± 0.8	203.1 ± 35.6	3.5 ± 1.1	26.0 ± 12.9	7.0 ± 5.7	5.2 ± 2.4
Willy	13	4.3 ± 0.8	236.2 ± 49.0	4.9 ± 0.3	24.4 ± 6.3	7.2 ± 4.1	5.6 ± 1.1
Durocs							
Dakota	42	3.7 ± 1.0	232.7 ± 45.0	3.6 ± 0.9	26.5 ± 9.7	11.5 ± 7.6	6.0 ± 2.0
TGA	41	4.3 ± 0.9	136.8 ± 20.3	4.0 ± 1.0	34.5 ± 13.2	6.8 ± 4.9	4.8 ± 2.1
Linse	23	3.3 ± 0.9	149.8 ± 45.6	3.3 ± 0.9	29.0 ± 14.5	15.9 ± 8.8	4.1 ± 1.8
D9-3	13	4.2 ± 1.0	155.4 ± 49.9	4.1 ± 0.8	33.1 ± 7.8	10.8 ± 6.3	4.9 ± 1.1

tween boars. The mean volume of the ejaculate ranged from 136.8 ml to 388.0 ml. Larger volumes were collected from those boars exhibiting greater libido. Boars that produced a lower volume of semen had a higher concentration of sperm per ml. The highest mean concentration for all boars was 37.9 x 10^7 sperm per ml., with the lowest mean concentration of sperm per ml. being 32.2 x 10^7. The sperm concentration in this experiment were similar to the average concentrations by Swierstra (3) and Barton *et al.* (4), 279 x 10^6 and 250 x 10^6 pr ml, respectively. Corrections were not made for the gelatin portion of the seminal plasma which was strained and discarded. This would tend to reduce concentration if used in determining concentration per ml of the gross ejaculate. The mean values reported for this study would also indicate temperature did not significantly alter sperm concentration per ml or the capacity of the testis to produce spermatozoa.

Motility of the semen and the percentage abnormalities were the main criteria used to ascertain the quality of the ejaculate. All boars in this experiment had good motility scores ranging from 3.5 to approximately 5. A motility score of four would be comparable to 80% motility. This was greater than the 58% value reported by Turkheimer *et al.* (5), but approximates the 80.2% motility reported by Gerrits *et al.* (6). The percentage of abnormalities ranged from 5.7 to 15.9%. All the semen samples had a relatively low percentage of abnormalities which were similar to values reported by Polge (7) and Gerritts *et al.* (6), 10 and 9.6%, respectively.

Swierstra (3) reported that as ambient temperatures decline, the quantity of semen tends to increase. Results observed in this study were contrary, in that the greatest mean semen volume of 240 ml was collected at the highest temperature. The concentration and total sperm increased only slightly from the medium to low temperature groups with values of 27.9 x 10^7 to 28.8 x 10^7 and 4.7 x 10^{10} to 6.0 x 10^{10}, respectively. These changes were not statistically significant and probably were due to chance alone.

Because volume and concentration were apparently not adversely affected by decreased tempeature the quality of the semen was evaluated to determine if temperature effects were present. Motility increased from 3.7 at high temperatures (above 32 degrees F.) to 3.9 at medium temperatures (32-0 degrees F.) to 4.1 at low temperatures (below 0 degrees F.). This suggested a trend toward greater motility as temperatures were reduced (Figure 1).

The percentage of abnormalities in the samples collected at the low temperature were almost half those of the semen ejaculated when temperatures were higher. The abnormalities declined steadily from 10.9 to 8.0 to 5.9% from high to medium and low temperatures, respectively (Figure 2). It would appear that an inverse relationship exists between the quality of semen and increasing temperatures. A similar relationship was reported by Swierstra (3).

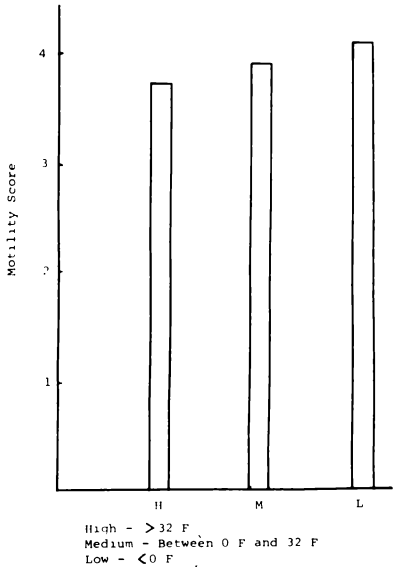

Figure 1. The Influence of Declining Temperature on Sperm Motility

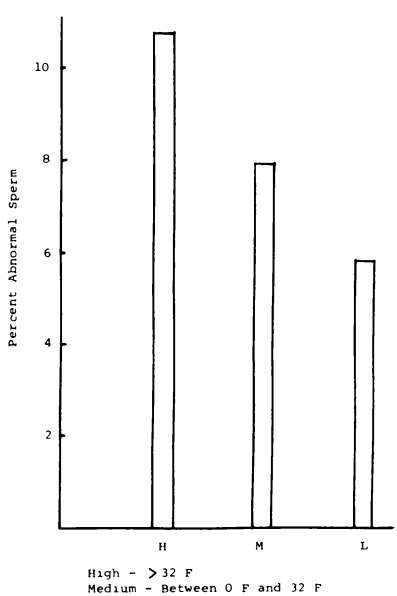

Figure 2. The Influence of Declining Temperatures on Percent Abnormal Spermatozoa.

The correlation coefficients between the minimum daily temperature on the day of collection and semen characteristics grouped within the three temperature classifications of high, medium and low are rported in Table 2. There were only three correlation coefficients which were statistically significant. These occurred when temperatures were equal to or greater than 32 degrees F. These express a negative relationship in that as temperatures increased, the motility and concentration decreased. However, the percentage of abnormalities increased as temperatures increased. There were no significant correlations between the effect of medium and low temperatures indicating that temperatures below freezing do not affect semen quality or quantity.

SUMMARY

Semen samples were collected from eight mature boars. Semen characteristics were evaluated and temperatures on the day of collection were recorded. No differences were found between breeds or between boars relative to the various characteristics studied. The data from the collections were grouped according to the minimum temperature on the day of collection and analyzed by correlation analysis. Three correlation coefficients were statistically significant (P .05), indicating a relationship exists between semen quality and ambient temperature. Concentration of sperm and motility improved as temperatures declined. As temperatures increased the percentage of abnormal sperm increased. It can be concluded that low ambient temperatures observed in this study does not interfere with sperm production or quality. In contrast, high ambient temperatures are known to reduce sperm motility and increase abnormalities.

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Table 2. Correlation coefficients between minimum daily temperature and observed semen characteristics.

Temperature[1]	Observations	Volume	Motility	Concentration	Percent Abnormalities	Total Sperm
High	94	0.12	-0.25[2]	-0.24[2]	0.22[2]	-0.13
Medium	84	0.10	-0.08	-0.02	0.18	0.05
Low	64	0.10	0.06	-0.20	-0.05	-0.06

[1]High - > 32 F
Medium - Between 0 F and 32 F
Low - < 0 F

[2] ($P < 0.05$)

ISOLATION OF THERMODURIC ESTEROLYTIC BACTERIA FROM RUMINAL DIGESTA

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ABSTRACT

Esterolytic ruminal bacterial strains were isolated by their heat tolerance and ability to hydrolyze tributyrin in a differential medium maintained under carbon dioxide. Dilutions from 10^{-1} to 10^{-9} of bovine ruminal digesta were heated at 80 C for 20 min. and inoculated into the medium. The medium was solidified in roll tubes and incubated at 39 C for 14 days. Thermoduric esterolytic colonies were observed at 10^{-6} dilution. Control samples held at 25 C for 20 min showed esterolytic colonies at 10^{-5} dilution. These were categorized by morphology and gram reaction into five groups. Bacterial isolates of these groups showed variations in proteolysis, lipolysis, or carbohydrate fermentation.

INTRODUCTION

Glycerides of long-chain fatty acids are present in most ruminant diets. Garton (1960) and Garton et al. (1961) have shown that mixed ruminal bacteria can hydrolyze fats and ferment glycerol. Ruminal bacteria have been isolated which are capable of hydrolyzing linseed oil (Hobson and Mann, 1961), peanut oil (Clark and Hawke, 1970) and of fermenting glycerol (Hobson and Mann, 1961; Johns, 1953). Therefore, lipolytic bacteria are an important constituent of the ruminal microflora. Only two esterolytic ruminal bacteria have been characterized, *Anaerovibrio lipolyticum* (Hungate, 1966) and *Butyrivibrio fibrisolvens* (Lanz and Williams, 1973).

This paper presents a method of isolating esterolytic thermoduric bacteria by thermal treatment of ruminal digesta prior to inoculation into a tributyrin differential medium. Numbers and characterizations of these esterolytic bacteria were determined. Tributyrin was chosen as a substrate for the detection of bacterial lipolysis since it is more readily hydrolyzed than other triglycerides (Alford and Steinle, 1966).

MATERIALS AND METHODS

Sample source. — Three animals were sampled for ruminal digesta: a permanently fistulated 7-year-old female Guernsey (No. 6), a 1½-year-old Holstein steer (No. 26) that had been removed from isolation (Williams and Dinusson, 1972) 4 months prior to sampling, and a one-year-old female Guernsey-Holstein (No. 27). Ruminal digesta samples were obtained via a fistula or with a stomach tube.

In addition, a portion of the ration (one part pelleted grain to two parts pelleted alfalfa) was analyzed to determine the numbers of thermoduric esterolytic bacteria.

Heat treatment and isolation of bacteria. — An inoculum was prepared by dispensing 11 g of each ruminal digesta or ration sample in 99 ml of an anaerobic diluting medium that contained the minerals, resazurin, Na_2CO_3, and cysteine -HCl of the 30% rumen fluid-trypticase-yeast extract (RFTY) agar medium (Table 1). The inoculum was mixed under a carbon dioxide atmosphere in a Waring blendor for 3 min. Serial dilutions to 10^{-10} were then made from each inoculum.

The dilutions were dispensed under 100% CO_2 in aliquots of 5 ml into sterile test tubes and incubated for 20 min at 80 C in a water bath (McClung and Lindberg, 1957). A portion of each dilution was held at 25 C for 20 min as a control. After heating, all the tubes were plunged into crushed ice for rapid cooling. Samples of these dilutions were then inoculated into tubes of spirit blue-tributyrin-rumen fluid agar medium (Table 1) (Starr, 1941) which had been melted previously and maintained at 45 C in a water bath. Roll tube preparations were made by rapidly rotating the inoculated tubes of agar in crushed ice. The preparations were incubated at 39 C for 14 days. Colony counts were made at 2, 4, 7, and 14 days. Colonies showing a zone of hydrolysis in the opaque tributyrin medium were considered esterolytic. Well-isolated colonies were picked from the roll tubes and transferred to tubes of 30% RFTY broth, which were incubated at 39 C.

MEDIA

Carbohydrate fermentation. — The basal medium was 30% RFTY agar medium without the agar, and glucose, cellobiose, and soluble starch were each reduced to 0.025%. Fructose, galactose, glucose, lactose, maltose, mannitol, sorbitol, and sucrose were millipore-filter sterilized before addition to the medium. Esculin, pectin, salicin, soluble starch, and xylan were autoclaved at 121 C, 15 lb pressure for 20 min with the medium. The final concentration of each carbohydrate was 1%. Uninoculated tubes served as controls. Carbohydrate fermentation cultures were incubated for 9 days at 39 C. Light absorbance was measured at 600 nm in 13- X 100-mm calibrated tubes with a Bausch and Lomb Spectronic 20 spectrophotometer. The pH of the midium was determined with a semi-micro combination glass electrode.

Spirit blue-lipase reagent-rumen fluid agar medium. — This medium contained the same components and concentrations as spirit blue-tributyrin-rumen fluid agar medium, with the exception that tributyrin was replaced by lipase reagent (Difco) at 1% concentration after the medium was autoclaved.

Egg yolk agar medium. — This medium was a modification of the 30% RFTY agar medium in that the $CoCl_2$, hemin, vitamins, glucose,

Table 1. Composition of 30% ruminal fluid-trypticase-yease extract agar medium[a]

Component	Percentage (w/v) in final medium	Component	Percentage (w/v) in final medium
K_2HPO_4	0.045	Thiamine HCl	0.0004
KH_2PO_4	0.045	Ca-D-Pantothenate	0.0004
$(NH_4)_2SO_4$	0.09	Nicotinamide	0.0004
NaCl	0.09	Riboflavin	0.0004
$MgSO_4$	0.009	Pyridoxal HCl	0.0004
$CaCl_2$	0.009	p-Aminobenzoic acid	0.0002
$CoCl_2$	0.001	Biotin	0.0001
Hemin	0.0003	Folic acid	0.0001
Resazurin	0.0001	Vitamin B_{12}	0.001
Trypticase	1.0	DL-thioctic acid	0.0001
Yeast extract	0.05	Clarified ruminal fluid	30.0
		Na_2CO_3	0.4
Glucose	0.05	$Na_2S \cdot 9H_2O$	0.05
Cellobiose	0.05	Cysteine·HCl	0.05
Soluble starch	0.05	Agar	1.8

[a] Medium was prepared with glass-distilled H_2O under 100% CO_2 at pH 6.8. Spirit blue-tributyrin-rumen fluid agar medium contained part of the above components with the addition of 3.5% spirit blue agar (Difco) and 1% tributyrin. Components deleted were $CoCl_2$, hemin, resazurin, trypticase, yeast extract, glucose, cellobiose, soluble starch, agar, and all of the vitamins.

Table 2. Mean esteroytic and nonesterolytic bacterial counts in samples of rumen contents and the animals' ration[a]

Bacteria/g of sample X	Reaction	Sources of Samples and Days at which the Inoculated Medium Was Counted															
		Animal Number												Ration			
		6				26				27							
		2	4	7	14	2	4	7	14	2	4	7	14	2	4	7	14
		Thermodurics															
10^3	E	45	51	44	45	19	91	107	116	92	TNTC	TNTC	TNTC	68	84	108	TNTC
	N	28	29	20	23	6	3	3	3	6	ND	ND	ND	8	6	2	ND
10^4	E	6	21	14	27	0	15	21	22	8	34	36	40	32	78	124	TNTC
	N	11	7	3	15	0	0	0	0	2	2	0	0	6	2	0	ND
10^5	E	0	1	2	4	0	2	4	3	0	2	2	6	0	28	40	42
	N	0	1	1	1	0	0	0	0	0	0	0	0	0	2	0	2
10^6	E	1	3	2	2	0	0	0	1	0	2	2	2	0	0	2	6
	N	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
		Controls															
10^5	E	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	2	2	18	26
	N	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0	0	0	0
10^6	E	247	TNTC	TNTC	TNTC	272	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	0	0	0	6
	N	73	ND	ND	ND	6	ND	ND	ND	ND	ND	ND	ND	0	0	0	0
10^7	E	37	62	52	TNTC	17	33	45	TNTC	46	114	124	272	0	0	0	0
	N	1	2	1	ND	0	2	6	ND	2	2	2	0	0	0	0	0
10^8	E	3	13	21	20	3	3	4	7	8	16	22	26	0	0	0	0
	N	0	0	2	2	0	0	0	0	0	2	0	0	0	0	0	0

[a] Counts were determined on spirit blue-tributyrin-rumen fluid medium incubated at 39 C for 14 days. E, esterolytic; N, nonesterolytic; TNTC, too numerous to count or colonies could not be differentiated due to extensive hydrolysis; and ND, not determined. Each count is a mean of three inoculated roll tubes.

Table 3. Lipolytic and photeolytic activities of five thermoduric ruminal bacterial strains and one ruminal strain that was not heated to 80 C for 20 minutes[a]

Medium	Reaction	Strain Number					
		1-C[b]	15-1	33-2	19-2	2-3	5-2
Egg yolk agar[c]	Lipolysis	-	-	±	-	+	-
Spirit blue-tributyrin-rumen fluid agar	Lipolysis	-	-	±	-	+	-
Spirit blue-lipase reagent-rumen fluid agar	Lipolysis	-	-	-	-	+	-
Gelatin	Proteolysis	-	-	-	+	+	-
Litmus milk agar	Proteolysis	+	-	+	+	-	-

[a] Hydrolysis, +; no hydrolysis, -; partial hydrolysis, ±. Results were observed at 72 hours after incubating at 39 C.

[b] Control strain heated at 25 C for 20 min.

[c] Lipolysis was indicated by a pearly layer and/or opalescence in the medium (Willis, 1960).

cellobiose, and soluble starch were deleted. Egg yolk enrichment (BBL) was added to sterilized melted agar medium to make a final concentration of 1%.

Gelatin. — The basal medium was 30% RFTY medium without glucose, cellobiose, soluble starch, but with the addition of 12% gelatin.

Litmus milk agar. — A solution of 10% litmus milk broth (Difco) was autoclaved and added to a sterile basal medium containing 30% rumen fluid that had been clarified by gauze filtering and centrifuging at 14,000 x g for 30 min., 1.8% agar, and glass-distilled water. Final concentration of litmus milk was 1%.

Blood agar. — Approximately 10 ml of portion of bovine blood were aseptically collected from the jugular vein of a mature ruminant in sterile tubes containing 0.5 ml of 20% sodium citrate. Blood was added aseptically to sterile basal medium containing a final concentration of 1.0% trypticase, 0.5% NaCl, 1.5% agar, 30% clarified rumen fluid, 0.4% Na_2CO_3, 0.5% $Na_2S \cdot 9H_2O$, 0.5% cysteine·HCl and 65.5% glass-distilled water. Final concentration of blood was 1%.

Loeffler's blood serum. — An 8% solution of Loeffler's blood serum (Difco) was prepared in warm water (42-45 C). Sodium sulfide, cysteine·HCl, and resazurin were added under 100% CO_2 and at a final concentration of 0.05%, 0.05%, and 0.0001%, respectively, to the serum solution. The serum was coagulated after dispensing by heating at 80 C for 6 hr (Skerman, 1959) and was sterilized at 85 C for 20 min on 3 successive days.

Semi-solid agar. — The basal medium was 30% RFYT agar medium with 0.5% agar.

The anaerobic methods of Hungate (1950) were used throughout this study. The dilution medium and culture media used, with the exception of Loeffler's blood serum, were prepared in a dispensing apparatus described by Williams (1966). The media were adjusted to pH 6.5 with 1 N HCl and heated to 90 C in a 100% CO_3 atmosphere. After cooling to 55 C, Na_2CO_3, $Na_2S \cdot 9H_2O$, and cysteine·HCl were added as solids. The media were dispensed into appropriate containers and sterilized at 121 C, 15 lb pressure for 20 min. Tubes were neoprene stoppered. Any medium that had substrates added aseptically was incubated for 48 hr at 39 C to detect contamination.

RESULTS AND DISCUSSION

Colony counts. — A summary of the esterolytic and nonesterolytic colony counts is shown in Table 2. The majority of the colonies that were counted were esterolytic. It was frequently observed that the nonesterolytic colonies counted at 2 days were subsequently found to be esterolytic. This accounts for the decrease in nonesterolytics in the 4-day and later counts.

The unheated samples did not have colonies at the 10^{-10} dilution but esterolytics colonies were present at 10^{-9} in cultures from animal No. 27. The cultures with dilutions of 10^{-1} to 10^{-8} had colonies that were too numerous to count (TNTC). Colonies from the animal cultures were TNTC in the 10^{-1} dilution but both esterolytic and nonesterolytic colonies could be counted in the ration cultures. These esterolytic and nonesterolytic rumen bacterial counts are in the range of those reported. Hobson and Mann (1961 observed lipolytic bacteria at 10^{-11} and 10^{-10} dilutions. Williams and Dinusson (1972) had esterolytic counts of 5 x 10^{9}/g and total anaerobic counts of 8 to 11 X 10^{9}/g.

Heated samples did not show colonies at greater than 10^{-6} dilution. Based on the average bacterial counts at 2 days of the 10^{-4} dilution for animal No. 6, the heated esterolytic count was 0.41% that of the control esterolytic count. Heating the samples reduced the numbers of colonies. Hobson and Purdom (1959) and Clarke (1961) sought to isolate spore-forming bacteria, and Sink et al. (1972), and Miskus et al. (1965) used heat to inactivate rumen cultures in ^{14}C-DDT metabolism studies.

It was expected that spore-forming microorganisms would be enriched in the heated samples, but from a total of 79 rumen digesta isolates, only 6 produced spores. However, the growth medium into which they were transferred may not have been favorable for spore formation. For example, *Clostridium perfringens* requires a specially formulated medium to induce sporulation (Freame, 1971). Spore-forming, or heat resistant, bacteria are frequently found in hay or feed (Appleby, 1955). The greatest themoduric esterolytic colony counts were from the heated pelleted ration samples. The lowest esterolytic count was from the control samples. The ration, therefore, could be the main source of the thermoduric esterolytic bacteria.

The highest esterolytic and nonesterolytic counts were from animal No. 27. These results agree with those found in calves free of ciliated protozoa (Williams and Dinusson, 1972). Animal No. 27 is in an isolation stall and has never been inoculated with protozoa. The esterolytic and nonesterolytic colony counts for animals No. 6 and 26 were similar in most cases.

Ruminal bacterial isolates.—The control esterolytic colonies were gram negative, curved or straight bacilli. The heated esterolytic colonies were categorized into five groups based upon gram stain and morphology. Groups I and II were gram positive bacilli (1.7 x 1.8 μ and 3.0 x 8.0 μ, respectively); group III, gram variable bacilli (2.3 x 6.5 μ); and groups IV and V, gram negative straight or curved bacilli (1.1 x 8.7 μ and 1.4 x 4.6 μ, respectively). A gram negative curved rod from a control sample and five isolates from the heated samples, corresponding to the groups described above, were chosen for further characterization.

Characterization of the bacterial isolates.—The results of some of the differential media reactions are given in Table 3. The ability

of the strains to degrade protein was tested with litmus milk agar, gelatin, and Loeffler's blood serum. Four of the strains were proteolytic, as evidenced by zones of hydrolysis surrounding colonies, but all failed to degrade Loeffler's blood serum. Strains 1 (control), 19-2, and 33-2 hydrolyzed litmus milk; gelatin was liquified by strains 2-3 and 19-2.

Egg yolk agar, spirit blue-tributyrin agar, and spirit blue-lipase reagent agar were employed to test for lipolysis. Although each of the isolates had been picked from esterolytic colonies on the spirit blue-tributyrin-rumen fluid roll tubes, only strains 2-3 and 33-2 produced lipolysis in the differential media. This indicated that lipolytic characteristics were lost upon subculturing.

Fructose, galactose, glucose, lactose, maltose, mannitol, pectin, sorbitol, sucrose, and xylan were not fermented. Phenolic glucosides, esculin and salicin, were fermented by strains 1 (control) and 5-2, and soluble starch was fermented by strain 1 (control). The final pH of the fermented carboyhdrate solutions ranged from 5.7 to 6.0, indicating limited fermentation occurred.

None of the strains were hemolytic and none produced indole or acetylmethylcarbinol. With the exception of strain 15-1, all other strains were motile.

Five morphological types of thermoduric esterolytic ruminal bacteria were isolated by temperature and differential media procedures. Although the spirit blue-tributyrin-rumen fluid medium was not completely selective for esterolytic bacteria, it permits differentiation of these bacteria from other bacteria in a mixed microbial population.

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LAND SNAILS OF NORTH DAKOTA: A PRELIMINARY REPORT

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ABSTRACT

In July and August, 1969, two east-west (along 46° 30′ and 48° 30′ N.) and two north-south transects (along 98° 00′ and 103° 00′ W.) were made across North Dakota for land snails. General localities along parallels (16) were at the intersections with whole meridians; those along meridians (14) were at the intersections with half-parallels. An open (no woody vegetation except wolfberry) and a covered (with woody vegetation) habitat were sampled from each locality. Twenty-one species were taken alive from the transects— 1 cionellid, 1 valloniid, 6 pupillids, 3 succineids, 3 endodontids, 1 limacid, and 6 zonitids. Three species, *Gastrocopta pentodon* (Say), *Vertigo binneyana* Sterki, and *Punctum minutissimum* (Lea), are newly reported for the state. More species and presumably more individuals occurred in the eastern one-fourth of the state, east of about 99° 00′ W. Two open habitats — area adjacent to marsh or slough and dry upland or area adjacent to ephemeral drainage — and four covered (wooded) habitats — aspen grove, farm grove. stream cutbank or slope and dense woods — are recognized. More species (up to 11) and generally more individuals occurred in any covered habitat than in any open habitat (up to 5 species). Low moisture and temperature extremes seem primarily to limit land snails in North Dakota.

INTRODUCTION

Little is known of the land snails of North Dakota. What is known consists mostly of species lists (Daniels, 1920; Winslow, 1921; and Tuthill, 1962, 1963) or simple notations of a species occurrence in the state (Pilsbry, 1946, 1948; Burch, 1962, and LaRocque, 1970). On the basis of four transects for land snails across the state, we are suggesting gross regional trends and major habitats with expected species assemblages, as well as an evaluation of certain ecologic factors responsible for these generalizations. Further collecting along additional transects will help decide the validity of these generalizations.

MATERIALS AND METHODS

Field work was done during July and August, 1969, a year with summer precipitation generally greater than normal and temperatures higher and lower than expected normals. Statewide average departures from the normal (based on the period 1931-1960) for precipitation for May, June, July, and August were -1.21 cm (-0.48 inches), +1.24 cm (+0.49 inches), +2.97 cm (+1.17 inches), and

+0.094 cm (+0.04 inches). Statewide average departures from the normal for temperature for May, June, July, and August were +1.30 C (+0.72 F), -10.51 C (-5.84 F), -5.00 C (-2.78 F), and +6.85 C (+3.81 F) (ESSA, U.S. Dept. of Commerce, 1970).

Land snails were collected from two east-west transects (along 46° 30′ and 48° 30′ N.) and two north-south transects (along 98° 00′ and 103° 00′ W.) across North Dakota. General localities along parallels

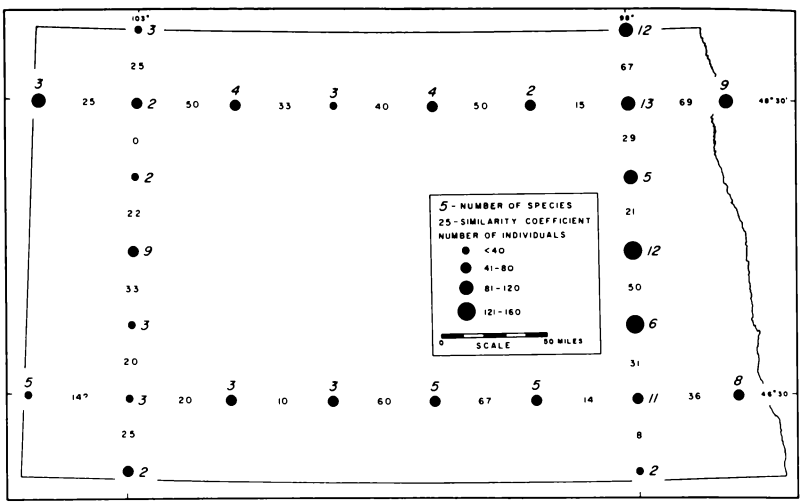

Figure 1. Land-snail localities (dots) along four transects across North Dakota. Species numbers are composites of two stations, at open (no woody vegetation except wolfberry) and covered (with woody vegetation) habitats. The similarity coefficient (Jaccard's) comparing species similarity between two adjacent localities has been multiplied by 100. The number of individuals are those live individuals collected from two stations in one hour by two collectors.

(16) were at the intersections with whole meridians; those along meridians (14) were at the intersections with half-parallels (Figure 1). Therefore, distances between general localities along parallels and meridians are about 77 and 55 km. An open (no woody vegetation except wolfberry and rarely rose) and a covered (with woody vegetation) habitat were sampled generally adjacent to each parallel-meridian intersection. All stations were within 14.4 km of each parallel-meridian intersection and more than three-fifths of the stations were less than 1.6 km from such an intersection.

A quarter of land in the section where a parallel-meridian intersection occurred was selected by a table of random numbers. The search for a suitable habitat began from the outside corner of the

selected quarter, and the choice of following an east-west or a north south road was established by the toss of a coin. The first relatively undisturbed habitat (open or covered) visible from the road was sampled. If no suitable habitat was available, another quarter was selected randomly and the above process was repeated until two stations were sampled. Each station was searched for snails for one-half hour by two collectors, and all live specimens observed were collected.

Most specimens were identified by comparison with voucher specimens. Others were identified by the use of Pilsbry (1946, 1948) and Burch (1962). Soil pH was determined for samples from 36 stations using the $CaCl_2$ method of Peech (1965, p. 923). Major habitat types, with associated land snail assemblages, were partly determined with the aid of cluster analysis using Jaccard's coefficient (Cheetham and Hazel, 1969, p. 1132). Clustering was accomplished by the unweighted pair-group method following the computer program of Bonham-Carter (1967).

All specimens collected are in the paleontological collection of the Department of Geology, University of North Dakota; they are accessioned within the numbers A910-A960.

RESULTS

Twenty-one species of land snails were collected from the transects (Table 1). The most frequently found species (in decreasing order) were *Vallonia gracilicosta, Catinella avara, Deroceras laeve, Euconulus fulvus,* and *Discus cronkhitei.* The least frequently found found species were *Pupilla blandi, Striatura milum, Vitrina limpida, Helicodiscus parallelus,* and *Punctum minutissimum.* Three species, *Gastrocopta pentodon, Vertigo binnyana,* and *Punctum minutissimum,* are newly reported for the state. One species, *Deroceras reticulatum* (Müller), did not appear in the transects but was collected in Grand Forks. (Twenty-nine species and subspecies have been reported from North Dakota in addition to the species presented here (Tuthill, 1962, 1963; LaRocque, 1970). No attempt has been made to evaluate the validity of these occurrences.)

Certain regional patterns are suggestive. Most species (20) occurred in the eastern transect, followed by the southern (17), northern (14), and western (11). Perhaps a faunal boundary occurs between 98° and 99° W. as suggested by the low similarity coefficients between these meridians (Figure 1). More species and possibly greater concentrations of individuals occurred east of this presumed boundary. Over the state, the association of more individuals with a greater number of species is suggested by Figure 2. Coefficients of similarity (Jaccard's) also indicate that greatest species similarity occurs between the south and east (0.80) and north and south (0.63) transects; least similarity occurs between the north and west (0.47) and east and west (0.48) transects.

Snail assemblages varied considerably with habitat. Generally 1-3 species (although up to 5) occupied an open habitat and 1-11 were found in a covered habitat (Figure 2). Only three species, *Vertigo ovata, Oxyloma retusa* and *Hawaiia miuscula,* seemed to prefer an open habitat (Table 1). Six specific habitats are tentatively recognized under the two general types (Table 2). Few species were

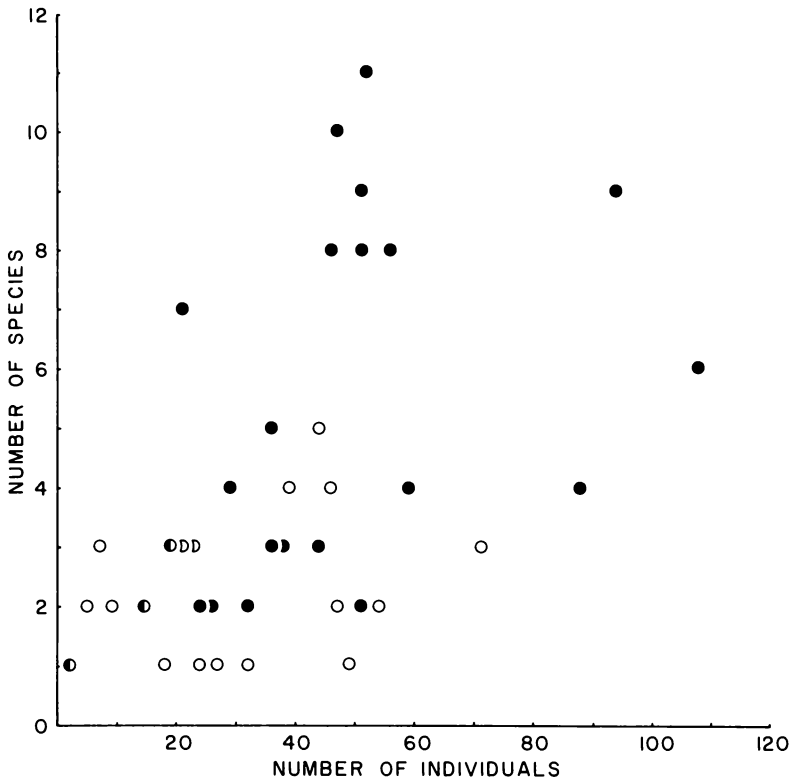

Figure 2. Scatter plot of number of species of land snails against total number of individuals of all species at each station. Open circles represent open habitats (no woody vegetation except wolfberry) and solid circles represent covered habitats (with woody vegetation). Half-filled circles represent plots of both open and covered habitats.

unique to a given habitat and species overlap commonly existed. However, the assemblage number and composition ought to be generally useful in characterizing a particular habitat. Two habitats,

Table 1. Systematic list of land snails recovered from four transects across North Dakota (Figure 1 gives location of transects and their preferred general habitats (expanded in Table 2).

Species	Preferred general habitat*
CIONELLIDAE	
1. *Cionela lubrica* (Müller) [All]**	Covered
VALLONIIDAE	
2. *Vallonia gracilicosta* Reinhardt [All]	Covered
PUPILLIDAE	
3. *Pupilla blandi Morse* [West]	Covered
4. *Gastrocopta armifera* (Say) [East, north, south]	Covered, open
5. *G. holzingera* (Sterki) [All]	Covered
6. *G. pentodon* (Say) [East, north, south]	Covered
7. *Vertigo binneyana* Sterki [East, west, south]	Covered, open
8. *V. ovata Say* [East, south]	Open
SUCCINEIDAE	
9. *Succinea ovalis* Say [East, north, south]	Covered
10. *Catinella avara* (Say) [All]	Covered, open
11. *Oxyloma retusa* (Lea) [East, south]	Open
ENDODONTIDAE	
12. *Discus cronkhitei* (Newcomb) [All]	Covered
13. *Helicodiscus parallelus* (Say) [East, south]	Covered
14. *Punctum minutissimum* (Lea) [East]	Covered
LIMACIDAE	
15. *Deroceras laeve* (Müller) [East, north, south]	Covered, open
ZONITIDAE	
16. *Retinella binneyana* (Morse) [All]	Covered
17. *Hawaiia minuscula* (Binney) [East, west, south]	Open
18. *Euconulus fulvus* (Müller) [All]	Covered
19. *Zonitoides arboreus* (Say) [All]	Covered
20. *Striatura milium* (Morse) [East, north]	Covered
21. *Vitrina limpida* Gould [East]	Covered

*Covered—with woody vegetation; open—no woody vegetation except wolfberry

**Names in brackets indicate transect (s) in which species was collected

Table 2. Habitats and land snail assemblages expected in each as based upon four transects across North Dakota (Figure 1 gives location of transects)

OPEN HABITATS (with grass forbs, possible wolfberry)

1. Area adjacent to marsh or slough
   Vertigo ovata, Catinella avara, Oxyloma retusa, Deroceras laeve
2. Dry upland or area adjaient to ephemeral drainage
   Vallonia gracilicosta, Vertigo ovata, Catinella avara, Deroceras laeve, Hawaiia minuscula, Euconulus fulvus

COVERED HABITATS (with woody vegetation)

3. Aspen grove
   Vallonia gracilicosta, Vertigo binneyana, V. ovata, Catinella avara, Deroceras laeve, Euconulus fulvus
4. Farm grove
   Vallonia grailicosta, Pupilla blandi, Gastrocopta armifera, Vertigo ovata, Catinella avara, Discus cronkhitei, Deroceras laeve
5. Stream cutbank or slope
   Cionella lubrica, Vallonia gracilicosta, Pupilla blandi, Gastrocopta holzingeri, Catinella avara, Discus cronkhitei, Deroceras laeve, Retinella binneyana, Euconulus fulvus
6. Dense woods
   Cionella lubrica, Vallonia gracilicosta, Pupilla blandi, Gastrocopta armifera, G. holzingeri, G. pentodon, Vertigo binneyana, V. ovata, Succinea ovalis, Catinella avara, Discus cronkhitei, Helicodiscus parallelus, Punctum minutissimum, Deroceras laeve, Retinella binneyana, Hawaiia minuscula, Euconulus fulvus, Zonitoides arboreus, Striatura milium, Vitrina limpida

"aspen grove" and "farm grove," are recognized because of their shortage of species. Only 1-2 species were observed in any one aspen grove and 2-4 species in a single farm grove.

Cluster analysis showed *Cionella lubrica* and *Retinella binneyana* with the highest similarity coefficient of occurrence (1.00). These two species were strongly associated with *Zonitoides arboreus* (0.75), and these three species were associated with *Succinea ovalis* by a coefficient of 0.63. *Discus cronkhitei* was associated with these four species by a coefficient of 0.59. All other species were associated by coefficients of 0.50 or less.

Values of pH (based on 13 samples from open habitats and 23 samples from covered habitats) of soils above which snails were collected varied from 5.5 (covered) to 8.1 (open). Of 10 species for

which five or more soil pH values were available, averages ranged only over the interval of 7.1-7.4. *Catinella avara* (the second most commonly found species) showed the greatest pH range (based on 19 values) or 5.5-8.1 with an average of 7.3.

DISCUSSION

Moisture and temperature seem to be the primary factors limiting the occurrence of land snails (Hunter, 1964, p. 104-105). That is, the primary physiological problems of these mollusks is control of water loss; therefore, high moisture content and equable temperatures are conducive to their existence. The occurrence of more species and presumably more individuals in the eastern one-fourth of the state is probably the result primarily of higher precipitation there (Bavendick, 1952, p. 60-61). Higher precipitation also results in more and better cover which tends to moderate extremes of air temperature. Superimposed on the regional trend, however, are somewhat anomalous areas as the Killdeer Mountains, which provide extremely suitable land snail habitat in a region generally unsuitable. For example, at one locality nine species (western transect, Figure 1) were collected in a region with generally fewer species.

The six specific habitats seem related primarily to moisture availability and temperature moderation. The two habitats of the open type have fewer species probably because less moisture is retained and higher temperatures may occur. Stream cutbank or slope and dense woods habitats of the covered type harbor considerably more species probably because of higher moisture retention and moderation of high air temperatures. The aspen grove and farm grove habitats are anomalous in their generally low number of species, possibly because of their intermediacy in moisture retention-temperature moderation, or because of the time factor. That is, neither of these two habitats may not have been in existence long enough at a given locality to have been populated by many species.

Soil pH does not seem to have any appreciable effect on land snail occurence as based on the few measured values. Calcium carbonate is presumably present in sufficient amounts for shell secretion.

The land snail fauna discussed here consists of 1 cionellid, 1 valloniid, 6 pupillids, 3 succineids, 3 endodontids, 1 limacid, and 6 zonitids. Dominated by pupillids and zonitids, this assemblage reflects the fact that much of North Dakota is in the Northern molluscan province of the Eastern Division (Burch, 1962, p. 10-12).

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firmed identifications of voucher specimens. Dr. F. G. Duerr, Department of Biology, University of North Dakota, critically reviewd the manuscript.

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NORTH DAKOTA FLEAS. IV. COLD TOLERANCE IN THE BIRD FLEA, *CERATOPHYLLUS IDIUS (JORDAN AND ROTHSCHILD)*

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ABSTRACT

Ceratophyllus idius is a common ectoparasite and nest inhabitant of purple martins, *Progne subis* (L.). Its cold tolerance and overwintering abilities were examined in experimental and natural situations. Over a period of 18 weeks at constant temperatures of +5 and -8C., greater survival occurred at the lower temperature, and at both, adult females out-survived males. Despite temperatures as low as -26C. and numerous days of freezing and thawing, adult fleas survived until mid-winter in large numbers. Subsequent winter collections from an unprotected birdhouse failed to yield living adults, thus leaving unanswered what stage and proportion of the population survives until spring.

INTRODUCTION

Although cold hardiness among insects was reviewed recently by Asahina (1969), it appears as if fleas have been largely ignored. Among the few reports is that of *Spilopsyllus cuniculi* (Dale), the European rabbit flea, which can survive for nine months at a refrigerator temperature of -1C., but dies at temperatures lower than -10C. (Rothschild, 1965). In North America *Orchopeas leucopus* (Baker) and *O. sexdentatus* (Baker) from small mammals are known to survive from one week to nearly three months at -10 to -15C. (Miller and Benton, 1970). The same authors noted that *O. howardii* (Baker) in nests of gray squirrels and *Ceratophyllus idius* (Jordan and Rothschild) in purple martin birdhouses tolerate freezing and severe winter temperatures. They also reported that viable adults of the later species were recovered from birdhouses during every winter month, despite exposures of -10 to -25C. for periods as long as three weeks.

In eastern North Dakota, purple martins, *Progne subis* (L.), have migratory dates which remove them from their fleas for 7-7½ months. However, large numbers of *C. idius* remain in the nest and some are presumed to survive the winter to reinfect returning birds. The present study was undertaken in order to ascertain some characteristics of winter and cold survival in these fleas.

MATERIALS AND METHODS

During a routine autumn cleaning of a purple martin birdhouse located in Grand Forks, North Dakota, its owner noticed numerous living adult fleas. About a cubic foot of infested nest material was given to this author in late October 1971. On November 1, 180 active

adult *C. idius* were selected at random from the nest material. These were placed in groups of 10 into small, cotton-stoppered test tubes. Half of these were placed in the freezing compartment of a refrigerator at -8C.; the others were placed in the main portion of the same refrigerator at +5C. During the next 18 weeks, tubes of fleas were removed at 2-week intervals and allowed to reach room temperature. Several small samples of additional nest material were removed from the birdhouse and examined throughout the fall and winter.

In all cases, sex and survival counts of the fleas were made, and all specimens were preserved, processed, and mounted on microscope slides.

RESULTS

Considerable fluctuations in survival occurred, but fleas held at the colder temperature lived longer than those which were kept above freezing (Figure 1). This consistent difference became apparent six weeks into the experiment. Fleas held at +5C. were rarely found alivde beyond the 10th week, while most of those at -8C. survived. The differences are more obvious if the data are pooled for the entire 18-week period (Figure 2). The survival at -8C. was twice as great as at +5C. (84.4% versus 42.2%).

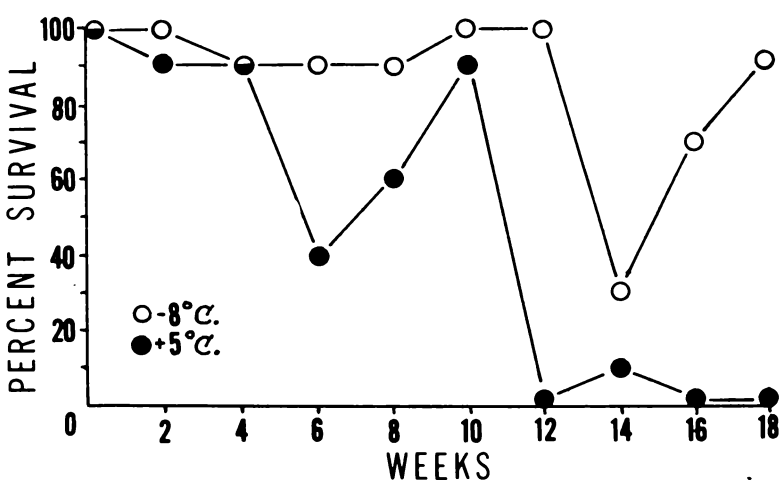

Figure 1. Differences in cold temperature survival of *C. idius* (10 fleas in each sample).

Comparisons between sexes indicate that females out-survived males at both temperatures (Figure 3). Although a small difference at -8C. (88% versus 80%), it was slightly more than double at +5C. (56.8% versus 28.3%).

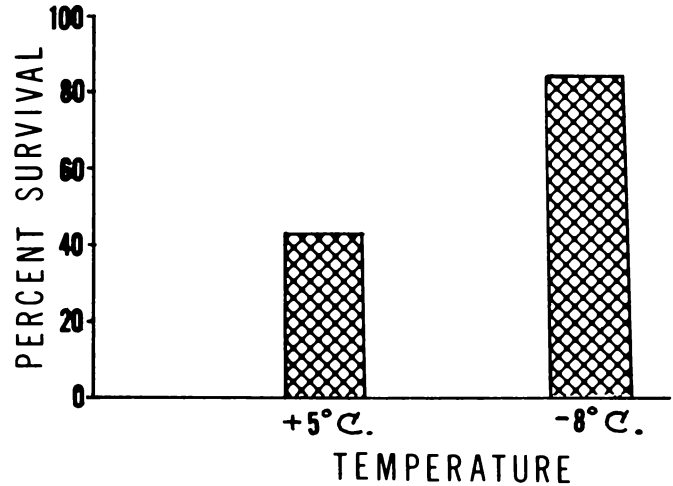

Figure 2. Pooled survival data for all *C. idius* used in experiment.

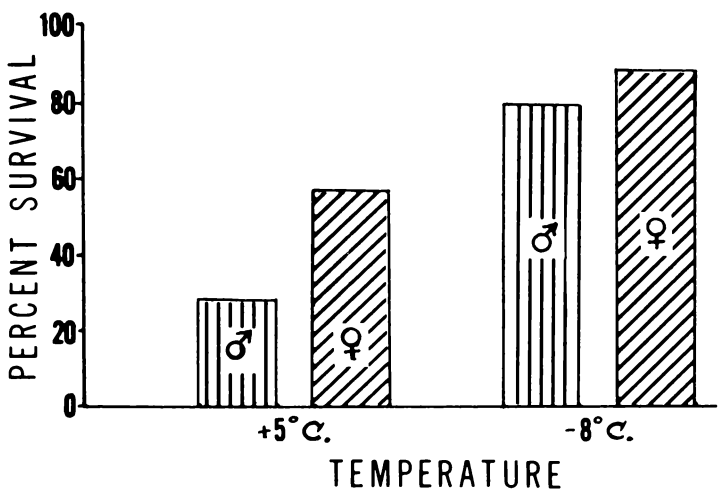

Figure 3. Pooled data for *C. idius* showing survival in relationship to sex and temperature.

After its autumn cleaning, the martin house rested atop a pile of bricks in the owner's backyard. Small samples of nest material continued to yield many living fleas until mid-winter. As late as

10 January, dead adults were seldom seen in frozen nest material. Two weeks later (and for the balance of the winter no living adults could be found. However, on 29 April the birdhouse was again raised on its pole amidst an active population of *C. idius*. These fleas appeared to be mainly in and around the pile of bricks, rather than in the birdhouse proper.

DISCUSSION

Results obtained from refrigerated fleas suggest two hypotheses: 1) lower metabolic rates at colder temperatures may account for greater survival at -8 than at +5C.; and 2) since females are larger than males, perhaps their better survival may relate to greater energy reserves in the form of fat bodies and egg materials.

Records compiled by the University of North Dakota weather station in Grand Forks from 1 November 1971 through 10 January 1972, list 19 days in which daily temperatures rose above or fell below 0C. Such freeze/thaw oscillations are probably stressful to the organism in two ways: 1) warmer temperatures increase metabolic rates, thus more rapidly depleting stored energy reserves, and 2) lower temperatures may cause formation of ice crystals. The recovery of numerous adult fleas from an unprotected birdhouse through 10 January implies tolerance of repeated freeze/thaw situation during which minimum temperatures dropped as low as -26C.

What transpired in the weeks following 10 January to cause a massive kill of adult fleas is uncertain, and is in variance with the winter survival reported by Miller and Benton (1970). By late April active adults were again common in and around the unraised birdhouse, thus implying that a portion of the population did survive the winter. Under less severe temperature conditions in northern England, it has been shown that a related bird flea overwinters in its cocoon as an unemerged adult (Humphries, 1969). Although a careful search was made for the same stage, only a single cocooned adult was found in my samples. However, that does not eliminate the possibility of overwintering by a pre-adult stage. Likewise, one cannot dismiss the possibility that a small percentage of *C. idius* do survive the winter as adults.

ACKNOWLEDGMENTS

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ZOOPLANKTON-PHYTOPLANKTON INTERRELATIONSHIPS IN HOOKER LAKE, ROLETTE COUNTY, N. DAK.

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ABSTRACT

Population levels of zoo- and phytoplankton in Hooker Lake, were investigated from June 1970 to May 1971. The organisms were identified in a Sedgwick-Rafter counting chamber. An inverse relationship was found between populations of zoo- and phytoplankton; when one was high the other was low. Blue-green algae (*Aphanizomenon flos-aquae* and *Oscillatoria* spp.) increased during the summer to maxima of 5000 cells/ml in July and 1100 cells/ml on 21 August, respectively. *Keratella cochlearis* increased to a maximum of 5300 organisms/l in November; there was a concomittant decline in phytoplankton. The zooplankters *Brachionus angularis* attained a population of 2200 organisms/l on 11 May and *K. cochlearis*, 1700 organisms/l on 27 May; the phytoplankton population at this time was minimal.

INTRODUCTION

Hooker Lake, in the Turtle Mountains near St. John in Rolette County, North Dakota has been used as a rearing lake for rainbow trout, *Salmo gairdneri* Richardson by the North Dakota Game and Fish Department. The lake is no longer satisfactory for rearing trout since it now has winter kills and summer kills of fish from time to time, because of oxygen deficiency.

The Game and Fish Department has supplied supplementary oxygenation to the lake by use of an Aqua-air system. This system, essentially an air compressor and a submerged perforated hose, was installed in August 1966 and has been run every winter and more or less continuously during each summer since that time.

Several types of chemical and biological studies have been made recently in Hooker Lake in order to evaluate the effects of this extensive artificial aeration. The physical and chemical parameters associated with these systems have been recorded for the previous

six years (Kreil, 1970). This study was undertaken to describe the plankton community of Hooker Lake in the period from June 1970 to May 1971 (Nordin, 1971).

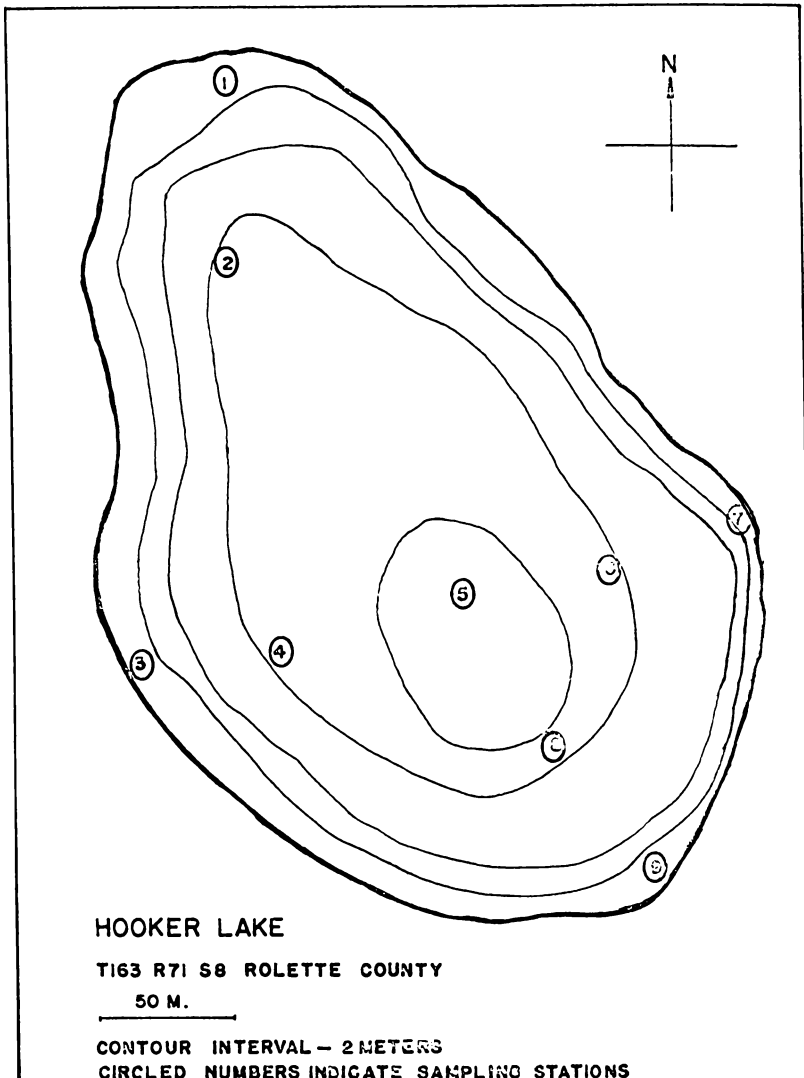

Figure 1. Outline map of study area.

There are numerous small lakes in the Turtle Mountains, none of which have been described in detail. Tubb (1966) studied the limnology and presented a preliminary description of the biota of several of these lakes. Moore and Carter (1923) conducted an algal survey of the area, and Young (1923) recorded the physiography of the area. The relationship between the phytoplankton and zooplankton have been described for Devils Lake by Anderson, (1966), and by Pennak (1946) and Anderson et al. (1955) for lakes in Colorado and in Washington respectively.

MATERIALS AND METHODS

Collections were made from 19 June to 14 December 1970 and from 4 May to 12 June 1971. Nine stations were established (Figure 1) and duplicate samples were obtained weekly June through September, bi-weekly October through December 1970, and weekly during May and June 1971. Samples were taken using a Clarke-Bumpus plankton sampler and a Kemmerer water sampler; the organisms concentrated through a No. 5 plankton net and diluted to a standard volume. Aliquots were examined in a live condition for identification purposes and were subsequently preserved in neutral 4% formalin. Enumerations were made using a Sedgwick-Rafter counting cell and a binocular microscope, 1000X magnification, equipped with an ocular micrometer. The principal authorities used for identification were Prescott (1951) and Pennak (1953).

RESULTS AND DISCUSSIONS

Hooker Lake is a warm (midsummer surface temperature is 25C.), alkaline (pH 7.8 - 8.4) body of water, with low nutrient content. The lake is normally stratified but an aeration system keeps the water in circulation. An oxygen gradient is present and the bottom can become devoid of oxygen under certain conditions. The lake is ideal for a study of this type because of its homogenous basin shape and lack of outlets or inlets.

The growth pattern of the organisms showed peaks of activity, the most noticeable was the "bloom" of *Aphanizomenon flos-aquae* during July (Table 1.). There was an inverse relationship between the numbers of the phytoplankton and zooplankton especially in fall, 1970 and in spring, 1971. During the periods when the algae were at high levels the zooplankton were at noticeably low levels; conversely when the zooplankton reached maximum numbers in spring and fall, phytoplankton numbers were relatively low. This pattern has been observed previously by Anderson et al. (1955).

Since a comparative study of the lake plankton without the effect of the aeration system was not available, the effect of this parameter cannot be evaluated. However, the installation of a second, larger pump during early August resulted in a resurgence of growth of *Aphanizomenon* and a concurrent deleterious effect on the fish life of the lake.

Table 1. Dynamics of phytoplankton and zooplankton organisms in Hooker Lake, North Dakota, June 1970 to June 1971.

Mean Surface C Temperature	23	24	23	17	13	7	4	10	21
DOMINENT PHYTOPLANKTON[1]									
Aphanizomenon flos-aquae	100	5000	158						40
Microcystis		100	1060	176					
Oscillatoria sp.				109	52	49	32		
Actinastrum hantszchii				120	48				
Synedra amphicephala								18	
Totay Phyto-plankton	260	5720	1390	620	180	92	42	41	63
DOMINANT ZOOPLANKTON[2]									
Daphnia pulex	200								
Naupli	210	600	25						450
Diaptomis Oregonensis		265	40	300					
Keratella cochlearis				610	832	1100	921		1760
K. quadrata						710	582		
Brachionus spp.								2200	
Filinia sp.								900	
Total Zoo-plankton	547	1012	110	1430	1120	2285	1830	3360	2805

[1] cells per ml.
[2] organisms per l.

ACKNOWLEDGEMENTS

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