Biochemical taxonomy of catostomidae and hybridization of Carpiodes species

Gene Raymond Huntsman
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Iowa State University of Science and Technology, Ph.D., 1966
Zoology

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BIOCHEMICAL TAXONOMY OF CATOSTOMIDAE AND HYBRIDIZATION OF
CARPIOIDES SPECIES

by

Gene Raymond Huntsman

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Fishery Biology

Approved:

In Charge of Major Work

Head of Major Department

Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1966
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INTRODUCTION

Taxonomical investigations at the biochemical level are at least a step closer to the genotype than are morphological and anatomical investigations. The DNA molecule, the bearer of genetic information, by means of a sequential three-unit code of four nucleosides is able to "program", through a system of enzymes, the structure of all life molecules. This system allows the building of multitudes of molecules, proteins, lipids, carbohydrates, etc., and from these molecules greater multitudes of life structures (Crick, 1963; Sibley, 1962). The ultimate goal of taxonomy is to delineate the similarity of genotypes in different groups of organisms. This goal will be realized when techniques enable us to characterize the DNA molecules for each species (Sibley, 1962). Until then our best indicators of genotypes are the molecular products of the biological synthesis mechanism.

Because of the conservative nature of proteins, and other life molecules, in respect to evolution one might hope to discover, through studies of these molecules, close relationships masked by divergent morphological features, or to uncover false relationships established on the basis of morphological features made similar by convergent evolution. Where morphological features are strongly environmentally influenced, studies of a conservative feature, such as proteins should be of use in characterizing and identifying species.

The catostomids are a very suitable group for studies in biochemical taxonomy. Much of their taxonomy has been well worked out by studies of morphological, anatomical, and meristic characters, and a
good background, therefore, exists to which to relate, and by which to evaluate the findings of biochemical studies. There are, however, many interesting taxonomic problems in the Catostomidae to which the application of biochemical studies may be of real value.

Carpsuckers, of the genus *Carpiodes*, are among the most numerous fish in many Iowa streams and figure prominently in the studies of the Iowa Cooperative Fisheries Research Unit. The morphology of these fish is highly plastic and identification of some adult individuals and of some small specimens is difficult, if not impossible, by ordinary means (Hubbs, Hubbs, and Johnson, 1943; Trautman, 1957). The problems encountered in this genus have inspired these studies in artificial hybridization and biochemical taxonomy.
NATURAL HISTORY AND TAXONOMY OF THE CATOSTOMIDAE

The position of the suckers within the animal kingdom is shown below:

- Phylum Chordata
- Subphylum Vertebrata
- Superclass Gnathostomata (Series Pisces)
- Class Osteichthyes
- Subclass Actinopterygii
- Order Cypriniformes
- Suborder Cyprinoidei
- Family Catostomidae.

The family is characterized among the soft-rayed fishes by the presence of the Weberian apparatus and a single row of 16 or more pharyngeal teeth (Lagler, Bardach, and Miller, 1962; Moore, 1957).

Of the 77 recognized species of suckers, only 2 are found outside North and Central America, *Myxocyprinus asiaticus*, a species endemic to China, and *Catostomus catostomus*, a species widespread in North America which has recently invaded eastern Siberia. Suckers are believed to have arisen from a cyprinid prototype in Asia, spread to the New World, and then to have declined in influence in the Asian fauna while prospering in North America. Suckers are found in every state of the continental United States, including Alaska, all the Canadian provinces, and range to Guatemala in the Atlantic drainage and into western Mexico on the Pacific slope (Miller, 1958).

The flesh of suckers is generally regarded as well-flavored but is often bony. The buffalo fishes are the least bony of the group and are important commercial fishes in some areas. Suckers are not considered sport fish but furnish much recreation in areas where white suckers and redhorses are speared during their spawning runs. Snagging redhorses
with an unbaited hook and line when they are "shoaling" in the spring is a time-honored Ozark custom. And to many farm boys the ubiquitous white suckers provide a first taste of the thrill of fishing as they are hauled flipping and splashing from a shade-splattered creek.

In the Great Lakes region white sucker minnows are a favorite bait for northern pike and are propagated extensively for that purpose (Dobie et al., 1956; Herter, 1965).

Perhaps the greatest importance of suckers lies in their position in the ecosystem. In many streams, such as the Des Moines River, suckers are among the most numerous, if not the most numerous, fishes. Sucker biomass often is greater than that of any other fish in some streams. Because many of the species feed on aquatic insect larvae, suckers can and do compete with game species, especially trout. On the other hand, small suckers can be important as forage fish for predaceous species. Lake chubsuckers may be useful as forage fish in farm ponds (Bennett and Childers, 1966).

Jordan (1878), following Gill (1861), recognized three subfamilies of the Catostomidae: Catostominae containing the genera Placopharynx, Quassilabia, Myxostoma, Erimyzon, Minytrema, Chasmistes, and Pantosteus; Cycliptinae containing Cycleptus; and Bubalichthyinae containing Carpiodes, Ichthyobus, Bubalichthyes, and Myxocyprinus. Jordan and Evermann (1896) retained this system but admitted the generic names Ictiobus to replace Ichthyobus and Bubalichthyes, Lagochila to replace Quassilabia, and Moxostoma to replace Myxostoma. Bubalichthyinae thus became Ictiobinae. They also added Xyrauchen to the Catostominae. Forbes and
Richardson (1909) used Jordan and Evermann's (1896) system in their classic work, *The Fishes of Illinois*. Fowler (1913) deviated from the thinking of Jordan and Evermann (1896) by placing the buffalo fishes in the genus *Amblodon* and naming the subgenus of the largemouth buffalo *Megastomatobus*. Fowler (1913) included the species of Jordan and Evermann's (1896) *Chasmistes* in two other genera, *Lipomyzon* and *Deltistes*. I have not found *Lipomyzon* used elsewhere. *Deltistes* persisted until Miller (1958) recognized it as a synonym of *Catostomus*. Jordan (1917) raised the *Catostomus* subgenus *Hypentelium* to a full genus and established a new genus, *Thoburnia*, for the fish named originally as *Catostomus rhothoeum*. *Lagochila* was shifted back to *Quassilabia*.

Carl Hubbs (1930) offered the following classification for suckers of the eastern United States:

**Subfamily Ictiobiinae**
- Genera
  - *Megastomatobus* bigmouth buffalo
  - *Ictiobus*
  - *Carpiodes*

**Subfamily Gycleptinae**
- Genera
  - *Gycleptus*

**Subfamily Gatostominae**
- Tribe *Moxostomatini*
  - Genera
    - *Moxostoma, Placopharynx, Lagochila*
- Tribe *Erimyzonini*
  - Genera
    - *Minytrema, Erimyzon*
- Tribe *Catostomini*
  - Genera
    - *Catostomus, Hypentelium, Pantosteus*
- Tribe *Thoburniini*
  - Genera
    - *Thoburnia*

In this treatment Hubbs established the tribe system for the Gatostominae, raised *Megastomatobus* to full generic status, and shifted back to the use of *Lagochila*. 
Tanner (1942) elevated Fowler's (1913) Notolepidomyzon, a subgenus of Pantosteus, to a full genus. Legendre (1942) established another sucker genus by the naming of a new redhorse Megapharynx valenciennesi, but reclassified this fish as Moxostoma hubbsi in 1952.

Nelson (1948) from intensive study of the Weberian apparati of catostomid fishes drew the following conclusions:

1. The Weberian apparatus of catostomid fishes has three main forms corresponding to the three recognized sucker subfamilies.

2. *Myxocyprinus* should be removed from the Ictiobinae and placed either in the Cycleptinae or its own subfamily.

3. *Myxocyprinus* and *Cycleptus* should be considered to represent the most primitive stocks. The *Myxocyprinus* line probably gave rise to the Ictiobinae, the *Cycleptus* line to the Catostominae.

4. Within the Catostominae, the Catostomini represent the stem form from which arose the Erimyzonini first and the Moxostomatini later.

5. Within the Catostomini, *Chasmistes* arose early from the catostomin stock, while *Pantosteus* and *Xyrauchen* have arisen from the *Catostomus* form.

6. *Minytrema* is less specialized than *Erimyzon*.

7. *Thoburnia* arose early from the moxostomatatin stock, while *Placopharynx* arose first and *Megapharynx* slightly later from *Moxostoma*.

8. *Hypentelium* is most likely of moxostomatatin, not catostomatatin, stock, although convergence may have masked a true affinity to the Catostomini.

Nelson, either by omission or design, did not recognize Fowler's (1913) Lipo@yzon or Deltistes, Tanner's (1942) Notolepidomyzon, or Legendre's
Hubbs (1955) designated Placopharynx and Megapharynx as subgenera of Moxostoma, and Megastomatobus as a subgenus of Ictiobus.

Raney and Robins (1956) reorganized Moxostoma and established 3 subgenera, Scartomyzon (Fowler, 1913), Megapharynx, and Moxostoma, regarded Hubbs' (1955) Placopharynx as synonymous with the subgenus Moxostoma, and agreeing with Nelson (1948) but disagreeing with Hubbs (1955) assigned Thoburnia to Moxostomatini.

Moore (1957) in his keys to fishes of the United States recognized these as the valid genera of American suckers: Cycleptus, Ictiobus, Carpiodes, Moxostoma, Lagochila, Minytrema, Erimyzon, Pantosteus, Ghasmistes, Thoburnia, Catostomus, and Hypentelium.

Fowler (1958) officially recognized the subfamily Myxocyprininae. Miller (1958) continued to recognize only the original three subfamilies. Miller presented a hypothetical evolutionary scheme for the Catostomidae slightly different from Nelson's (1948) in not recognizing the origins of Ictiobinae and Catostominae from, respectively, myxocyprinine and cycleptine stocks. Miller concurs with Raney and Robins (1956) in the synonymy of Placopharynx and Megapharynx with Moxostoma.

Bailey (1959) discovered a third Thoburnia [Raney and Lachner (1946) had named a second], but after studies of the similarities of Thoburnia to the Moxostoma subgenus Scartomyzon, decided Scartomyzon was at least as similar to Thoburnia as to the other Moxostoma subgenera and, consequently, named Thoburnia as a subgenus of Moxostoma. Bailey doubted the generic status of Lagochila and felt it was descended from, and,
perhaps, best included in Moxostoma. The American Fisheries Society Committee on the Names of Fishes (1961), chaired by Bailey, recognized the same genera as Moore (1957) except Thoburnia, which was included in Moxostoma.

Nelson (1961), on the basis of swimbladder morphology, agreed to the subgeneric stature of Lagochila but felt Hypentelium and Thoburnia were well-distinguished genera. Miller and Evans (1965) after studying catostomid brains agreed with the generic stature of Thoburnia, (at least until a study of "relatively non-plastic features" showed otherwise), and the subgeneric stature of Lagochila. Unfortunately, evidence indicates that Lagochila had become extinct before final clarification of its taxonomy. Miller and Evans feel that if Thoburnia is subgeneric to Moxostoma, Pantosteus might better be subgeneric to Catostomus.

In attempting to summarize the status of the genera of Gatostomidae in 1966 I place Cycleptus in the subfamily Cycleptinae, and Myxocyprinus in this subfamily or in Myxocyprininae. Ictiobinae contains Ictiobus and Carpiodes. Catostominae contains three tribes: Erimyzonini with Erimyzon and Minytrema; Moxostomatini, (some authors use Moxostomini), with Moxostoma, Hypentelium, and, (depending upon whether or not you agree with Bailey) Thoburnia; and Catostomini with Catostomus, Pantosteus, Chasmistes, and Xyrauchen.

Because the ictiobine species, especially the carpsuckers, are of interest to fishery workers at Iowa State University I felt a brief summary of these species and the history of their taxonomy would be of value. The species of Ictiobinae have labored under a number of generic names
including Sclerognathus, Ictiobus, Ichthyobus, Amblodon, Bubalichthyes, Megastomatobus, Carpiodes, and even Catostomus. There have been a multitude of species names applied since most of the species have been named two, three, or more times. Hubbs (1930) established the nomenclature of the United States' Ictiobinae essentially as it is today by naming three buffalo fishes, Megastomatobus (now Ictiobus) cyprinellus, Ictiobus niger, and I. bubalus, and four carpsuckers, Carpiodes carpio, G. velifer, G. cyprinus, and G. forbesi. Hubbs was not positive of the validity of G. forbesi and suggested it might be a hybrid of G. carpio and G. cyprinus. Of the 12 species names of Carpiodes listed by Jordan, Evermann, and Glark (1930), five, cutisanserinus, labiosus, elongatus, microstomus, and meridionalis, remained unsynonymized after Hubbs' (1930) revision.


Two other ictiobine species listed as Carpiodes labiosus and G. meridionalis by Meek (1904) occur in Mexico and Guatemala. These species are apparently both referable to Ictiobus (personal communication, Dr. Robert Rush Miller, Museum of Zoology, University of Michigan, Ann Arbor, Michigan).
HYBRIDIZATION EXPERIMENTS

Literature Review

The presence in the Des Moines River of adult carpsuckers whose characters appear intermediate to those of two of the recognized species of *Carpiodes* suggests the possibility of hybridization among species of this genus. Hubbs, Hubbs, and Johnson (1943) felt the difficulties encountered in identifying some *Carpiodes* specimens were probably not due to hybridization but instead to the characterization of the species by two or more traits all of which must be taken into consideration when identifications are made. Hybridization takes place within and among several other catostomid genera (Hubbs, Hubbs and Johnson, 1943; Hubbs and Hubbs, 1947; Hubbs and Miller, 1953; Hubbs, 1955; Trautman, 1957). Trautman apparently accepts the possibility that carpsuckers may hybridize for he identified some Illinois River, Illinois carpsuckers as *Carpiodes cyprinus* x *C. forbesi* hybrids (Starrett and Fritz, 1965). Starrett and Fritz, however, chose to follow Bailey and Allum (1962) and called all the fish *C. cyprinus*.

Artificial hybridization of the carpsuckers was attempted in 1964 and 1965 to see if such hybridization was genetically possible, and, if possible, to furnish hybrid fish for studies in biochemical taxonomy.

Materials and Methods

Hybridization attempts were begun in May, 1964 by collecting ripe adult carpsuckers from the Des Moines River. A boat-mounted 230-volt alternating-current shocking device was used to stun fish for capture.
Fish were considered ripe if eggs or milt could easily be expressed from their vents by pressure on their sides or belly. Ripe eggs were approximately 1 mm in diameter, translucent, and very pale yellow. Occasionally eggs could be forced from unripe fish, but these eggs were smaller, paler, and more opaque. Such eggs were not used in crosses. In the case of another sucker, *Catostomus commersoni*, experience has shown that eggs forced from fish with heavy pressure will not yield young (Dobie et al., 1956). Eggs were collected in a plastic dishpan which had been immersed in water, and then drained immediately prior to the stripping of the eggs. Eggs and milt were mixed by stirring with the fingers. Several rinses of water removed the excess milt from the eggs. The diameter of the eggs increased to 1.5 to 2 mm upon contact with water.

The fertilized eggs are free flowing until the rinsing process at which time they become very adhesive and tend to clump in large masses. Such clumping can cause the death of eggs on the interior of the masses because the inner eggs are not in free exchange with the water. Corn starch, river mud, and bentonite were used in attempts to coat the eggs and stop this aggregation. Of these the most successful was river mud which had been dried, pulverized, and then, prior to use, rewet to the consistency of heavy cream. It was difficult to achieve a creamy, homogeneous mixture of bentonite. Though these additives were used, the eggs retained some tendency to clump.

The fertilized eggs were decanted into quart jars full of water for hardening and transportation. Fertilized eggs were incubated in 13.5 in. x 14.0 in. x 4.5 in. wooden-framed, screened containers which were
floated in Squaw Creek, north of the Iowa State campus. Plastic screening was used since it seemed less likely to harm the eggs than metal. The 1.5mm x 1.5mm mesh held the eggs well but was too coarse to trap any young that might hatch within the boxes. In 1965, a plastic screening of 0.8 mm x 0.8 mm mesh was used. This was small enough to hold larval fish but tended to become clogged with suspended matter and to prevent free circulation of the water within the container. The containers had 13.5 in. x 14.0 in. screened lids which were held on with hooks and eyes. The lids were easily removable for inspection and care of the eggs.

These containers were cheap, easily constructed, and enabled me to use the abundant supply of suitable hatching water available in the creek. The design of the boxes, however, caused the eggs to eddy together into masses. The use of the creek as a hatching site also had some disadvantages. Dramatic rises in the water level occur in the creek after heavy spring thunderstorms. Such rises are usually accompanied by a drop in the water temperature and an increase in turbidity although neither of these seemed to harm the eggs. One might suspect that carpsucker eggs would be resistant to the effects of such changes. Boxes left in the creek, unless properly protected, are also fair game for curious children and wayfaring raccoons. The only really easy way to check the eggs involved wading in the creek, a process not always unpleasant, but sometimes inconvenient. The most serious drawback to the use of the stream water was the abundance of fungus organisms present therein. It is possible that the fungus first attacked the dead eggs in the center of some of the egg clumps and then spread to live eggs.
In 1965, attempts were made to develop a hatching system which could use tap water and avoid the problems of using creek water. The use of tap water for hatching fish eggs poses certain inherent problems. Chlorine, the concentration of which in the University water supply was less than 0.05 ppm, was removed by bubbling air through the water as it flowed downward through a column of glass beads. Tap water temperatures averaged around 17 °C, slightly less than the 20 °C temperature extant in the streams at spawning time. Aquarium heaters, infra-red and incandescent light bulbs, and a coil of aluminum tubing through which was passed hot water were used in attempts to warm the hatching water as it was held in an elevated 20-gallon plastic refuse can. The hot water coil was most useful for it had the greatest heating capacity and could be most easily regulated.

A siphon delivered water from the reservoir into 0.6-liter apothecary jars through drawn glass nozzles. The nozzles increased the velocity of the water enough to impart the desired rolling action to the eggs. The shape of the jar was also conducive to the rolling of the eggs. No eggs were hatched in this apparatus but the eggs that did hatch in 1965 spent 12 hours in one of the jars before being transferred to a box in the creek, indicating that eggs could survive for at least a while in the hatching jars.

One of the greatest problems encountered in the hybridization experiments was the obtaining of ripe parent fish. Ripe males of all species of carpsuckers were easier to acquire than fully ripe females. Near ripe females were far more common than fully ripe fish of the same sex.
In 1965 injections of carp pituitary gland were given to near-ripe female carpsuckers, which had been captured and held in wading pools, in the hope that these fish could be stimulated to ripen and become female donors to the crosses. Ripe males of all species were held also but were not given pituitary injections. The males would retain their potency for about a week and then would no longer yield milt. Pituitary injections might have stimulated the males to retain their potency, but since ripe males were fairly abundant this was not deemed necessary.

Carp pituitaries were collected, dehydrated in acetone, and then stored dry. Before injection, the pituitaries were ground, and mixed with 0.7 percent saline in the ratio of one gland to 1.5 ml of saline. Intraperitoneal injections of 1 ml of this mixture were made through the venter of the fish. Intraperitoneal injections cause fewer complications than do intramuscular injections (Ball and Bacon, 1954).

Free flow of eggs was usually obtained the day after injection of fish in which eggs could be seen at the vent upon squeezing the sides of the fish but from which eggs would not flow freely. Usually a smaller quantity of eggs could be obtained on the second day after pituitary injection from fish which had been stripped the previous day. Only one fish failed to respond to pituitary injections.

Only one cross using eggs from pituitary-injected females hatched. Though the eggs flowed freely after pituitary injections, they may not have been mature. The eggs that did hatch in 1965 were larger, yellower, and more translucent than those eggs which did not hatch. The viable eggs also had a much greater tendency to clump than those eggs which did not hatch, and were in appearance and response entirely similar to the viable
Eggs of 1964. The pituitary injections may have caused the freeing of eggs, but not their maturation. Such a phenomenon is fairly common when pituitary injections are used (Pickford and Atz, 1957). The dosages administered to the carpsuckers (1/3 gland) seem approximately in accord with those recommended by de Azevedo and Canale (1938). If the dosages had been reduced and the number of injections increased, higher quality ova might have resulted.

Results and Discussion

Details of the attempted crosses made in 1964 and 1965 are given in Table 1.

Development of fertilized carpsucker eggs is rapid; in 1965 *G. velifer* x *G. carpio* larvae hatched after spending 12 hours in hatching jars at about 23°C and 60 hours in creek water at 20°C. In 1964 *G. carpio* x *G. cyprinus* larvae began hatching after 119 hours. In 1964, heart beating and embryo movement was evident 49 hours after fertilization. At 62 hours unpigmented eyes were visible. At 76 hours the eyes were pigmented and very active movement was evident in the egg. At 100 hours one embryo appeared to have stuck its tail through the chorion. This fish had been attacked by a fungus, however. At 110 hours pre-hatching activity was evident in many eggs. This activity consisted of violent movement by the larvae which resulted in the eventual rupture of the chorion. The fish hatched tail first so that the hatching eggs bounded along the bottom of the jar propelled by the flagellating, extended tail. The larvae eventually escaped from the egg. The prolarval carp-
Table 1. Attempted hybridization of *carpiodes* species

<table>
<thead>
<tr>
<th>Date</th>
<th>Female parent</th>
<th>Male parent</th>
<th>Pituitary used</th>
<th>Hatching method</th>
<th>Comments</th>
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<tr>
<td>1964</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 16</td>
<td><em>C. carpio</em></td>
<td><em>G. cyprinus</em></td>
<td>No</td>
<td>Incubated in creek boxes</td>
<td>Fungus attacked by May 19. Fungused eggs removed. Nearly 100% of non-fungused eggs hatched producing about 50 young.</td>
</tr>
<tr>
<td>May 16</td>
<td><em>C. carpio</em></td>
<td><em>G. cyprinus</em></td>
<td>No</td>
<td>Boxes in lab tank</td>
<td>No development</td>
</tr>
<tr>
<td>June 3</td>
<td><em>C. carpio</em></td>
<td><em>G. cyprinus</em></td>
<td>No</td>
<td>Boxes in lab tank</td>
<td>No development</td>
</tr>
<tr>
<td>June 3</td>
<td><em>G. cyprinus</em></td>
<td><em>G. velifer</em></td>
<td>No</td>
<td>Boxes in creek then in lab</td>
<td>Fungus attacked eggs in creek. No development.</td>
</tr>
<tr>
<td>June 18</td>
<td><em>C. carpio</em></td>
<td><em>G. velifer</em></td>
<td>No</td>
<td>Hatching jars</td>
<td>No development</td>
</tr>
<tr>
<td>1965</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 10</td>
<td><em>C. carpio</em></td>
<td><em>G. velifer</em></td>
<td>June 9</td>
<td>Hatching jars</td>
<td>No development</td>
</tr>
<tr>
<td>June 10</td>
<td><em>C. carpio</em></td>
<td><em>G. cyprinus</em></td>
<td>June 9</td>
<td>Hatching jars</td>
<td>No development</td>
</tr>
<tr>
<td>June 11</td>
<td><em>C. carpio</em></td>
<td><em>G. velifer</em></td>
<td>June 9</td>
<td>Hatching jars</td>
<td>No development</td>
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<td>(used on 10)</td>
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<td>June 12</td>
<td><em>C. carpio</em></td>
<td><em>G. cyprinus</em></td>
<td>June 9</td>
<td>Hatching jars</td>
<td>No development</td>
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<td><em>C. carpio</em></td>
<td><em>G. velifer</em></td>
<td>June 23</td>
<td>Hatching jars</td>
<td>No development</td>
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<tr>
<td>June 25</td>
<td><em>C. carpio</em></td>
<td><em>G. velifer</em></td>
<td>June 25</td>
<td>Hatching jars</td>
<td>No development</td>
</tr>
<tr>
<td>June 26</td>
<td><em>G. velifer</em></td>
<td><em>G. carpio</em></td>
<td>June 25</td>
<td>Hatched on June 29, 12 hrs. Box in creek 2 days</td>
<td>About 50 young.</td>
</tr>
<tr>
<td>July 8</td>
<td><em>G. sp.</em></td>
<td><em>G. carpio</em></td>
<td>No</td>
<td>?</td>
<td>No development</td>
</tr>
<tr>
<td></td>
<td>(velifer?)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
suckers were translucent except for the large dark eye. They were about 3-4 mm long at hatching, had rounded heads, stubby blunt tails, and bore elongate yolk sacs about 2/3 of their length.

After hatching the larvae lay on the bottom of the container. After 15 to 30 minutes they began swimming vertically upward for 6 to 8 inches with a violent wriggling motion. At the top of the ascent they ceased swimming and sank slowly to the bottom. Touching the bottom seemed to stimulate the upward swimming again. After this up-down motion was continued for several cycles, the fish would lie on the bottom as if to rest. The fish began swimming in more or less horizontal planes within 2 to 3 days after hatching.

Since I had no knowledge of the dietary requirements of larval carpsuckers, a "shotgun" approach to feeding them was adopted. Algae scraped from aquaria walls and water from parasitologists' snail-rearing tanks containing algae, rotifers, and protozoa were added to the containers holding the young fish. About 3 days after hatching, on May 25, 1964, the larvae appeared dark in the gut region indicating that they were feeding. The fish survived until early June when they began to die of unknown causes. The last four carpsuckers were lost to an aquarium filter on June 3, 1964.

In 1965 attempts were made to provide better rearing facilities for larval hybrid carpsuckers. Three steel-walled, plastic-lined wading pools were erected in the court of the Science Building on the Iowa State Campus. They were filled with tap water in late April and allowed to stand for about 2 weeks. On May 13 each of these pools was fertilized with about a pint of composted sheep manure and then stocked with mud and water from the Izaak Walton pond near Ames to provide an inoculation of aquatic organisms. These
pools were first used as holding ponds for carpsucker breeding stock. One
was selected as a rearing pond.

The *Carpiodes velifer* x *C. carpio* hatch occurred on June 29, 1965.
The larvae were transferred to the aerated wading pool on June 30. Indi­
vidual larvae were seen on July 1 and 2 but never after that.

The hybrid fish could have died of disease, lack of proper food and/or
living conditions, or congenital insufficiencies. The young fish were
not deformed, seemed to behave and swim in a normal fashion, apparently
even began feeding, and showed outwardly no sign of genetic defect.

Even had hybrid carpsuckers survived to adulthood, this would not have
been evidence for natural hybridization. Hubbs (1955) stated, "Species that
rarely or never hybridize in nature can often be crossed, sometimes very
freely, in captivity. Merely placing a male of one species into an
aquarium with a female of another species may lead to a crossing".

Judging from the amount of written knowledge available, little is known
about carpsucker spawning. Harlan and Speaker (1956) state *Carpiodes*
*forbesi, C. cyprinus,* and *C. carpio* spawn in April or May, and *C. velifer* in
May. Our experiences on the Des Moines River have shown that the peak
month for the spawning of *C. carpio, C. velifer,* and *C. cyprinus* is June
with some spawning activity in late May and early July. Carpsuckers,*  
*C. cyprinus,* in breeding condition were observed in the Maquoketa River at
Manchester, Delaware Co., Iowa on May 7, 1965. Variability in the spawning
season is possibly related to stream conditions. *Carpiodes cyprinus* sup­
posedly spawn in the quiet waters of streams and overflow ponds over sand
or mud bottoms, and *C. velifer* in shallow areas of streams and overflow
ponds (Harlan and Speaker, 1956). In the Des Moines River the ripest individuals of all three species of carpsuckers were found in and around gravelly riffle areas indicating that these areas might be spawning sites. The aforementioned Maquoketa River carpsuckers were bunched in what may have been a breeding group and could be seen to run up on a rocky riffle and exhibit behavior which appeared very similar to that of spawning red-horses.

Several factors may promote hybridization. Where spawning areas are limited and breeding individuals are forced into close proximity, the likelihood of chance meeting of eggs and sperm, or of individuals choosing a mate of the wrong species increases. The situation in which a few individuals of one species cohabit with a multitude of individuals of another species is likely to engender hybridization (Hubbs, 1955).

Whether the carpsuckers spawn on riffle areas or in backwaters there is a limited amount of spawning area available in the Des Moines River in Boone Co., Iowa. River carpsuckers far outnumber highfin and quillback carpsuckers in the Des Moines River. Assuming similar times and areas of spawning for the different carpsuckers, it seems likely in terms of Hubbs (1955) proposals, that some hybridization would occur.

Still another factor that might favor hybridization is the predominance of fully ripe males of all species of carpsuckers during the spawning season. Almost every male carpsucker will yield some milt during the spawning season, yet very few fully ripe females are taken at any one time.

Hybridization among the carpsuckers has been neither proved nor disproved. The evidence available leads me to the belief that hybridization is at least as likely as not.
Paper chromatography, a chemical separatory technique, has been shown useful in studying the taxonomy of many kinds of organisms (Huntsman, 1964a). Barry and O'Rourke (1959) using paper chromatography of the whole external body mucus separated closely related species of Sebastes and species of Gadus. Chromatograms of whole body mucus viewed with ultraviolet light (2537 Å) showed an absorption band near the origin in all suckers studied except Hypentelium nigricans and three more absorption bands for Carpiodes carpio, C. cyprinus, and C. velifer, and three fluorescence bands for Hypentelium nigricans, Catostomus commersonii, Moxostoma erythrurum, M. anisurum, and M. macrolepidotum (Huntsman, 1964b).

Wessler and Werner (1957) working with Pleuronectes platessa, Gadus callarias, G. merlangus, Anguilla vulgaris, Esox lucius, Raja sp., and Myxine glutinosa showed that the sugars of the body mucus of these fishes included ribose, fucose, mannose, galactose, and glucose. Enomoto and Tomiyasu (1961) made chemical analyses of the mucuses of Anguilla japonica, Uranoscopus japonicus, Gnathagnus elongatus, Lagocephalus lunaris, Pseudorhombus cinnamomeus, Raja hollandi, and Dasyatis akajei and determined the presence of a hexuronic acid in all fishes examined except Pseudorhombus, glucosamine, galactosamine, fucose, ribose, and galactose in all species examined, and glucose in Lagocephalus only.

Neither the study of Wessler and Werner, nor that of Enomoto and Tomiyasu were taxonomically oriented. Wessler and Werner's study did
include two species of *Gadus*. Mucous sugars of these fish were identical qualitatively and, at least, very similar quantitatively. They were different from those of other fishes studied.

Investigations of the mucous sugars of some catostomid fishes were undertaken to assess the taxonomic value of this technique.

**Materials and Methods**

In the summer and autumn of 1964, body mucus was collected for later analysis from *Ictiobus bubalus*, *I. cyprinellus*, *Carpiodes carpio*, *C. cyprinus*, *C. velifer*, *Hypentelium nigricans*, *Moxostoma anisurum*, *M. erythrurum*, *M. macrolepidotum* and *Catostomus commersoni*, from two sites, Boone and Luther, on the Des Moines River, Boone Co., Iowa, and from the South Fork of the Iowa River, Hardin Co., Iowa. Mr. Bill Welker, Iowa State Conservation Commission biologist, collected mucus from Missouri River blue suckers, *Cycleptus elongatus*, at Sioux City, Woodbury Co., Iowa, and Commission biologist Roger Schoumacher collected mucus from spotted suckers, *Minytrema melanops*, from the Mississippi River, Guttenberg, Clayton Co., Iowa. Mr. David Vanicek furnished mucus of *Pantosteus delphinus* and *Catostomus latipinnis* taken from the Green River in northern Utah. Dr. Earl Herald of the Steinhart Aquarium, San Francisco, California kindly allowed me to collect mucus from a *Xyrauchen texanus* in the aquarium.

Because *Carpiodes carpio* was to be used as a reference species, records of the sex and age of some of these fish were made. Aging was by the scale method.
Mucus was collected by scraping the fish with a dull knife or laboratory spatula. When a small mass of mucus had accumulated on the scraper, the scraper was stirred in a container of 70 per cent ethanol until the mucus protein was denatured. Denatured mucus, its adhesiveness gone, easily fell off the scraper into the container. The mucus was stored in 70 per cent ethanol. Further drying of the samples was effected by at least 24-hour treatments with ethanol and with ether. Before hydrolysis the mucus was evaporated dry, and then ground to a powder. The samples were weighed and then placed in hydrolysis ampules with 2N sulfuric acid in a ratio of 10 mg dried mucus per ml of acid. The samples were hydrolyzed after 1 hour, a 2-hour hydrolysis was tried to increase the sugar concentration in the hydrolyzate. As was shown by Wessler and Werner (1957) a 2-hour hydrolysis reduced the concentration of the pentose without compensating benefits. Using congo red as an indicator, the pH of the samples was adjusted to about 5 by the addition of barium carbonate. Excess barium carbonate, which remained undissolved, and insoluble barium sulfate, formed by the reaction of barium carbonate and sulfuric acid, were removed by centrifugation.

Foam produced by the reaction of carbonate and acid often trapped some of the sample and speeded its evaporation. In some of the very small samples the danger existed of losing all the sample to the foam. These small samples were diluted with distilled water before neutralization and evaporated to their preneutralization concentration afterward. Less intense foaming occurred in these dilute samples and less liquid was lost. A few drops of isopropanol were added to each sample as a preservative.
The samples were spotted on Whatman No. 1 paper and run, using descending chromatography, from 24 to 36 hours. Enomato and Tomiyasu (1961) made two chromatograms of each sample using in one a phenol: water system and in the other a butylacetate: ethanol: acetic acid system to separate sugars which exhibited near equal movement in one of the systems. Wessler and Werner (1957) achieved good separation of the sugars they found by using a lutidine: n-amyl alcohol solvent system. I chose a butanol: pyridine: water (8:4:3 v/v) solvent because it separates excellently galactose and glucose, which many systems will not, and gives adequate separations of the other sugars involved.

Preliminary chromatographic comparisons of mucus hydrolyzate with xylose, arabinose, rhamnose, glucose, galactose, fucose, mannose, and ribose indicated that it was sufficient to use the latter five sugars as controls. Control sugars were placed on both left and right sides of the chromatograms to detect uneven flow of the solvent front and allow interpretation of the sample spots when the front was uneven.

The developed chromatograms were allowed to dry, sprayed with aniline hydrogen phthalate reagent (Partridge, 1949), again allowed to dry, and then heated for 5 minutes at 100 C to develop color. Aniline hydrogen phthalate stains pentoses, such as ribose, red, and hexoses (glucose, galactose, and mannose among others) and methylpentoses (fucose among others), greenish brown. The stained spots are more apparent under ultraviolet light.
Results and Discussion

Chromatograms indicate that the carbohydrate content of sucker body mucus is very low. The main constituent of the mucus is probably a simple protein that contains little or no carbohydrate (Wessler and Werner, 1957). The carbohydrate is likely associated with minor glycoprotein constituents (Wessler and Werner, 1957). Even for *Carpiodes* species where mucus is relatively abundant, the sugars in single fish mucus samples are scarcely sufficient to produce visible spots on chromatograms. In suckers, such as the redhorses, which have scant mucous secretion, single-fish mucus samples are nearly useless. Because of the low concentration of sugar in mucus, a series of chromatograms was run to assess the dangers of pooling mucus samples. River carpsuckers, because of their abundance and their copious mucous secretion, were used in these experiments.

A chromatogram bearing single-fish mucus samples from seven females, one age V, five age IV, and one of unknown age, showed no qualitative individual differences. Another chromatogram bearing samples from four females, one age V, one age VII, and two age IV (also run on the previous chromatogram), and one age IV male gave the same results. All fish used on the two previous chromatograms were from the Luther collecting area on the Des Moines River, June 18, 1964. On the basis of the previous experiment, a chromatogram was run bearing samples pooled in respect to age, sex, and collection date. These seven pooled samples included 1 age IV female, 3 age V females, 3 age IV males from the Luther collecting area on August 18, 1964, and 10 age IV females, 8 age IV males, 3 age V females, and 7 age V males taken from the Luther area July 28 and 31, 1964 (collections
combined). No sex or age qualitative differences were shown by these pooled samples. Mucus of river carpsuckers of both sexes and all ages were pooled with respect to date for collections from the Boone area on July 22 and 24, 1964 (combined collections), August 10, 1964, and October 8, 1964, and from the Luther area on September 3, 1964. These samples were pooled in the field and the exact numbers of fish represented are unknown, but there are almost certainly five, and probably over ten, fish in each sample. The chromatograms for these showed no qualitative temporal changes, or differences between the areas.

Even in the best of the chromatograms, the spots were pale and often hard to interpret. However, since the comparisons made in the previous experiment were of spots essentially side by side, I feel the results are valid.

These chromatograms indicated that pooling the samples, and the possible masking of age, sex, or temporal differences or differences associated with proximate collecting areas on the same stream do not pose a serious obstacle to comparisons between species.

The mucus of the catostomid fish studied contains sugars whose stained spots correspond to those of glucose, galactose, mannose, fucose, and, approximately, to ribose. It should be noted that even if two spots match in color and migration, they do not necessarily represent the same substance. Such matching is regarded as a good indicator of identity. Since this study was comparative, complete identification of the various sugars was deemed unnecessary. I have used as names of sucker mucous sugars the names of the control sugars with which the mucous sugars most closely
agreed in staining reaction and migration. The pentose in river carp-sucker mucus seems to migrate slightly less than ribose, and may or may not be that sugar. In other species studied the pentose and ribose migrate about equally.

Mucus samples of many of the species studied were too small to furnish enough sugar to make enough distinct spots on the chromatograms, and results presented in Table 2 are tentative.

The patterns show no phylogenetic significance. Differences shown are from single chromatograms and may be confounded with batch effects. Only Moxostoma anisurum did not show a galactose spot. Further study may show galactose for this species.

The study of mucous sugars for taxonomic purposes requires either a more sensitive identifying technique or much larger samples of mucus.

Table 2. Sugars in the mucus of some catostomid fishes

<table>
<thead>
<tr>
<th>Species</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Mannose</th>
<th>Fucose</th>
<th>Ribose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycleptus elongatus</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Ictiobus cyprinellus</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ictiobus bubalus</td>
<td>?</td>
<td>?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carpiodes carpio</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Minytrema melanops</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moxostoma erythrurum</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moxostoma macrolepidotum</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Moxostoma anisurum</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catostomus latipinnis</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

^Numbers in parentheses indicate a minimum number of fish in the pooled samples.

^These species were not collected by me. The amount of mucus indicates at least five fish per species.

^Though more than 50 Carpiodes carpio were used in the study no single hydrolyzate represented more than about ten fish.
Samples for one Hypentelium nigricans, one Catostomus gommersoni, one Xyrauchen texanus, and an unknown number of Pantosteum delphinus contained too little sugar to make visible chromatographic spots.

Inept spraying ruined the chromatograms for Carpiodes velifer and C. cyprinus.
ELECTROPHORESIS

Electrophoresis in Biochemical Taxonomy

Electrophoresis, a technique which separates proteins on the basis of their ionic charges allows the study of protein mixtures for taxonomic purposes.

Fish hemoglobins (Smith, 1963; Schumann, 1957; Tsuyuki and Gadd, 1963; Tsuyuki, Roberts, Vanstone and Markert, 1965), and fish eye lens proteins (Smith, 1962) have shown some taxonomic value. Fish serum proteins (Starr and Fosberg, 1957; Engle, et al., 1958; Fujiya, 1961; Gunter, et al., 1961; Sulya, et al., 1961; Tsuyuki and Roberts, 1966) show species specific patterns with some electrophoretic techniques. Sanders (1964) and Tsuyuki and Roberts (1965) found salmonid hybrids display serum protein patterns similar to those of one parent.

Another source of protein for electrophoretic studies of fish taxonomy is skeletal muscle extract. Hamoir (1951a, b) made the first electrophoretic studies of fish skeletal muscle and (1951c) reviewed the knowledge of fish muscle proteins at that time. Connell (1953a) investigated the low ionic strength extract of codling (Gadus morhua) muscle, and then extended his electrophoretic study to include 20 fish species including three Pleuronectes and three Raja species. Electrophoretic patterns of low ionic strength muscle extracts were species specific. Nikkila and Linko (1955), using both the older moving-boundary and paper electrophoresis, compared the low ionic strength extracts of 10 species of fish and demonstrated both species specificity of the extract patterns and the superior resolution of paper electrophoresis.

Thompson (1960) of the U.S. Food and Drug Administration found species
specificity of distilled water extracts of flesh from perch, cod, haddock, whiting, pollock, halibut and flounder, and proposed this technique for use in detecting illicit substitutions of fish in the commercial market. Lillevik and Schloemer (1961) performed the first taxonomic study of muscle extracts by examining these extracts from 10 centrarchids. All patterns were similar but species differences were evident.

Most prominent in the study of fish muscle myogens is a group centered around H. Tsuyuki and E. Roberts at the Technological Station of the Fisheries Research Board of Canada, Vancouver, British Columbia. Tsuyuki, Roberts, and Gadd (1962a) in column chromatographic studies of low ionic strength fish muscle extracts demonstrated differences between four species of Oncorhyncus and the ling cod, Ophiodon elongatus. Similar extracts from five North American Oncorhyncus and Salmo gairdneri when analyzed by starch gel electrophoresis yielded species-specific patterns that showed relationships closely correlated with phylogenetic relationships derived from morphological studies (Tsuyuki, Roberts, and Gadd, 1962b). Muscle myogen patterns did not vary with sex and varied only slightly with the sexual maturity of the fish. Tsuyuki and Roberts (1963) extended their electrophoretic studies of salmonid muscle myogens to include steelhead, brown, and brook trouts and found similar results.

Hewitt et al. (1963) found paper electropherograms of the water extracts of poeciliid fish muscles showed differences at the genus, tribe, species, and population levels.

Tsuyuki, Roberts, and Vanstone (1965) examined the muscle myogen patterns of 50 fish species including elasmobranchs, a holocephalan, and tele-
osts. Starch gel electropherograms of all species but *Anaploma fimbria* and two *Sebastodes* were species specific. The three patterns exhibited by *Anaploma fimbria* were believed caused by hybridization or by genetically isolated populations. *Sebastodes polyspinis* and *S. ciliatus* each show two patterns which were the same in the two species. The authors felt that this resulted from problems relating to identification of the species. Further investigations into the muscle myogens of *Oncorhyncus* showed no difference in electrophoretic patterns of *O. nerka* from 27 racial stocks, of fish of different sex, stage of spawning, or from environmentally different spawning areas (Tsuyuki, Roberts, Vanstone and Markert, 1965). Minor differences did appear between the patterns of two groups of fall chinook, *O. tshawytcha*, taken at different hatcheries, and yearling *O. nerka* showed minor variation from the adult patterns. These variant patterns did not, according to the authors, exceed the bounds of species specificity. Tsuyuki and Roberts (1966) found, as they had hypothesized, that the pattern of *Oncorhyncus masou* was most similar to that of the *Salmo*. Tsuyuki and Roberts (1966) also disclosed that they had encountered three myogen patterns, apparently two parental and one hybrid, for Great Lakes populations of *Stizostedion vitreum* and of *Catostomus catostomus*.

Lane et al. (1966) proposed the substitution of cellulose acetate for starch gel as the electrophoretic medium used in species determination of fish flesh by the Food and Drug Administration. The cellulose acetate method is easier and faster, yet still yields species specific patterns of muscle extracts.

Studies on the enzymes of fish muscle indicate some sources of the

Electrophoresis of muscle extracts has been shown, with few exceptions, to yield species specific patterns and is proving to be a useful tool in taxonomy of fishes and identity checks of fish flesh in the commercial market.

Materials and Methods

Fish for electrophoretic studies were collected with boat-mounted shocking gear and with a smaller electrofishing system powered by a 115-volt alternating current Homelite generator and utilizing hand-held electrodes. A 20 foot x 6 foot nylon seine with 1/4 inch mesh was used at times. Collections were made at the following sites and dates:


Species studied are listed below. Common and scientific names are as given in Special Publication No. 2 of the American Fisheries Society. The capital letters following each name are the "initials" of the species as used in later illustrations. The collections in which each species appeared are indicated by the numbers (taken from above) following the species name. The species are:

- *Ictiobus cyprinellus* (Valenciennes), bigmouth buffalo, BMB, (1,2,4,12).
- *Ictiobus niger* (Rafinesque), black buffalo, BB, (8).
- *Ictiobus bubalus* (Rafinesque), smallmouth buffalo, SMB, (1,8,12).
- *Carpiodes carpio* (Rafinesque), river carpsucker, RCS, (1,2,5,6,7,9,13,15).
- *Carpiodes velifer* (Rafinesque), highfin carpsucker, HF, (1,4,5,6,7,9).
- *Carpiodes cyprinus* (LeSueur), quillback, QB, (1,3,4,5,6,7,9,13,17).
- *Minytrema melanops* (Rafinesque), spotted sucker, SPT, (15).
- *Moxostoma erythrum* (Rafinesque), golden redhorse, GRH, (1,2,4,5,9,10,13,14).
- *Moxostoma macrolepidotum* (LeSueur), northern redhorse, NRH, (3,4,6,9,12,14).
- *Hypentelium nigricans* (LeSuerr), northern hogsucker, HOG, (9,10,11,13).
- *Catostomus commersoni* (Lacepede), white sucker, WHT, (4,6,12,13).
The 12 species studied represent 7 of the 12 (or 11, 10, or 9) genera and 2 of the 3 (or 4) subfamilies of Catostomidae, and the 3 tribes of Catostominae.

Sex and length were recorded for the 187 fish sampled for blood, or muscle, or usually both.

Cardiac punctures made in the field with Luer-lock syringes and no. 21 needles provided blood for the study. To reduce hemolysis, needles were removed from the syringes before expelling the blood into storage containers. Syringes and needles were used repeatedly but were rinsed with 0.7 per cent saline solution several times between each use. Centrifuging the clotted blood sample for 10 to 15 minutes compacted the clot so that the serum could be decanted. Occasionally a second centrifugation was necessary to remove cells not caught in the clot. Serum samples were kept frozen until used. Most samples were pale straw color indicating only slight hemolysis.

Muscle samples were taken from above the lateral line in the anterior portion of the left side of the body. Efforts were made to prevent inclusion of red muscle from the lateral line region. Samples were wrapped in polyethylene, frozen by dry ice in a styrofoam cooler, and kept frozen in the laboratory until used. Some small fish were frozen whole, and then skinned and sampled in the laboratory.

Muscle samples were homogenized with twice their volume of 0.05 ionic strength phosphate buffer at pH 7.5. Buffer was made by mixing equal volumes of 0.0156 M K₂HPO₄ and 0.0035 M KH₂PO₄ (Connell, 1953a). The same buffer was used for dialysis. Glassware and buffer were prechilled.
to 0°C and the muscle samples remained at least partially frozen until homogenized. A Virtis "23" tissue homogenizer did not warm the samples and was judged superior to the Waring Blender.

Baby food jars fitted with one-hole rubber stoppers were efficient, inexpensive, readily available, homogenization flasks which fit the Virtis apparatus well, allowed the use of a separate jar for each sample, (standard Virtis homogenization flasks cost $5.25 each), and enabled 22 samples to be homogenized per day.

Homogenized samples sat for at least an hour at 0°C and then were centrifuged. Because glass tubes often broke in the centrifuge, nalgene centrifuge tubes were used. Muscle extract was dialyzed in cellulose dialysis tubing against the phosphate buffer for approximately 48 hours.

The first samples processed were frozen for later electrophoresis, but Tsuyuki (personal communication) advised that freezing would precipitate some components of the extract and later samples were electrophoresed immediately upon completing dialysis. Upon thawing, large, flocculent precipitates appeared. Electrophoretic patterns of frozen muscle extracts appeared qualitatively the same as those of unfrozen extracts. Paired "t" tests comparing frozen and unfrozen extracts from the same individuals (of several species) showed no differences in any of the four bands compared. Inspection of other data did not indicate noticeable effects of freezing on percentage of protein found in principal bands. I included frozen samples in later analyses. The precipitate in thawed samples is probably a non-migrating or cathodic migrating substance.
Early electrophoresis methods involved migration in a liquid medium. Electrophoresis on a solid medium such as filter paper, or cellulose acetate strips gives better resolution of fractions. Gel electrophoretic techniques, such as starch gel and polyacrylamide disc gel, because of a sieving effect at the molecular level resolve more fractions and show sharper boundaries than do other techniques. Polyacrylamide gels, which are used in this study, are superior to starch gels because the former are thermostable, transparent, strong, relatively inert chemically, can be prepared with a large variety of pore sizes, and are non-ionic (Ornstein, 1964).

Because the resolution of bands is improved if all the ions are concentrated in a thin zone before actual separation begins, disc electrophoresis, which uses a system of sample, spacer and separation gels and a discontinuous buffer system to cause stacking of the ions, provides very good resolutions (Ornstein, 1964). Raymond (1964) and Clarke (1964) contend that the discontinuous gel system is unnecessary for stacking the ions. Raymond claims that though discontinuous buffer systems improve resolution we are incapable, at the present, of using such high resolution, and the extra effort involved in preparing discontinuous buffer systems is unwarranted.

One major disadvantage of disc electrophoresis is that one can study only the anodic, or the cathodic portion of a sample in a single run. This is not usually a problem in electrophoresis of serum because most investigators are interested only in the anodic serum proteins. Tsuyuki, in his studies of muscle myogens, places samples in the middle of starch gels and simultaneously separates both anodic and cathodic fractions. Raymond's (1964) vertical acrylamide gel slab could be used similarly. Two separate
runs were necessary to examine both fractions when using disc gel electrophoresis. Studies of Nikkila and Linko (1955), Thompson (1960), Hewitt, et al. (1963), and Lane, et al. (1966) emphasize only the anodic portions of muscle extracts. In many of the groups studied by Tsuyuki, Roberts, and Vanstone (1965) the cathodic portion of the muscle myogens was slight compared to the anodic fraction, although some groups, especially the Salmonidae, showed considerable cathodic fractions.

This study has been on the anodic portions of sucker muscle myogens and sera. The procedures, reagents, and equipment used were essentially those described by Davis (1964). Square 7 inch x 7 inch x 1 3/4 inch dishes were made of 1/4 inch plexiglass. Twelve 17/32 inch holes spaced equidistantly on the perimeter of a 4 3/8 inch diameter circle were drilled in the bottom of the upper dish. Red rubber serum stoppers, their centers removed, were fitted to the holes and accommodated 12 7 mm OD x 63 mm gel tubes. This apparatus allowed me to run 12 samples, usually including one control, at a time. These 12 samples constituted a batch.

Platinum wire and glass electrodes as described by Davis (1964) were used. I found wire (20 gauge) heavier than the 30 gauge recommended by Davis much less likely to flex and, as a result, break after several uses.

A set of serum stoppers, their "skirts" removed, glued on small boards held the gel tubes while they were being filled and facilitated the processing of several samples at once. This last idea and design of the dishes were suggested by Mr. Thomas Wright, Laboratory of Limnology, University of Wisconsin, Madison, Wisconsin.

A Buchler Model 3-1014A regulated power supply was used.
Electrophoretic migration is related to the protein's ionic charge. Changes in the pH of the medium will affect the charge and, therefore, the migration of the protein. The tris-glycine buffer (pH 8.3) described by Davis (1964) was used for electrophoresis of muscle extracts and sera. Buffers with pH values around 8.5 are usually used for electrophoresis of serum, and Tsuyuki, Roberts, and Gadd (1962b) found this pH to give best separation of muscle myogens. Thompson (1960) and Lane, et al. (1966) used buffers of pH 8.6. Nikkila and Linko used a buffer of pH 7.55, ionic strength 0.05. When I tried this buffer, muscle myogens migrated very slowly. Hewitt, et al. (1963) used a pH 6.5 buffer when studying poeciliid muscle extracts.

Sample doses were on the order of 4 to 5 microliters of serum and, 6 to 8 microliters of muscle extract since the latter were more dilute. A 0.001 per cent solution of bromphenol blue was used as a marker dye. A current of 5 ma per tube was passed through the gel tubes until the marker dye was about 5 mm from the end of the tube. Electrophoresis required from 55 to 75 minutes. The technique employed by Tsuyuki and his cohorts requires 18 hours (Tsuyuki, Roberts, and Gadd, 1962b). The 5 ma current was sufficient to cause some heating of the gels. Excessive ohmic heating may inactivate some enzymes.

After electrophoresis the gels were removed from the gel tubes and fixed and stained in a solution of amido black and seven per cent acetic acid. Chilling the tubes in cold water facilitated removal of the gels. Gels remained in the stain at least 8 hours, and then were removed and destained electrophoretically. Amido black will stain all proteinaceous material and other materials with an affinity for the dye (Tsuyuki,
Destained gels were stored in seven per cent acetic acid and scanned with a Photovolt Densicord Densitometer (model 5099). A Photovolt Integraph recorded the area under the resulting curves as a series of blips.

Measurements of the gels were used to separate bands on the tracings where such separations were indistinct. The percent protein in each band was calculated by dividing the number of blips under a curve by the total number of blips.

Figure 1 which shows gels of RCS A (a standard placed in each batch) from batches 21, 26, 27, 29, 32, 33, and 37 illustrates the reproducibility of patterns between batches.

Electrophoresis of Serum Proteins

Results

For most species studied, the wide range of intraspecific variability of disc electrophoretic patterns of serum proteins prohibited the discernment of a typical species pattern. The patterns of a single species could sometimes be grouped into two or three groups that were internally similar, but such grouping was subjective, and often intermediate patterns existed.

For the northern redhorse, Moxostoma macrolepidotum, what appeared to be a species-specific pattern existed. The 16 fish examined were of both sexes, ranged from 10.0 to 16.8 inches long, and were from four widely separated collecting sites, the Des Moines River, the Iowa River, the Big Sioux River, and the Iowa River. Of these 16, only one fish, a 15.1 inch female from the Big Sioux, showed a pattern difference. Figure 2 shows six "normal" northern redhorse patterns on the left and the aberrant
Figure 1. Disc gels of RCSA serum (a control) from batches 21, 26, 27, 29, 32, 33 and 37.

Figure 2. Six normal (left) and one aberrant (right) electrophoretic patterns of Moxostoma macrolepidotum serum. Note the single band near the origin in the normal pattern that is replaced by two in the other.
RCS A serum

Moxostoma macrolepidotum serum
pattern on the right. The single band near the origin in the usual patterns is replaced by two bands in the aberrant pattern. Though a member of the same subgenus as the northern redhorse, the golden redhorse hardly showed any two patterns alike. This phenomenon is unexplained.

Discussion

Thomas and McCrimmon (1964) in a study of lamprey (Petromyzon marinus) serum proteins found that total protein decreased with starvation and the relative amount of protein in various fractions was affected by the life history stage, sex, and disease. Booke (1964) presented an extensive review of the literature of fish serum proteins, and stated that sex, maturity, starvation, pathogens, age, anadromy and temperature had been shown to affect serum proteins. Booke (1965) found increased serum globulin levels with age in lake whitefish. Bouck and Ball (1965) using paper electrophoresis demonstrated oxygen levels in the water affected bluegill and largemouth bass serum protein patterns, but not those of bullheads. The amount of variation was deemed within the range of species specificity as they had demonstrated it with these and 20 other species.

Disc gels of serum proteins of various species of Oncorhyncous show a good deal of variation and Tsuyuki and Roberts (1966) found it difficult to recognize phylogenetic relationships on the basis of these patterns. Tsuyuki and Roberts (1965) found apparent species specific disc electrophoretic patterns of sera from some marine fishes, but patterns from freshwater fishes from the Great Lakes region were much more variable. Some species they examined seemed to show a different pattern for every individual examined. Tsuyuki and Roberts (1965) felt the cause of this dif-
ference in variability between freshwater and marine species was explained by Hubbs (1955) who proposed that more hybridization had occurred in the evolution of freshwater fishes than in that of marine species. Freshwater species are supposedly more diverse genetically than marine fishes.

Serum protein patterns obtained by disc electrophoresis may be too sensitive to individual genetic differences, and to environmental and physiological effects on the serum proteins to be used in taxonomy. The many-banded gels are difficult to compare and a "forest from the trees" situation results when one attempts to recognize species patterns and establish phylogenetic relationships. Less sensitive techniques such as paper electrophoresis seem to provide a better tool for taxonomic studies of serum proteins. Because of its high resolution, disc gel electrophoresis may be useful in studies at the infra-species level. Studies of particular portions of the serum proteins, such as haptoglobins (Koehn, 1966) or transferrins (Dessauer and Fox, 1964) might use disc electrophoresis to advantage.

Electrophoresis of Muscle Extracts

Results

Disc electrophoretic patterns of sucker muscle extracts showed up to five main bands and several smaller bands which tended to remain near the origin (Figures 3 to 10). Homologies between species were decided on the basis of similar migration of the bands in gels of the same batch in which the marker dye had run equal distances. Similar migration does not guarantee that two substances are the same, but in these extracts, obtained by identical procedures and from relatively closely related species, I believe I can assume that like migration indicates homology. In all
Figure 3. Diagrammatic representations of disc electrophoretic patterns of muscle extracts of some catostomid fishes.
Figure 4. Disc gels of muscle extracts from *Ictiobus cyprinellus*, *I. bubalis*, *I. niger*, and *Carpiodes carpio*.
Figure 5. Disc gels of muscle extracts from *Carpiodes velifer* and *Carpiodes cyprinus*. 
Carpio
des velifer

Carpio
des cyprinus
Figure 6. Disc gels of muscle extracts from *Erimyzon oblongus*, and *Minytrema melanops*. 
Brinysen oblongus

Minytrema melanops
Figure 7. Disc gels of muscle extracts from *Moxostoma macrolepidotum* and *M. erythrum*. 
Moxostoma macrolepidotum

Moxostoma erythrurum
Figure 8. Disc gels of muscle extracts from *Hypentelium nigricans* and *Catostomus commersoni*. 
Hypentelium nigricans

Catostomus commersoni
Figure 9. Representative densitometer tracings of disc electrophoretic patterns of muscle extract from some ictiobine fishes. Abbreviations for names are as shown on page 33.
Figure 10. Representative densitometer tracings of disc electrophoretic patterns from some catostomine fishes. Abbreviations for names are as shown on page 33.
species examined, except *Hypentelium nigricans*, all the main bands are homologous to main bands in other species. The hogsucker pattern shows, in addition to main bands shared by other species, a prominent band near the origin, designated the Z band, which may or may not be homologous to one of the minor bands in other species.

Because of the abundance of river carpsuckers, tests of the effects of length, sex, collecting area, and experimental technique were to have been made using that species. Approximately 40 muscle samples were collected from river carpsuckers. Unfortunately, about twenty of these samples were left out of the freezer during a several day absence of the author. The thawing and decomposition of these samples caused me much mental anguish and my cohorts much olfactory dismay.

To make the originally intended tests, I had to choose species whose data on the independent variable furnished the contrasts needed. In the tests made there are sometimes confounding effects. In most tests made I have had to assume that effects other than the ones being tested are randomly allotted to both sides of the contrasts. I regarded as meaningful, differences significant at the 0.05 level.

Because reagents, or my technique, may have varied from day to day, tests were made to evaluate any batch effect. Means of bands 2, 3, 4, and 5 of seven chubsuckers in batch 32 compared by t tests to the equivalent means for four chubsuckers in batch 34 showed no significant differences even at the 0.10 level. The greatest difference was 6.9 per cent in band 4. Similar tests of the means of bands 1, 2, 3, and 4 for five white suckers each in batch 28 and batch 31 showed no difference even at the 0.30
level. Student's t tests of the means of bands Z, 2, 3, and 4 for seven hogsuckers in batch 24, three in batch 25, and three in batch 28 showed no significant differences at the 0.30 level. The means of band 1 showed some differences.

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<th>Standard error</th>
</tr>
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<td>20.0</td>
<td>1.00</td>
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<tr>
<td>Batch 25</td>
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<td>Batch 28</td>
<td>3</td>
<td>25.7</td>
<td>0.493</td>
</tr>
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</table>

A Duncan's multiple range test indicates these means are different at the 0.05 level. Because the tests conducted for chubsuckers and white suckers, and for hogsucker bands Z, 2, 3, and 4 did not show significant batch effects I am tempted to assign the cause of the differences found in band 1 to small sample sizes in batches 25 and 28. Values of band 1 in batch 24 range from 16.9 to 22.7 and, thus, closely approach the means in the other batches. Although I do not feel the batch differences shown are meaningful, the possible existence of batch differences prompted me to make paired-comparison t tests on the basis of batches where this was desirable and possible.

No significant effects could be related to sex in a test of five male river carpsuckers versus seven females, all of which were taken from the Des Moines River on October 2, 1965. The means for band 4 showed the greatest difference, 2.9 per cent. The t value for this difference, 2.048, exceeded the tabular value at the 0.10 level, but did not exceed the 0.05 value. In tests of five male versus six female hogsuckers collected from the South Fork of the Iowa River, November 9, 1965, no significant sex
differences appeared.

Though length and age are not necessarily well correlated, especially when dealing with fish from different waters, I felt that the length measurement provided a measure of maturity adequate for this study. A size range of sampled fish wide enough to permit good analyses of the effects of length on the muscle protein patterns did not exist in all species. Scatter diagrams of length versus per cent protein in the main bands were made for 14 white suckers, 9.2 to 15.1 inches long; 18 quillbacks, 4.2 to 17.2 inches long; and 19 river carpsuckers, 6.5 to 16.5 inches long. In these 16 diagrams only white sucker band 2, and quillback bands 4 and 5 gave any suggestion of varying with length. Regression coefficients (Snedecor, 1956) computed for these bands were: white sucker band 2, \( b = -0.99 \), not significant; quillback band 4, \( b = -0.49 \), significant at the 0.05 level; quillback band 5, \( b = -0.25 \), not significant. The \( b \) values were tested against zero.

The length trends for quillback bands 4 and 5 are caused primarily by the high values for these bands observed in a group of small quillbacks taken in one seine haul from Bluff Creek, Boone Co., Iowa. The effects of length are confounded with those of collection area (Figure 11), batch, and possible genetic similarity. The collection occurred so near the Des Moines River collections that it is doubtful that these fish represent a different geographical stock. The six Bluff Creek quillbacks ranged from 4.2 to 5.1 inches long and possibly came from the same spawning. The uniformly high values of bands 4 and 5 might represent genetic similarity of the fish. The samples for these small fish were all run in batch 34 and the possibility exists that the high values of bands 4 and 5 are batch
Figure 11. Scatter diagram of percentages of protein in band 4 versus length for quillbacks from four different collections.
caused. However, batch 34 was shown not to differ, at least, from batch 32 when chubsucker bands were compared. The obvious factor, immaturity, may have caused these high values. Tsuyuki, Roberts and Gadd (1962b) noticed greater intensity of one band of the patterns of mature coho and sockeye salmons when compared to patterns of immature fish of the same species.

Because fishes from different geographic areas may represent different genetic stocks, or may have differences in the proteins because of environmental factors, tests for the effect of area were made where possible. I have assumed random allotment of the effects of sex, length, and other factors. Student's t tests comparing the percentages of protein in the different bands were made for 13 river carpsuckers from the Des Moines River, October 2, 1965 versus 3 from the Maquoketa River, October 6, 1965; 4 white suckers from the Maquoketa, October 6, 1965 versus 10 from the Iowa River, November 12, 1965; 4 hogsuckers from the Castor River, October 30, 1965 versus 12 from the South Fork of the Iowa, November 9, 1965; 6 quillbacks from the Des Moines River, October 2, 1965 versus 6 from the Maquoketa, October 6, 1965; and 4 northern redhorses from the Big Sioux River, November 12, 1965 versus 6 from the Iowa River, November 12, 1965.

Of the 21 tests made, a difference of 3.0 per cent between the mean of band 4 in the Castor River hogsuckers and the band 4 mean in South Fork hogsuckers was significant at the 0.10 level, a difference of 6.4 per cent between the band 2+3 mean of Des Moines River quillbacks and the band 2+3 mean of Maquoketa quillbacks was significant at the 0.05 level (for quantitative comparisons, the region between bands 1 and 4 on the quillback pattern was designated band 2+3 since band 3, if not missing is much reduced),
and a difference of 8.5 per cent between Big Sioux and Maquoketa northern redhorse band 4 means was significant at the 0.025 level. The differences shown were achieved through comparison of relatively small samples and though the statistical tests made supposedly compensate for small sample size, I would feel the differences more meaningful had the samples been larger.

Tsuyuki, Roberts, Vanstone, and Markert (1965) could not visually assess racial or geographic differences in the patterns of Oncorhyncus nerka or O. tshawytscha, except that patterns of O. tshawytscha taken from two different hatcheries varied slightly. The quantitative approach may be more sensitive to area differences. Area differences might represent environmental or, hopefully, genetic differences between the populations. If the muscle myogen patterns are capable of showing small genetic differences, these patterns may be of value in the study of racial stocks.

Though tests comparing patterns of fish species within collections, or within batches, or within both would be more sensitive than comparisons of the combined samples, I felt that the small sample sizes encountered and the extra effort involved would negate any advantages derived by "within" comparisons. I have based most species comparisons on the combined data. The larger sample sizes achieved are statistically desirable.

No species had all its samples in one batch. Only the chubsucker samples are taken from one collection, and only the highfins come from one collection area. (The black buffalo and the spotted sucker, for which there is one sample each, are exceptions to the above statement.) Thus the factors which earlier tests have shown might affect the patterns can probably be safely assumed as randomly allotted.
The pooling of diverse data is most likely to increase within species variability and make between species tests less sensitive. Since my interests lie mainly in differences between species, pooling the data seems conservative.

I have presented the species comparisons graphically (Figures 12 through 17) in a scheme similar to that suggested by Hubbs and Perlmutter (1942) and modified by Hubbs and Hubbs (1953). (The values for quillback band 2+3 are compared to the summed values of band 2 and band 3 in other Carpiodes). Such presentations allow a sort of visual t test to be made between species. A sample of at least nine fish is necessary for the attachment of statistical validity to such visual comparisons (Hubbs and Hubbs, 1953). Also, if all possible comparisons on a graph are made, these tests are no longer independent, and the usual confidence statements about the comparisons do not hold. Rothschild (1963) presented several criticisms of this method of graphical representation and recommended instead a graphical representation on Tukey’s test. Tukey’s test requires equal sample sizes, although an approximation (Steel and Torrie, 1960) exists which allows use of unequal samples.

Duncan’s multiple range test (Table 3) although it cannot be presented graphically, has a specific modification for use with unequal samples (Kramer, 1956) and is somewhat less conservative than Tukey’s test. The modification makes Duncan’s test more conservative than it would otherwise be. Duncan’s test with the modification was used to compare species means.

In general the differences shown by Duncan’s test agree with those shown by the graphs. Where the Hubbs-Hubbs graphs indicated significance,
Figure 12. Hubbs-Hubbs diagrams of percentages of protein in band 1 for some catostomid fishes. The upright line indicates the mean. The hollow rectangle indicates one standard deviation on each side of the mean. The solid rectangle indicates two standard errors on each side of the mean. The long horizontal line ended by small vertical lines indicates the range. Where the range is exceeded by one or both of the rectangles the lines protruding downward indicate the end of the range. Numbers in parentheses indicate sample sizes.
Figure 13. Hubbs-Hubbs diagrams of percentages of protein in band 2 for some catostomid fishes. The upright line indicates the mean. The hollow rectangle indicates one standard deviation on each side of the mean. The solid rectangle indicates two standard errors on each side of the mean. The long horizontal line ended by small vertical lines indicates the range. Where the range is exceeded by one or both of the rectangles the lines protruding downward indicate the end of the range. Numbers in parentheses indicate sample sizes.
Figure 14. Hubbs-Hubbs diagrams of percentages of protein in band 3 for some catostomid fishes. The upright line indicates the mean. The hollow rectangle indicates one standard deviation on each side of the mean. The solid rectangle indicates two standard errors on each side of the mean. The long horizontal line ended by small vertical lines indicates the range. Where the range is exceeded by one or both of the rectangles the lines protruding downward indicate the end of the range. Numbers in parentheses indicate sample sizes.
ICTIOBUS
CYPRINELLUS (4)
ICTIOBUS
NIGER (1)
ICTIOBUS
BUBALUS (3)
CARPIOIDES
CYPRINUS (20)
CARPIOIDES
VELIFER (5)
ERIMYZON
OBLONGUS (11)
MOXOSTOMA
ERYTHRURUM (14)
MOXOSTOMA
MACROLEPIDOTUM
HYPENTELIUM
NIGRICANS (19)
CATOSTOMUS
COMMersoni (15)
Figure 15. Hubbs-Hubbs diagrams of percentages of protein in band 4 for some catostomid fishes. The upright line indicates the mean. The hollow rectangle indicates one standard deviation on each side of the mean. The solid rectangle indicates two standard errors on each side of the mean. The long horizontal line ended by small vertical lines indicates the range. Where the range is exceeded by one or both of the rectangles the lines protruding downward indicate the end of the range. Numbers in parentheses indicate sample sizes.
Figure 16. Hubbs-Hubbs diagrams of percentages of protein in band 5 for some catostomid fishes. The upright line indicates the mean. The hollow rectangle indicates one standard deviation on each side of the mean. The solid rectangle indicates two standard errors on each side of the mean. The long horizontal line ended by small vertical lines indicates the range. Where the range is exceeded by one or both of the rectangles the lines protruding downward indicate the end of the range. Numbers in parentheses indicate sample sizes.
ICTIOBUS
CYPRINELLUS (2)
ICTIOBUS
NIGER (1)
ICTIOBUS
BUBALUS (3)
CARPIOIDES
CARPIO (20)
CARPIOIDES
VELIFER (5)
CARPIOIDES
CYPRINUS (2)
MINYTREMA
MELANOPS (1)
ERIMYZON
OBLONGUS (12)
MOXOSTOMA
MACROLEPIDOTUM
(2)

PERCENT PROTEIN IN BAND 5
Figure 17. Hubbs-Hubbs diagrams of percentages of protein in band 2+3 for some catostomid fishes. The upright line indicates the mean. The hollow rectangle indicates one standard deviation on each side of the mean. The solid rectangle indicates two standard errors on each side of the mean. The long horizontal line ended by small vertical lines indicates the range. Where the range is exceeded by one or both of the rectangles the lines protruding downward indicate the end of the range. Numbers in parentheses indicate sample sizes.
Table 3. Duncan's multiple range tests of species differences of percentage protein in bands 1, 2, 3, 4, 5, and 2+3*

<table>
<thead>
<tr>
<th>Band 1</th>
<th>Species</th>
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<th>RCS</th>
<th>HF</th>
<th>HOG</th>
<th>WHT</th>
<th>QB</th>
<th>GRH</th>
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<tr>
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<td>23.8</td>
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<th>CHB</th>
<th>SMB</th>
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<tr>
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<th>WHT</th>
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<tr>
<td>Mean</td>
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<td>12.7</td>
<td>12.8</td>
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<th>HF</th>
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<td>45.4</td>
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*Lines drawn under the ranked means indicate groups of means not significantly different from one another. Because sample size is accounted for in these tests, occasionally a mean which is significantly different from the largest mean in a given comparison will be underlined by a line passing from the large mean to one smaller than the significantly different mean. I have indicated these situations by broken lines under the significantly different mean. Species for which certain bands were not present or were sporadic are not included in the Duncan's tests for those bands. Initials of the fish are taken from page 33. Differences indicated are significant at the 0.05 level.
but the Duncan's test did not, t tests were applied. In band 2 values of t calculated from the differences between the means of hog suckers and of highfin carpsuckers and between hog suckers and smallmouth buffalo both exceed the 0.05 tabular value of t. The river carpsucker means for bands 3 and 4 were significantly different at the 0.01 level from the means of highfin carpsuckers for the same bands. River carpsuckers were significantly different from smallmouth buffaloes at the 0.01 level in band 3 and at the 0.05 level in band 4. Though the patterns of river carpsuckers and smallmouth buffaloes appear similar there seem to be real quantitative differences. A larger sample of smallmouth buffaloes is needed.

In band 5 t tests show river carpsuckers significantly different from quillbacks at the 0.05 level and from highfins at the 0.10 level, although the calculated t for the river carpsucker-highfin test (1.95) almost reaches the 0.05 tabular t (2.069).

In addition to the above tests, paired comparisons of golden and northern redhorse samples in the same batches were made. When more than one sample of one or both species occurred in the same batch I averaged the values for a species within a batch. The paired comparisons were then between averages, averages and single values, or single values. Despite the effort to remove batch effects and the fact that much variability was hidden in the averages, none of the four bands showed a significant difference between the species.

Some small traces of band 5 were noted in a few northern redhorses but in no golden redhorses. It is possible that a faint trace of band 5 substance may be present in all northern redhorse samples and separate
these from those of the golden redhorse. Some of the minor bands might also be important in separating these species. The cathodic fraction of the muscle extracts might provide the differentiation desired.

No quantitative tests could be made for the two species for which only one sample each was available. The pattern of the spotted sucker looks superficially similar to that of the quillback. *Minytrema* had 44.6 per cent of its protein in band 1. This is considerably above the quillback average of 36.6 per cent but not outside the quillback range. Only three out of 21 quillbacks sampled exceeded 40.0 per cent in band 1. One fish showed 42.7 per cent, another 44.7 per cent, and a very aberrant sample showed 71.1 per cent. More spotted suckers are needed before valid statements can be made about their pattern.

*The black buffalo* pattern looked very similar to those of the bigmouth buffalo. In a confusing genus like *Ictiobus*, the chance of misidentification of specimens always exists. It is probably more common to confuse *I. niger* with *I. bubalus* than with *I. cyprinellus*. Identification was made on the basis of Moore's (1957) key to the species of *Ictiobus* and both David Behmer (Department of Zoology and Entomology, Iowa State University) and I concurred in the identification. The fish in question was most unlike other bigmouth buffaloes (the common *Ictiobus* in the Des Moines River) taken by us and our first inclination was to call it *I. bubalus*. More specimens are needed.

Table 4 summarizes the differences in patterns between species. Numbers refer to bands of difference. The black buffalo and spotted sucker are omitted from this table.
Table 4. Bands showing a difference between species

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<th></th>
<th>WHT</th>
<th>HOG</th>
<th>NRH</th>
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<th>CHB</th>
<th>QB</th>
<th>HF</th>
<th>RCS</th>
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<td>1,2+3,4,5</td>
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<tr>
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<td>1,2+3</td>
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<tr>
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Discussion

At least one goal of the study has been accomplished. The three species of *Carpiodes* can be adequately distinguished on the basis of electrophoretic patterns of muscle myogens. The patterns were different enough that even considerable variation in the pattern would not mask the identity of the sampled fish. Hubbs (1930) felt that many of the problems encountered in identifying *Carpiodes* specimens were caused by failure of the investigator to evaluate the specimen on the basis of several criteria. The muscle myogen pattern should allow identification on the basis of a single character. Krumholz (1943) felt that the Weberian apparatus of *Carpiodes* species was diagnostic. Nelson (1948) felt, however, that catostomid Weberian apparatus could not be used to distinguish units below the tribal level.

The electrophoretic patterns seem to be correlated with accepted notions of relationships in the Catostomidae. On the basis of band 5 the patterns seem to fit the evolutionary scheme proposed by Miller (1958) and Nelson (1948). All of the Ictiobinae studied show at least a measurable trace of band 5. Both members of Erimyzonini, which Miller depicts as arising only slightly later than the Ictiobinae from the catostomid stem, also show band 5. The Moxostomatini and Catostomini show only a trace (*Moxostoma macrolepidotum*), or no evidence (*M. erythrurus*, *Hypentelium nigricans*, and *Catostomus commersoni*) of band 5.

In the Ictiobinae, the two most similar patterns are those of *Carpiodes carpio* and *Ictiobus bubalus*. This could indicate that these
species, of those studied, are nearest the ictiobine stem stock. The smallmouth buffalo is the most Carpiodes-like of the United States' buffaloes and has been named as a Carpiodes species at least once. The two Central American buffaloes have also been called Carpiodes species. The knowledge of their electrophoretic pattern would be especially interesting.

Within Ictiobus, I. cyprinellus and I. niger presented patterns similar to each other's and markedly different from that of I. bubalus. This phenomenon does not support the actions of Fowler (1913) and Hubbs (1930) who placed the bigmouth buffalo in a separate subgenus and genus, respectively. Hubbs (1955) later decided the differences merited only subgeneric recognition. Drawings of the Weberian apparati by Krumholz (1943) show a greater resemblance of I. bubalus and I. niger apparati to one another than to that of I. cyprinellus and support the grouping of I. bubalus and I. niger. Jordan and Evermann (1896) could not always distinguish I. niger (then urus) from I. cyprinellus and were not sure these were distinct. Though modern authors agree to the validity of I. niger, the electrophoretic patterns indicate a close relationship of this species to I. cyprinellus. The morphological changes associated with the plankton eating habits of Ictiobus cyprinellus may mask a true affinity to I. niger.

In the genus Carpiodes, the patterns of C. velifer and C. carpio are most similar as is true of the morphology. Carpiodes cyprinus has apparently secondarily lost band 3 and may be a more "advanced" member of the genus.

In the Erimyzonini, the pattern of the single spotted sucker examined,
because of the small amount of band 5 present and the predominance of bands 1 and 2, might indicate that this species was closer than Erimyzon to the catostomine stock. This would agree with Nelson's (1948) conclusion. If, as Miller (1958) depicts the Erimyzonini, of the catostomine tribes, arose from the stem stock closest to that point from which the Ictiobinae branched, then the strong band 5 of Erimyzon would indicate that this genus was actually less "specialized". Nelson (1948) found the Weberian apparatus in Erimyzon similar to that of the ictiobine fishes but regarded the similarity a result of convergence. The similarity of the electrophoretic patterns of Erimyzon muscle extracts to the patterns of ictiobine fishes seems parallel to the similarity of the Weberian apparati. The coincidence of this evidence from two sources leads me to believe that Erimyzon may actually be the least specialized of the Erimyzonini.

The resemblance of the patterns of the spotted sucker to that of the quillback is probably of no significance and seems an exception to the general rule of phylogenetic significance of the patterns.

The two Moxostoma species show the most similar muscle myogen patterns of any of the fishes studied. Since both redhorses are members of the same subgenus, Moxostoma, the close agreement of patterns is not surprising and strengthens the contention that myogen patterns do reflect phylogenetic relationships.

The qualitative patterns of the main bands of Hypentelium nigricans and Catostomus commersoni are similar to those of Moxostoma, but numerous quantitative differences exist between these patterns. Not enough species were available in the Moxostomatini and Catostomini to establish tribal
trends.

The protein percentages for Hypentelium lay between those of Catostomus and Moxostoma and closest to Moxostoma for bands 3 and 4. For bands 1 and 2, Hypentelium percentages were closest to Catostomus, but Catostomus was closer to Moxostoma than was Hypentelium. The available evidence from protein patterns does not show whether Hypentelium is most closely related to the Moxostomatini or the Catostomini.

The strong Z band of Hypentelium was not seen for any other sucker studied. This phenomenon and the overall similarity of the main bands of Hypentelium, Moxostoma, and Catostomus closely parallel the results of chromatographic studies of sucker body mucus (Huntsman 1964b) described earlier.

The inclusion of more individuals of some of the species studied, of more species of suckers, and the investigation of the advantages of other electrophoretic techniques should be goals of future studies.
SUMMARY

1. Biochemical taxonomy utilizes sources of evidence that are more closely related to the genotype than are morphological features. The Catostomidae are particularly well suited for studies in biochemical taxonomy because much of the taxonomy of this group has been worked out on the basis of morphological structures, and yet interesting problems remain.

2. The 77 species of Catostomidae are found chiefly in North America south to Guatemala. There is one Asiatic species, Myxocyprinus asiaticus, and the North American Catostomus catostomus ranges into eastern Siberia.

3. The following scheme of classification of the Catostomidae is in general use today:

- **Genera**
  - Subfamily Myxocyprininae: Myxocyprinus
  - Subfamily Cycleptinae: Cycleptus
  - Subfamily Ictiobinae: Ictiobus, Carpiodes
  - Subfamily Catostominae:
    - Tribe Erimyzonini: Erimyzon, Minytrema
    - Tribe Moxostomatini (Moxostomini): Moxostoma, Hypentelium, Thoburnia (?)
    - Tribe Catostomini: Catostomus, Pantosteus, Chasmistes, Xyrauchen

4. At present, eight species are recognized in the Ictiobinae:
   - Ictiobus cyprinellus, I. bubalus, I. niger, I. labiosus, I. meridionalis,
   - Carpiodes carpio, C. velifer, and C. cyprinus.
5. To see if hybridization between Carpiodes species is genetically possible, artificial hybridization was attempted in 1964 and 1965. Despite difficulty in finding ripe females and despite fungus attacks on incubating eggs, two Carpiodes carpio x C. cyprinus crosses produced about 50 young in 1964. In 1965 one C. velifer x C. carpio cross produced about 50 young.

6. Carpsucker eggs hatched in as few as 72 hours, producing prolarvae with elongate yolk sacs.

7. Injections of carp pituitary into female Carpiodes caused release of apparently non-viable eggs in six of seven instances and viable eggs once.

8. C. carpio x C. cyprinus hybrids swam normally and began feeding, but all were dead within 14 days of hatching. C. velifer x C. carpio larvae also appeared normal, but it is not known if they fed before their demise.

9. Ecological factors in the Des Moines River should promote hybridization of carpsuckers.

10. Chromatography of whole fish mucus has shown some taxonomic value. The sugars of fish mucus might indicate phylogenetic relationships.

11. Sucker mucus was collected, dehydrated, powdered, hydrolyzed in 2 N sulfuric acid for 1 hour at 100 °C, and neutralized. The hydrolyzate was chromatographed for about 36 hours using descending chromatography and a butanol:pyridine:water solvent. Chromatograms were stained with aniline hydrogen phthalate.
12. The sugar content of sucker mucus is very low and chromatograms of single fish mucus samples stained very weakly. A series of chromatograms of samples from river carpsuckers showed no obvious age, sex, or temporal differences, nor differences associated with proximate locations in the same stream. Such samples may thus be pooled for study.

13. Patterns, though lightly stained, were obtained for *Cycleptus elongatus*, *Ictiobus cyprinellus*, *I. bubalus*, *Carpiodes carpio*, *Minytrema melanops*, *Moxostoma macrolepidotum*, *M. erythrurum*, *M. anisurum*, and *Catostomus latipinnis* and indicated the presence of sugars whose stained spots correspond to those of galactose, glucose, mannose, fucose, and ribose in the mucus of one or more species of suckers. River carpsucker mucus may contain a pentose other than ribose.

14. Polyacrylamide disc electrophoresis was chosen for study of catostomid serum proteins and low ionic strength muscle extracts. Only the anodic portions of serum proteins and muscle extracts were examined.

15. In the electrophoretic study, 187 fish of the following species were sampled for blood, or muscle, or usually, both: *Ictiobus cyprinellus*, *I. niger*, *I. bubalus*, *Carpiodes carpio*, *C. cyprinus*, *C. velifer*, *Erimyzon oblongus*, *Minytrema melanops*, *Moxostoma macrolepidotum*, *M. erythrurum*, *Hypentelium nigricans*, *Catostomus commersoni*.

16. Blood was obtained by cardiac punctures, allowed to clot, centrifuged, and the resulting sera was frozen until used.

17. Muscle samples were homogenized with twice their volume of pH 7.5 phosphate buffer (ionic strength 0.05). The homogenate was centrifuged and the extract dialyzed for 48 hours against the extracting buffer.

18. Some muscle extracts were frozen after dialysis and a large
precipitate appeared in these samples upon thawing. Electrophoretic patterns of frozen and unfrozen extracts were not significantly different.

19. Electrophoresis of both serum and muscle extracts was carried out at pH 8.3, at 5 mA per gel, for 55 to 75 minutes.

20. Serum protein patterns, except for those of *Moxostoma macrolepidotum*, were not obviously species specific.

21. The muscle extract patterns consist of prominent "main" bands which are apparently homologous in the different species and minor bands which may or may not be homologous in different species. A maximum of five main bands occurred for a species. *Hypentelium nigricans* shows a prominent band shown by no other species studied.

22. The percentage protein for each major band for each fish was determined by scanning the disc gels of muscle extracts with a Photovolt model 5099 densitometer and an electronic integrator.

23. Muscle extract patterns were significantly different at the 0.05 level for each of the species examined except the two *Moxostoma*, which showed the same pattern.

24. Student's t tests showed the possible existence of batch and length effects, and effects related to collection area. The reality of batch effects is doubted, and the length effects are confounded with possible batch, area, and genetic effects. The area effects occur in more than one species and indicate electrophoretic patterns of muscle extracts might be capable of distinguishing different racial stocks. Sex effects were not apparent.

25. The electrophoretic patterns of muscle extracts seem correlated with accepted phylogenetic affinities in the Catostomidae. Band 5 is
present in all the Ictiobinae examined and is much reduced or absent in all the catotomine fish examined except the Erimyzonini. Carpiodes carpio and Ictiobus bubalus, of the species examined in their respective genera, seem closest to the ictiobine stem stock. Ictiobus cyprinellus and I. niger seem more closely related to each other than to I. bubalus. Erimyzon, rather than Minytrema, may be the most primitive member of the Erimyzonini. The extra prominent band in the muscle extract pattern of Hypentelium nigricans is of particular significance since this species lacks a prominent band on the chromatographic pattern of its whole body mucus which was shown by all the other suckers studied.
American Fisheries Society

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