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Paper Chromatograms of Body Mucus of Some Suckers (Family Catostomidae)

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Literature Cited

Brown, E. H. Jr. 1961. Trans. Am. Fisheries Soc. 90:449-456.
Cleary, R. 1958. Iowa Conserv. Comm. Biol. Rept. 10(4):4-5.
Eschmeyer, P. H. and W. R. Crowe. 1955. Misc. Publ. No. 8. Mich. Dept. Conserv. 32 pp.
Gerking, S. D. 1959. Biol. Rev. 34:221-242.
Houser, A. 1955. Netting, trapping, and tagging—Fort Gibson. Job Completion Rept. Proj. No. F-4-R-2. Job No. 2. Okla. Game and Fish Dept. 8 pp. (Mimeo rept.).
Hubley, R. C. Jr. 1963. Trans. Am. Fisheries Soc. 92:165-168.
McCammon, G. W. 1956. Calif. Fish and Game 42:323-335.
Muncy, R. J. 1957. Distribution and movements of channel and flathead catfish in Des Moines River, Boone County, Iowa. Unpubl. Ph.D. thesis. Iowa State Univ. Library. Ames, Iowa. 118 pp.
Reynolds, J. B. 1963. Life history of the smallmouth bass, *Micropterus dolomieui* Lacépède, in the Des Moines River, Boone County, Iowa. Unpubl. M.S. thesis. Iowa State Univ. Library. Ames, Iowa. 85 pp.
Rose, E. T. 1958. Iowa Conserv. Comm. Biol. Rept. 10(4).10-14.
Schmulbach, J. C. 1959. Factors affecting the harvest of fish in the Des Moines River, Boone County, Iowa. Unpubl. Ph.D. thesis. Iowa State Univ. Library. Ames, Iowa. 195 pp.

Paper Chromatograms of Body Mucus of Some Suckers *(Family Catostomidae)*[1]

GENE R. HUNTSMAN

Abstract. Phenol:water and butanol:acetic acid:water solvent systems were used with horizontal and descending paper chromatography of the body mucuses of *Carpiodes, Catostomus, Ictiobus, Moxostoma,* and *Hypentelium.* Mucus could be sampled in the field, applied to the chromatography paper, allowed to dry, and kept for several days without refrigeration. Chromatograms of fresh and dried mucus appeared the same. Horizontal runs were faster but were abandoned for the greater separation possible with descending techniques. Ninhydrin-stained descending chromatograms showed differences between some genera within a run. Descending chromatograms run in butanol:acetic acid:water and viewed with short wave ultraviolet light showed differences between most genera studied. The pattern seen depended on the mucus and the intensity and the wavelength of the ultraviolet light. There seemed to be no effect of age, sex, or area of collection of the fishes on the pattern. Chromatograms of the mucuses of *Catostomus, Hypentelium,* and *Moxostoma,* members of the subfamily Catostominae, all showed prominent fluorescent spots under ultraviolet light, while the chromatograms of the *Carpiodes* species studied (subfamily Ictiobinae) lacked this fluorescence.

Morphological characteristics are often insufficient for distinguishing the four species of *Carpiodes*, the carpsuckers, espec-

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ally in the case of smaller specimens. Paper chromatography, a separatory technique, can furnish information on the chemical affinities of substances. Paper chromatography of metabolic substances has been used as a taxonomic aid in both plant and animal taxonomy. Buzzati-Traverso and Rechnitzer (1953) first applied paper chromatography to fish taxonomy by making chromatograms of fish muscle extract and showing that degree of similarity of the chromatographic pattern is correlated with the degree of taxonomic relationship as established by conventional morphological techniques. Dannevig (1955), Rechnitzer (1956), Viswanathan and Pillai (1956), and Carlson (1961) also used chromatography of muscle extracts as a key to taxonomic relationships of fishes.

Barry and O'Rourke (1959) separated closely related species of *Sebastes* (rosefishes), and *Gadus* (cods) by horizontal paper chromatography of the body mucus. Since mucus is easily sampled and the sampling does not require killing the fish, mucus seemed to offer more potential for use in practical situations than did muscle extracts.

Before attempting to separate the four *Carpiodes* on the basis of the chromatographic patterns of their body mucuses, I undertook a study of the body mucuses of several genera of catostomid fishes including: *Ictiobus, Carpiodes, Catostomus, Moxostoma,* and *Hypentelium.*

Mucus is primarily composed of a simple protein, which has a similar amino acid content in several species of fish studied, and variable amounts of one or more glycoproteins. The sugar fraction of the glycoprotein may be galactose, glucose, fucose, ribose, glucosamine, galactosamine, or sialic acid (Wessler and Werner, 1957, Enomoto, Nagao, and Tomiyasu, 1961; Enomoto, Izumi, and Tomiyasu, 1961; Enomoto and Tomiyasu, 1961a,b). Nucleic acids are present in the mucus, possibly as the result of mucus cell breakdown (Wessler and Werner, 1957). O'Rourke (1961) demonstrated, with immuno-diffusion techniques, antigen-antibody reactions of mucus of *Sebastes marinus* and of *Gadus callanias* and, therefore, the apparent presence of serum protein antigens in the mucus.

The presence of different proteins, polysaccharides, nucleic acids, and serum protein antigens provide a basis for species specificity of fish mucus which might be detected by paper chromatography.

Materials and Methods

Hubbs (1930) grouped the species used in this study into the following subfamilies and tribes: The scientific and common

names are as given by the American Fisheries Society (1960).

Class Teleostei
Order Cypriniformes
Family Catostomidae
Subfamily Ictiobinae
Ictiobus sp. buffalofishes
Carpiodes carpio (Rafinesque) river carpsucker
Carpiodes velifer (Rafinesque) highfin carpsucker
Carpiodes forbesi Hubbs plains carpsucker
Carpiodes cyprinus (Le Sueur) quillback
Subfamily Catostominae
Tribe Moxostomatini
Moxostoma anisurum (Rafinesque) silver redhorse
Moxostoma erythrurum (Rafinesque) golden redhorse
Moxostoma macrolepidotum (Le Sueur) northern redhorse
Tribe Catostomini
Catostomus commersoni (Lacepede) white sucker
Hypentelium nigricans (Le Sueur) northern hogsucker

Fishes for study were captured from the Des Moines River in Boone County with an electric shocking device and with seines from the South Fork of the Iowa River in Hardin County.

Mucus was sampled by lightly scraping the fish with a dull knife or laboratory spatula. Mucus can be applied to the chromatography paper in the field, allowed to dry, and kept for several days without refrigeration. Chromatograms of fresh and dried mucus appeared the same. In the field, I maintained cleanliness of the chromatography paper by keeping it in plastic bags or in aluminum foil. Mixing of the mucus, which might be caused by the rubbing together of fish, was avoided by eliminating crowding during capture or handling. The mucus behaves more or less as an integral part of the fish, and I doubt that light contact of the fish in the water would cause mixing.

A 26-cm diameter Kawerau capillary tripod circular chromatography dish (Kawerau, 1956) for horizontal chromatography and a 20-inch by 8-inch by 20-inch all glass tank, with a removable top for two dimensional and descending chromatograpny were used in this study. Both were manufactured by Shandon Scientific Co. Ltd., 65 Pound Lane, London, N.W. 10, England, and distributed in the United States by Consolidated Laboratories Inc., Chicago Heights, Illinois. Horizontal chromatography in the circular dish is rapid (3 to 4 hours per run) and allows several samples to be run at once, but, because the greatest length of run is only about 12 cm., effective separations were not made with any of the solvents tested.

Two-dimensional chromatography, in which a substance is separated along one axis of a sheet of paper by one solvent system and then along the other axis by another solvent system is time consuming (24 to 48 hours or more), and only two samples could be run at once. Early trials showed little promise and were discontinued.

Descending chromatography on 1 by 18-inch paper strips was used for most of the present study. Whatman No. 1 and No. 3 filter papers for chromatography both gave good resolution of spots. Whatman No. 3 is a heavier paper and was easier to handle in the field and when wet.

The selection or creation of a suitable solvent system is a requisite for successful chromatographic separations. Barry and O'Rourke (1959) used three solvent systems: (1) butanol:acetic acid:water, (2) phenol:water, and (3) phenol-ammonia in their studies of the mucuses of the rosefishes and the cods. I chose these for this initial work on the mucuses of catostomid fishes.

Phenol:water (8:2 v/v) was least satisfactory and caused the patterns to blur, yet I did separate genera with it. I have not yet fully evaluated the phenol:ammonia solvent system. Butanol: acetic acid:water (100:22:50 v/v) gave the clearest, most reproducible patterns.

Though the word chromatography implies color, the substances analyzed are often colorless, and various means are used to make visible the substances on the chromatogram. A solution of 0.45 grams of ninhydrin (triketohydrindrene hydrate) and 1.5 ml. 2,4,6 collidine in 100 ml. ethanol was applied by means of a hand-operated insect sprayer to deveop chromatograms, after which the chromatograms were dried for 30 minutes at 55°C. to develop color (Block, Durrum, Zweig, 1958). Ninhydrin will stain proteins, peptides, amino acids, and primary amines (Block, Le Strange, Zweig, 1953). Color development of ninhydrin-stained spots varies with temperature, humidity, and the composition of the atmosphere (Carlson, 1961). Since facilities for control of these variables were lacking, I had much variability between runs and considered comparisons between runs risky when using ninhydrin. Ninhydrin-stained chromatograms were often difficult to interpret because many of the spots intergrade. Ninhydrin-stained spots begin to fade almost immediately and must be marked at the first opportunity.

A solution of ammoniacal silver nitrate (equal volumes of 0.1 N $AgNO_3$ and 0.5 N NH_4OH) (Litwack, 1960) showed that some spots were related to sugar-like substances.

Vitamins A and E, purines, pyrimidines, flavenoids, and steroids fluoresce or absorb under ultraviolet light (Block, Durrom, Zweig, 1958). Ulltraviolet light was the most effective means tested for detection of spots. Absorption and fluorescence patterns were usually distinct and the fluorescing and absorbing substances persist on the chromatogram for at least 4 months, allowing repeated examination of the chromatograms. Not all lamps are equally effective in revealing spots.

The patterns seen under ultraviolet light depend on both the wave length and the intensity of the light emitted by this lamp. Shortwave (2537 Å) ultraviolet light gave the patterns most useful in this study.

Results

Horizontal chromatography, using either the phenol:water or the butanol:acetic acid:water solvent systems, would not separate the four species of carpsuckers. Ninhydrin stained chromatograms of different species within a run had the same pattern. Variation in the pattern occurred between runs even though the same species were used. In chromatograms run in butanol:acetic acid:water three to seven ninhydrin staining spots appeared for *Carpiodes carpio* in 31 chromatograms, two to eight for *C. velifer* in 17 chromatograms, and three to eight for *C. cyprinus* in 14 chromatograms. Phenol:water gave five to eight in 5 chromatograms of *Carpiodes carpio*. Other catostomid fishes were not included in this study.

Since other chromatographic methods did not separate the species of *Carpiodes*, horizontal chromatography may have a greater potential for the study of mucus than was thought at the time of its abandonment. The great length of run used to produce understandable patterns with the descending technique would not be possible with apparatus used for horizontal chromatography. Varying of the solvents might make horizontal chromatography a more useful technique.

Descending chromatograms run in phenol:water or butanol: acetic acid:water and stained with ninhydrin separated some genera within a run. One such run, made in phenol:water and allowed to descend 14 inches, compared *Carpiodes carpio*, *Moxostoma erythurum, M. anisurum* and an *Ictiobus* (Figure 1). The fact that spots present in some chromatograms are absent in other chromatograms for the same species is possibly the result of the variability in ninhydrin staining.

The comparison of Figure 2, which was made in phenol:water allowed to descend 11.5 inches, with Figure 1 illustrates the variability between runs. In this instance some of the difference might be ascribed to the greater separation in the longer run.

Figure 3 illustrates a run made in butanol:acetic acid:water for 22 hours and compares *Carpiodes carpio* and *Moxostoma eythrurum*. A single fish furnished the mucus samples for all the chromatograms of each species here. Other runs showed that chromatograms of different individuals of the same species gave similar patterns within a run. Data for several other runs are not given here, but they show similar patterns.

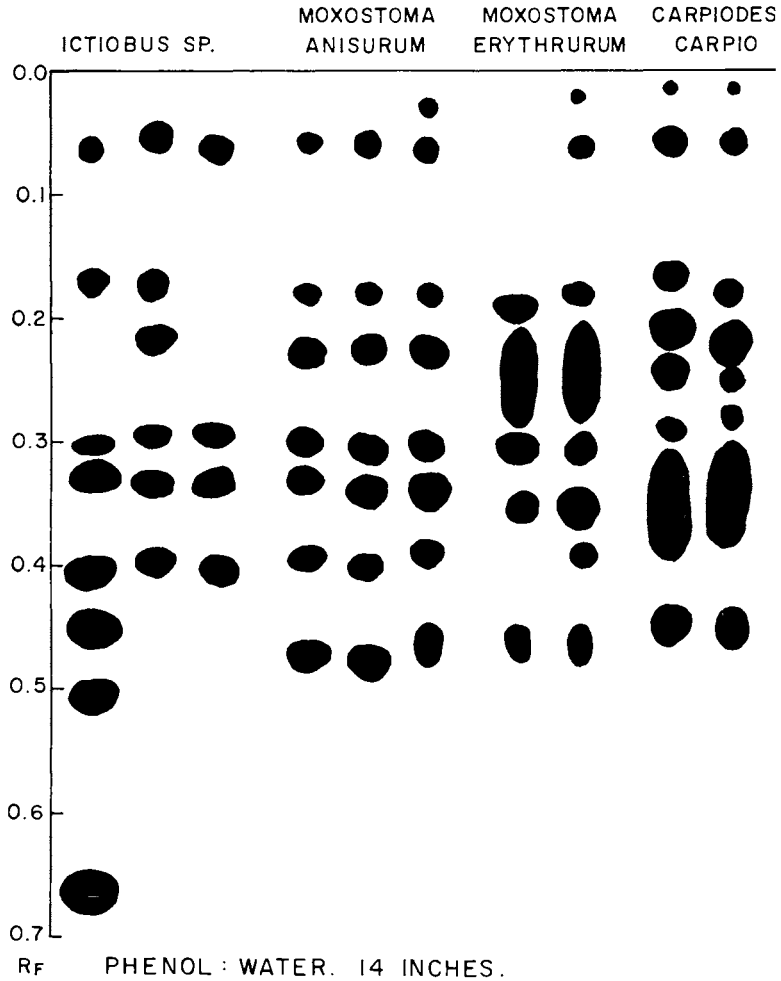

Figure 1. A schematic representation of descending chromatograms of the body mucus of four species of catosomid fish run in phenol: water (8:2 v/v) for 14 inches on a sheet of Whatman No. 1 filter paper for chromatography then stained with ninhydrin.

$$(R_f = \frac{\text{distance traveled by a spot}}{\text{distance traveled by the solvent front}})$$

A 24-hour descending run in butanol:acetic acid:water (100:22:50 v/v) with examination of the dried chromatograms with ultraviolet light was the best method of study used. Variability between runs, so often experienced with ninhydrin staining, was reduced to a minimum. Seventy-four chromatograms were developed and examined in this manner: 16 for *Carpiodes carpio*, 12 for *C. cyprinus*, 9 for *C. velifer*, 3 for unidentified *Carpiodes* specimens, 11 for *Moxostoma macrolepidotum*, 4 for

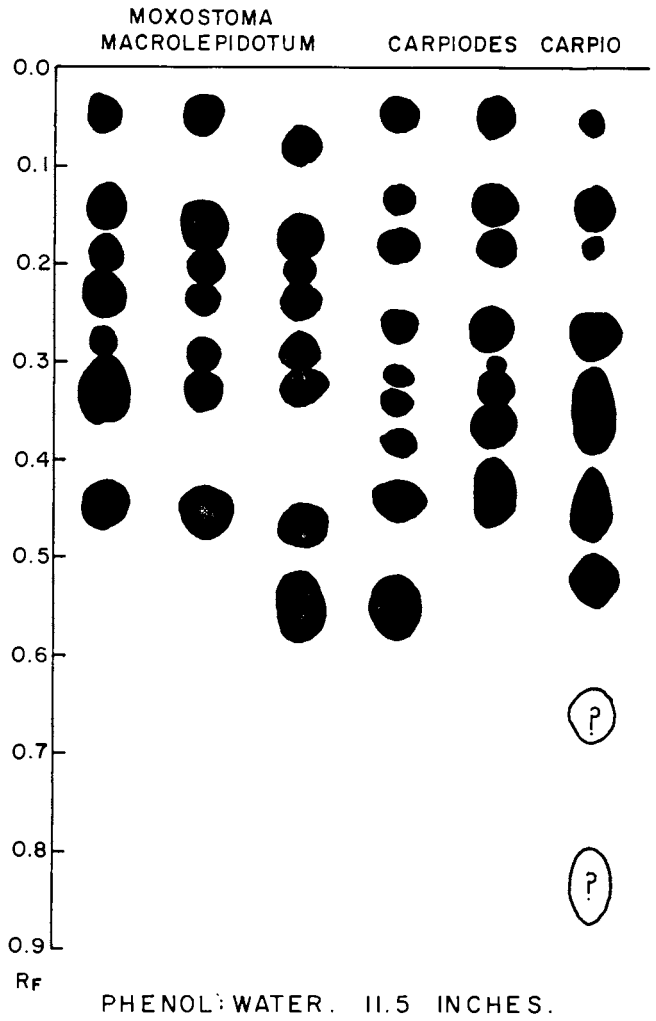

Figure 2. A schematic representation of descending chromatograms of body mucus of *Moxostoma macrolepidotum* and *Carpiodes carpio* run in phenol:water (8:2 v/v) for 11.5 inches on a sheet of Whatman No. 1 filter paper for chromatography. Solid spots represent spots actually present on the chromatogram. Circles with spots indicate spots which were very faint on the original chromatograms and which may not be part of the true chromatographic pattern.

M. anisurum, 7 for *Hypertelium nigricans*, 8 for *Catostomus commersoni*, and 4 for *Ictiobus* sp.

The patterns of all these fish were in general similar (Figure 4). There was usually a narrow absorption band near the point of application and three wider bands of fluorescence or absorption farther along the strip.

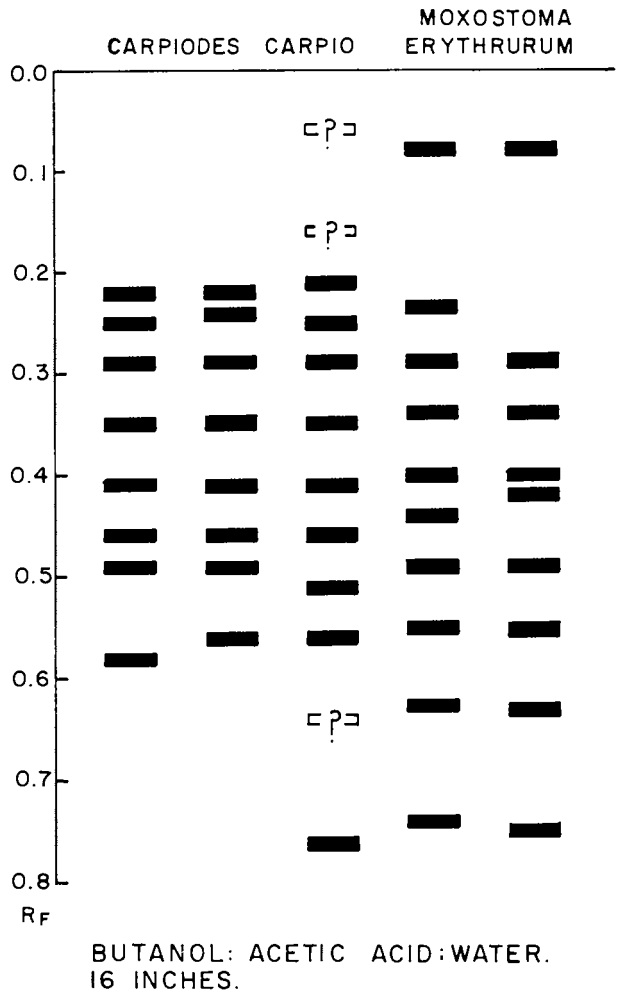

Figure 3. A schematic representation of descending chromatograms of body mucus of *Carpiodes carpio* and *Moxostoma erythrurum* run in butanol: acetic acid: water (100:22:50 v/v) on strips of Whatman No. 1 filter paper for chromatography for approximately 16 inches, then stained with ninhydrin. Solid bars represent spots actually present on the chromatograms. Bars with question marks represent spots which were very faint on the original chromatograms and which may not be part of the true chromatographic pattern. The third chromatogram for *Carpiodes* appears more similar in pattern to those of *Moxostoma* than was actually the case. The colors of the spots were more typical of *Carpiodes* than of *Moxostoma*.

Since these chromatograms were created by allowing the solvent front to run off the end of the paper, Rf values could not be calculated. I instead calculated Rx values, the ratios of the distances traveled by the spots to the distance traveled by the fastest moving substance.

Using these values, one cannot separate the chromatograms of *Moxostoma* and *Catostomus*. Both possess the dark absorption

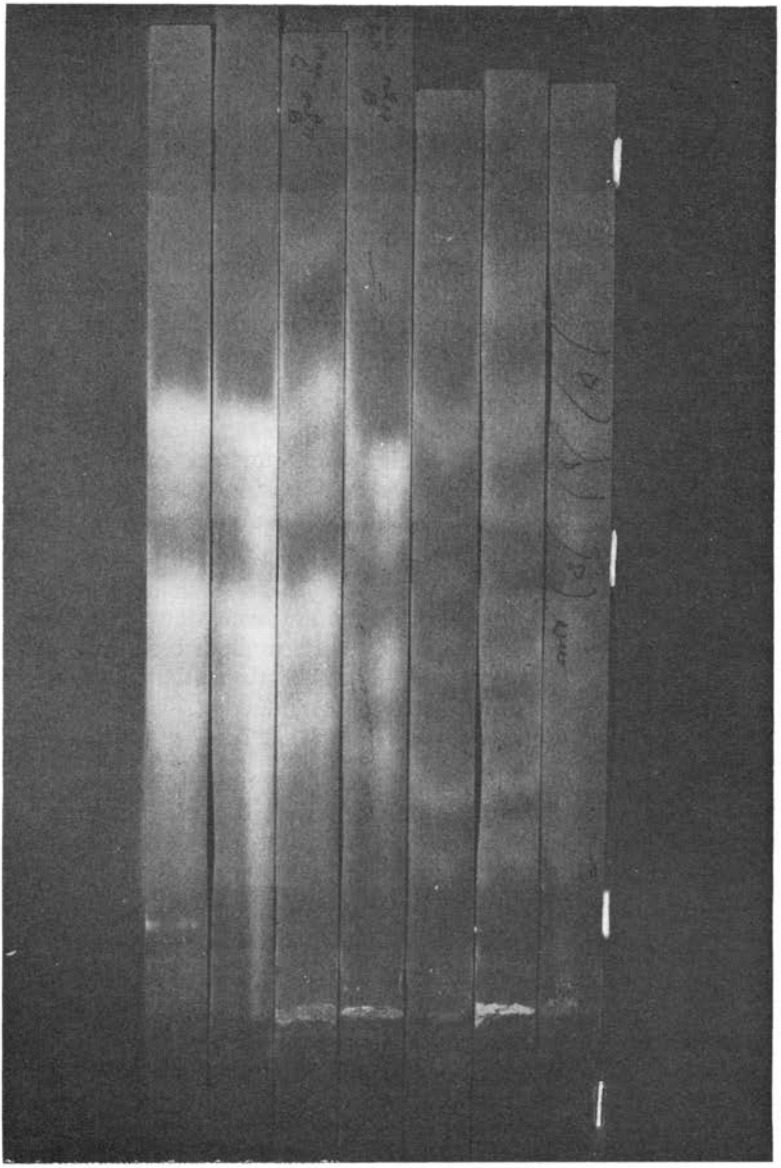

Figure 4. A photograph of descending chromatograms under ultraviolet light (3600Å) of the body mucus of five catostomid species run in butanol: acetic acid: water (100:22:50 v/v) for 24 hours on strips of Whatman No. 3 filter paper for chromatography. From left to right are chromatograms of *Hypentelium nigricans. H. nigricans, Moxostoma erythrurum, Catostomus commersoni, Carpiodes carpio, C. carpio,* and *C. cyprinus*. The pencil markings on the chromatogram of *Carpiodes cyprinus* mucus mark the location of some of the absorption bands seen under shorter wave ultraviolet light. The three regular dark bars which pass across all the chromatograms and the fourth across the bottom of the strips are caused by tape used to hold the chromatograms for photographing. The similarity of the two chromatograms for *Hypentelium*, each from a different run, illustrates the reproducibility afforded by this method.

band at approximately Rx 0.3 then three fluorescent bands at Rx 0.5, 0.7, and 1.0.

Carpiodes carpio has a pattern with the narrow absorption band positioned as in *Catostomus* and *Moxostoma* and three more absorption bands at about Rx 0.7, 0.8 and 1.0, respectively. *Carpiodes velifer* and *C. cyprinus* had patterns essentially identical to that of *C. carpio.* I had no data for *C. forbesi.* In one batch of chromatograms, I found one chromatogram each for *Carpiodes velifer* and *C. cyprinus* which showed some fluorescence bands not unlike those of *Moxostoma* and *Catostomus.* I believe these to be a reflection of some mistake on my part and not to represent the true situation.

The chromatogram of *Hypentelium nigricans* is very distinctive in that the prominent dark absorption band near the origin, consistently present in the chromatograms of other suckers studied, is lacking. *Hypentelium* does possess three fluorescent bands in about the same position as the upper three bands of *Moxostoma* and *Catostomus.*

Twelve chromatograms examined with ultraviolet light then stained with ninhydrin failed to show any consistent coincidence of fluorescing or absorbing spots with ninhydrin-stained spots except in the genus *Carpiodes* where the absorption band at Rx 0.7 always had a ninhydin-staining counterpart. This is an indication of a proteinaceous nature for that spot.

Ammoniacal silver nitrate staining of two chromatograms for *Moxostoma* and six for *Carpiodes* after ultraviolet viewing revealed a correlation of a stained spot with the dark absorption band near the origin which occurs in these two genera. This spot is then apparently related to sugar-like substances.

Discussion

Mucus samples were taken from fishes approximately 5 to 13 inches long and therefore, presumably, for several age classes. Although no fish were sexed, I assumed both sexes were included in the study. The butanol:acetic acid:water chromatograms viewed with ultraviolet light were made from August through November, and no seasonal effect was evident. Fishes from both the Des Moines River and the South Fork of the Iowa River gave the same patterns.

In a study of this nature, in addition to looking for characters which will distinguish species, one is always hoping to find similarities which will show phylogenetic affinities. The fluorescence and absorption patterns on chromatograms of mucus run in butanol: acetic acid:water seem to show phylogenetic trends. Chromatograms of *Catostomus, Moxostoma,* and *Hypen-*

telium, all members of the subfamily Catostominae (Hubbs, 1930), show a set of similarly positioned fluorescent bands. *Carpiodes*, a member of the subfamily Ictiobinae, has a chromatogram characterized by absorption bands. However, these chromatograms do not follow Hubbs' (1930) tribal arrangement, for *Moxostoma*, whose chromatogram is most similar to that of *Catostomus*, is in the tribe Moxostomatini, while *Catostomus* and *Hypentelium*, whose chromatograms differ quite a little, are placed together in the tribe Catostomini.

To draw conclusions about the abilities of these chromatograms to show phylogentic tendencies would be hasty until a more complete study is done on the genera and species of the Catostomidae.

The original goal of this study is yet unfulfilled. The four *Carpiodes* species cannot yet be distinguished by using paper chromatography of the mucus. However, this study on the species of *Carpiodes* and relationships in the Catostomidae is continuing. Better control of the temperature during chromatographic runs and more refinement in the preparation of solvent systems may give greater consistency to the ninhydrin staining technique. There are ninhydrin stains which are less affected by atmospheric conditions than the one described herein. There are several common solvent systems which are yet untested. Attempts to hybridize the carpsuckers may give information on the source of variability in this genus. Another tool of the biochemist, electrophoresis, may yield information on the relationships of the species of *Carpiodes* and genera of catostomid fishes.

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Literature Cited

American Fisheries Society. 1960. A list of common and scientific names of fishes from the United States and Canada. Second edition. Am. Fish. Soc. Spec. Publ. No. 2.

Barry, J. M. and Fergus J. O'Rourke. 1959. Nature 184:2039.

Block, Richard J., Emmet L. Durrum and Gunter Zweig. 1958. A manual of paper chromatography and paper electrophoresis. Second edition. Academic Press Inc. New York, N.Y.

———, Raymond LeStrange and Gunter Zweig. 1952. Paper chromatography. Academic Press Inc. New York, N.Y.

Buzzati-Traverso, Adriano A. and A. B. Rechnitzer. 1953. Sci. 117:58.
Carlson, Bruce M. 1961. J. of Exptl. Zool. 147:43-56.
Dannevig, E. H. 1955. Tidsskrifter for Hermetikindustrie 41:121-123.
Enomoto, Noriyuki, Tokio Izumi and Yukio Tomiyasu. 1961. Japanese Society of Sci. Fish. Bull. 27:606-608.
Enomoto, Noriyuki, Tadaaki Nagao and Yukio Tomiyasu. 1961. Japanese Soc. of Sci. Fish. Bull. 27:143-146.
——— and Yukio Tomiyasu, 1961a. Japanese Soc. of Sci. Fish. Bull. 27:609-612.
——— and ———. 1961b. Japanese Soc. of Sci. Fish. Bull. 27:613-616.
Hubbs, Carl L. 1930. Materials for a revision of the catostomid fishes of eastern North America. Univ. of Mich. Museum of Zool. Misc. Publ. No. 20.
Kawerau, E. 1956. Biochemical J. 61: 32.
Litwack, Gerald. 1960. Experimental biochemistry. John Wiley and Sons, Inc. New York, N.Y.
O'Rourke, Fergus J. 1961. Nature 189:4760.
Rechnitzer, Andreas B. 1956. Intern. Congress of Zool. Proc. 14:333-335.
Viswanathan, R. and V. Krishna Pillai. 1956. Indian Acad. of Sci. Proc. 43, Sec. B:334-339.
Wessler, Erland and Ivan Werner. 1957. Acta Chemica Scandinavica 11: 1240-1247.

Age, Growth, Fecundity and Food Habits of Fantail Darters in Boone County, Iowa[1]

James R. Karr

Abstract. Although 117 fantail darters (*Etheostoma flabellare*) were collected from Bluff Creek, Boone County, Iowa, during summer 1962 and winter 1962-63, only 1 was found in the Des Moines River. Slenderhead darters occupy the similar riffle areas in the river. Female fantail darters were 18.7, 34.8, and 42.4 mm at the first through the third annuli, respectively, and male darters were 18.5, 36.7, 59.0, and 62.0 mm at the first through the fourth annuli, respectively. No females were found that had formed a fourth annulus. Average coefficient of condition for 104 fantail darters was 1.749. Length-weight relationships for males and females were statistically the same. Food of the fantail darter was 93% insects. The peak of the spawning period is probably in May.

Darters were collected from the Des Moines River and its tributaries in Boone County, Iowa during the summer of 1962 and winter of 1962-63. Karr (1964) presented data on slenderhead darter, *Percina phoxocephala,* blacksided darter, *P. maculata,* and johnny darter,*Etheostoma nigrum,* collected during the summer. All except 1 of the 118 fantail darters, *E. flabellare,*

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