CLEAR TECHNICAL REPORT NO. 66

INTROGRESSIVE HYBRIDIZATION OF THE COMMON CARP (*CYPRINUS CARPIO*) AND THE GOLDFISH (*CARASSIUS AURATUS*) IN THE WESTERN BASIN OF LAKE ERIE

by

Ronald L. Crunkilton

Department of Zoology

THE OHIO STATE UNIVERSITY
CENTER FOR LAKE ERIE AREA RESEARCH
COLUMBUS, OHIO

July 1977

THE OHIO STATE UNIVERSITY

PREFACE

The following report was prepared by Ronald L. Crunkilton as partial fulfillment for the Master of Science Degree in the Department of Zoology at The Ohio State University. Research for this thesis was conducted at the Franz Theodore Stone Laboratory in Put-in-Bay, Ohio and The Ohio State University Museum of Zoology in Columbus. Dr. Ted M. Cavender served as advisor; other members of the reading committee were Drs. Tim M. Berra, Milton B. Trautman and James R. Triplett.

On behalf of the Center for Lake Erie Area Research, I am pleased that we are able to reproduce this research document and make it available to other scientists.

Charles E. Herdendorf
Director

Center for Lake Erie Area Research / Biological Sciences Bldg. / 484 West Twelfth Avenue / Columbus, Ohio 43210 / Phone (614) 422-8949

TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

Acknowledgements

The author would like to thank the numerous individuals that assisted the development of this project. Dr. T. Cavender, Dr. T. Berra, Dr. M. Trautman and Dr. J. Triplett's critical evaluation of the manuscript was greatly appreciated. I would like to acknowledge the use of the facilities of The Ohio State University Museum of Zoology and the Franz Theodore Stone Laboratory. Sincere thanks to my wife, Lila, for her assistance and patience throughout all phases of this project. I would like to express sincere gratitude to my advisor Dr. Ted Cavender for making this pursuit possible.

PART I: INTRODUCTION

Hybridization of the common carp with the goldfish (Cyprinus carpio x Carassius auratus) is a frequent phenomenon reported for the Western Basin of Lake Erie. Trautman (1957) believed that as many as 30-90% of the carp-goldfish catch in the Maumee and Sandusky Bays consisted of introgressed fish. A study was begun in the Spring of 1975 in order to resolve the questions stimulated by this phenomenon of alleged introgressive hybridization. Hybridization, although generally rare, is not in itself unusual. Two closely related species often hybridize, but are nearly always eliminated from the gene pool by hybrid sterility. Contrasting hybridization, introgression implies the incorporation of genetic material into a population of backcrosses. The appearance of fully fertile hybrids constitutes introgression. It is a rare biological event and generates considerable interest. Hybrid fertility in part violates the conventional "species" concept. If a hybrid form is fully fertile then the species definition should be modified or the taxonomic relation between the parents must be reassessed and possibly reduced to at least the subspecific level. Through the failure of isolating mechanisms backcrossing may occur and result in an introgressed population of crosses. If this population becomes reproductively isolated from their parentals or if the parentals are lost through introgression, a new species may be formed. This has been postulated as a mechanism of speciation but little experimental evidence is available to substantiate it.

If indeed as many as 90% of the carp and goldfish in Lake Erie are introgressed, a number of questions are generated: (1) Is introgression taking place or is it simple hybridization? (2) If it is the former, what initiated this massive introgression? (3) What is perpetuating the introgression? (4) Are the crosses reproductively isolated from the parentals? (5) Are the parentals lost? (6) What are the ecological implications of this introgression?

With this thesis I will attempt to analyze several of these questions in order to resolve this phenomenon. The direction of this study will follow the sequence of:

1. Review of taxa present in Lake Erie.
2. The relationship between introgression and the dissemination of carp and goldfish in North America.
3. Statistical analysis of suspected Lake Erie introgressed populations.
4. Descriptive analysis of Lake Erie populations.
5. The relationship between isolating mechanisms and introgression.
6. A central hypothesis of introgression.
7. Conclusions.

A. Systematics of Taxa Studied

Two distinct subspecies of carp are widely recognized, the European wild carp, Cyprinus carpio carpio Linnaeus and the Eastern Asian wild carp, Cyprinus carpio heamatopterus Temminick et Schlegel from Siberia and China. The two forms are separated by gill raker counts of 21-29, usually 23-26, and 17-25 respectively (Berg, 1964). The ranges of Lake Erie carp fall entirely within the former. The pharyngeal tooth formula of known wild European populations is 1,1,3-3,1,1. Deviations from this formula 1,3-3,1,1 / 1,3-3 / 1,1,3-3,1 / 3-3 / 1,3-3,1 in wild carp are said to be a result of remote interbreeding with the crucian carp Carassius carassius (Linnaeus) (Berg, 1964). Lake Erie carp pharyngeal teeth are generally 1,1,3-3,1,1 but with over 40% variation. Eastman and Underhill (1973) reported only a 5.8 percent variation in a sample of Minnesota carp. This may indicate that Lake Erie carp may have at some time prior to or during the early days of cultivation in the United States introgressed remotely with the crucian carp.

Vertebrae in European carp populations 36-38 usually 37, are more variable in Lake Erie populations, 36-39 usually 38, and may indicate that the American carp is somewhat more genetically variable. Other characteristics are too variable to be of use in detecting differences between American and European carp.

The distribution of wild carp is believed to range over the basins of the Mediterranean, the Black, Caspian, and Aral Seas, the Amur Basin and in China (Nikolskii, 1961). Worldwide distribution is known for cultivated varieties. The leather and mirror carp, scaleless and partially scaled varieties arising solely from domestication of European subspecies were considered to be only morphs by Hessel (1878). This conclusion was more recently substantiated by Makino (1939) who found that there were no chromosome differences among those varieties.

Carassius a. auratus (Linnaeus), the Chinese goldfish, is believed to be a color variety of Carassius a. gibelio (Bloch), the Prussian carp. There are few morphological differences. Both types are believed to have been introduced into Lake Erie. Introductions of ornamental varieties have been continuous, however, their bright coloration is often lost when released into the wild.

The crucian carp, Carassius carassius, is similar to the goldfish and Prussian in shape and form. Berg (1964) differentiates the crucian from the Prussian by the following characters:

C. carassius: Gill rakers on first arch 23-35, mostly 26-31. LL 32-35. Vertebrae 31-34. Anal fin usually with 6 branched rays. Body rounded in profile, back thick, scales weakly sculptured. Serrations on last unbranced ray in dorsal and anal small, numerous (up to 30), commencing not far from base of ray. Young usually having a dark spot on caudal peduncle. Peritoneum light colored.

C. auratus gibelio: Gill rakers first arch 39-50. LL 29-31. Vertebrae 29-31, mostly 29 and 30. Anal fin usually with 5 branched rays. Body shape angular, back compressed, scales rough. Serrations on the last unbranched ray in dorsal and anal coarse, few (10-15), commencing farther from the base of the ray. Young never having a dark spot on the caudal peduncle. Peritoneum usually black.

Numeric characters of Lake Erie goldfish populations generally agree with those of *Carassius auratus* although the possibility of remote backcrossing with *Carassius carassius* at some previous time can not be ruled out. Gill rakers on the first arch (Lake Erie populations) are 37-43. LL 27-31. Vertebrae 29-31, mostly 30.

C. carassius is distributed throughout Europe and is encountered with *C. auratus* except in Sweden and Finland. Both are distributed in Europe, North Asia, in the Aral Sea Basin and in East Asia.

The comparison of Lake Erie populations of carp and goldfish with European populations of carp and goldfish reveals that the American carp is slightly differentiated possibly because of remote interbreeding with the crucian carp. Lake Erie goldfish appear to be identical to the European or Chinese types. However, the exact nature of the relationship between Lake Erie and European populations cannot be determined since both may have been changed substantially by cultivation.

Paleontological evidence of fossil *Cyprinus* indicates that an ancestor of *Cyprinus carpio* may have evolved as early as the Miocene in Eurasia (Romer, 1966). Additional evidence (Orlov, 1967) records fossil *Cyprinus* from the Paleogene through the Holocene in Europe, Siberia, and China. The immediate ancestor of the contemporary wild carp, *Cyprinus carpio*, evidently spread from the area of the Caspian Sea at the end of the Pleistocene (Balon, 1974). Post-glacial climatic conditions allowed the migration of some strains from the Danube into the Black Sea, the Aral system and into Eastern Asia about 8,000-10,000 years b.p. The incorrect belief that the carp was only an East Asian species arose from a probable introduction of carp by man from

China to Europe in the Middle Ages. Although the carp may not be of East Asian origin, the Chinese can rightfully claim responsibility for its original domestication which began more than 2000 years ago.

The ancestor of the carp may have risen through polyploidy. This has been a topic of considerable discussion among taxonomists. Cyprinus carpio, Carassius carassius and Carassius auratus have a chromosomal compliment nearly twice that of other cyprinids indicating a tetraploid condition (Ohno, 1967). Cyprinus carpio and Carassius carassius probably shared a common tetraploid ancestor that could have risen when the chromosomal sets of two species were combined by hybridization or when four haploid chromosome sets of a single species formed a zygote. Carassius auratus is a more recent addition thought to have been derived from Carassius carassius. The goldfish is often considered to be the Eastern form of Carassius carassius that differentiated slightly during geographical isolation in Eastern Asia. Fossil records of Carassius are reported as early as the Pliocene of Eurasia (Romer, 1966), and the Neogene of Siberia, and the Pliocene of China (Orlov, 1967).

The interaction among these three species since the Pleistocene has been a balance between maintaining species integrity and introgression. Crosses between these fish have been recorded but reported hybrid sterility at least in the males has probably reduced the chances for massive introgression. Natural hybrids between Cyprinus carpio and Carassius auratus have been reported to be numerous in Europe (Finsch, 1882), but absent from Eastern Asia and Japan. It is of particular significance that populations of carp and goldfish of Japan are not introgressed nor even hybridized while Western populations may be. Perhaps in the geographical areas of no hybridization the populations were sympatric for a sufficient amount of time thus maintaining all isolating mechanisms. This is compatible with the hypothesis that natural hybridization between carp and goldfish in Lake Erie is a result of two closely related, previously allopatric species being thrown together (Woodruff, 1973). Indeed this may be a possibility since the carp and goldfish at one time in their evolution may have been allopatric but now are living under completely sympatric conditions in many parts of the world. Invariably where hybridization occurred it was preceded by an introduction of fish that were previously allopatric. This point is supported by Woodruff (1973) who stated that the hybridization of carp and goldfish in Lake Erie is of the artificial sympatric type which is diagrammed in Figure 1. But this argument is not entirely adequate. The causal relationship has been overlooked. I maintain that the argument of two previously allopatric populations being thrown together is more coincidental than causal since hybridization in the carp and goldfish was always preceded by domestication regardless of whether the fish were previously sympatric or allopatric. Natural hybridization is not found in populations of carp and goldfish known to be naturally sympatric like those of Japan. They will, however, readily produce F_1 hybrids under confined conditions (Ojima, 1973). This indicates that hybridization is not necessarily as simple as two previous allopatric populations being thrown together under natural conditions as suggested by Woodruff (1973) and Hubbs (1955) but is more likely related with the artificial

Figures 1-4. Conventional and proposed models of hybridization.

Fig 1.

SYMPATRIC HYBRIDIZATION

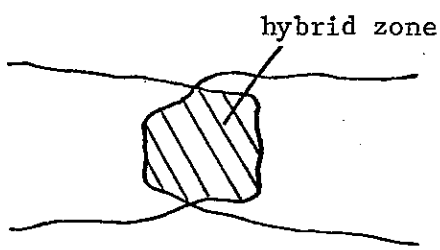


Fig 2

ENVIRONMENTALLY INDUCED
HYBRIDIZATION

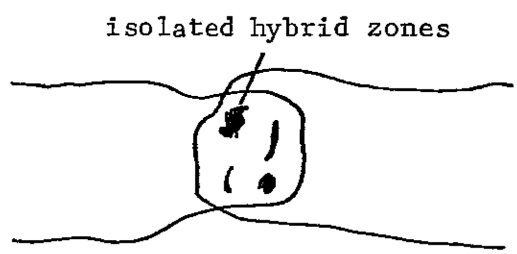


Fig 3

ALLOPATRIC HYBRIDIZATION

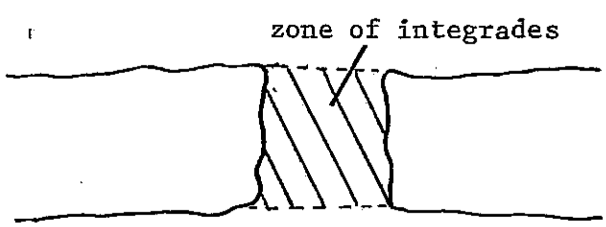
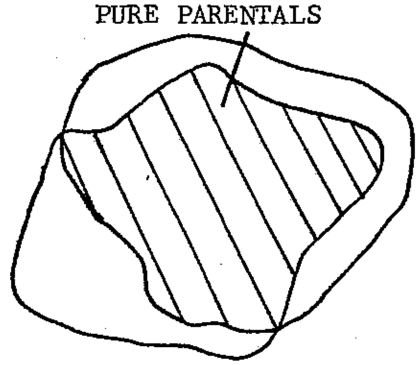


Fig 4

ZONE OF HYBRIDS, INTERGRADES AND
PURE PARENTALS



confinement associated with domestication.

Another type of hybridization that could explain the phenomenon is diagrammed in Figure 2. This localized hybridization is caused by habitat alteration and could account for unusually high numbers of hybrids in some areas of Lake Erie, but again this statement is not entirely adequate as discussed in a later section.

Furthermore, both diagrammatic zones of overlap (Figures 1 and 2) conventionally represent hybrid zones without introgression while the term allopatric hybridization is restricted to populations that are introgressing (Figure 3) (Woodruff, 1973). Allopatric hybridization is described when two populations are separated completely by a zone of introgressed individuals, but in Lake Erie the carp and goldfish are fully geographically sympatric and are believed to be introgressed.

Each of the three previous models of hybridization alone do not describe the phenomenon of hybridization in Lake Erie. A combination of all three more nearly represents an adequate model of the relationship between the carp and goldfish in Lake Erie. The model in Figure 4 represents the hypothetical situation that exists in Lake Erie. It is a proposed model and will be tested in subsequent sections.

Summary of Genetic Forms

1. Cyprinus carpio carpio--common European wild carp, German carp or Danube carp

2. Cyprinus carpio heamatopterus--East Asian wild carp

3. Carassius a. auratus--Chinese ornamental goldfish

4. Carassius auratus gibelio--Prussian carp

5. Carassius carassius--crucian carp

6. *Cyprinus carpio*--domesticated varieties, mirror, leather, and scale carp. (Often remotely hybridized with *C. carassius* and, or, *C. auratus*)

The original introduction of North American carp (*Cyprinus carpio*) consisted of domesticated European varieties of *Cyprinus carpio carpio* first stocked by the U.S. Fish Commission in the 1870's. Evidence suggests that North American goldfish are represented by both subspecies, *Carassius a. gibelio* and *Carassius a. auratus*. This discussion will refer to all North American forms of *Cyprinus* as carp and all North American forms of *Carassius* as goldfish.

B. The Introduction and Dissemination of Carp and Goldfish into Lake Erie

The first introductions of carp into the Great Lakes were said to coincide with the Fish Commission's first distribution of carp in November of 1879 (Cole, 1905). Smiley (1886) reported the first catch of carp in Lake Erie occurred December 10, 1883, close to the mouth of the Raisin River, near Monroe, Michigan. Any one of the numerous recipients of 1880 located in the Lake Erie drainage of Pennsylvania, Ohio, Michigan, and New York listed by Smiley (1884b) could have been responsible for the first introduction. Regardless, all fish distributed were derived from the original stock obtained from Europe first bred by the U.S. Fish Commission in Washington ponds in 1878.

One of the first lots sent from Washington in 1879 went to Fremont, Ohio on the Sandusky River. Another group of fish were released directly into the Maumee River and Ten Mile Creek by the Ohio Fish Commission, another to Kellys Island and to Catawba Peninsula, all in 1881. Fish Commission introductions in Ohio continued on a large scale until 1887. At this time private concerns began propagating fish for distribution. Many of the fish intended for pond culture escaped into the river systems and eventually into Lake Erie. Cole (1905) stated that no other body of water in the U.S. appeared to be so well stocked with carp as Lake Erie and that Sandusky Bay was one of the most intensely populated areas of the lake. The shallow marshy habitat of western Lake Erie initiated a reproductive response by the carp that was likened to the spread of the English sparrow. The shallow basin coupled with extensive marshes of the Sandusky and Portage Rivers provided conditions ideal for the feeding and spawning of carp. Today the carp is one of the most visible fish in these regions.

There are no early introductions of goldfish directly into Lake Erie, however McDonald (1887) reported that 11 applicants in Ohio received Federal fish in 1885-86. Goldfish were probably first introduced in the Hudson River in 1831, (DeKay, 1842) and may have spread into Lake Erie before the activities of the Fish Commission in the 1870's (Trautman, 1957). Goldfish were used for ornamental purposes and undoubtedly reached Lake Erie at the time of the carp introductions or shortly after.

Natural hybridization between the carp and goldfish in Lake Erie was not documented until Trautman (1957) stated that large populations existed in several areas of western Lake Erie. Museum specimens housed at The Ohio State University Museum of Zoology are known from the 1930's.

The earliest areas of carp introduction, the Maumee River and Sandusky Bay coincide with the areas where large numbers (30-90%) of hybrids have been reported, while other waters in Ohio generally produce less than 10% hybrids. This may suggest that hybridization in these areas is related to the genetic makeup of fish that the U.S. Fish Commission distributed or possibly that the lake is a disturbed habitat. North American carp and especially Lake Erie carp may have genetically differentiated into a form that more easily hybridizes than its progenitors. The nature and origin of the original U.S. Commission fish first stocked in 1879 is discussed in the following section.

C. Introgression of the History of the Dissemination of Carp and Goldfish in North America

The dissemination of the carp and goldfish is one of the best documented and deliberate actions of exotic species introduction that has occurred in North America. It is also one of the most perplexing. This condition arises from extensive efforts beginning in 1831 and continued in 1877 by the United States Fish Commission to distribute a newly obtained food fish from Europe for pond culture. This fish previously believed to be a pure line of German carp, *Cyprinus c. carpio*, may have been a hybrid form. Historic evidence in part suggests a hybrid form but no one to my knowledge has ever posed the question that the common carp as we know it today may have risen from hybrid progeny in the 1800's. A review of the original literature on the introduction of the carp supports the belief that the fish in question may not have been pure forms. If this contention is true the relative ease of hybridization between the carp and goldfish today in Lake Erie as discussed in this thesis can be explained.

The first reported introduction of carp-like fish into North America occurred in 1831 when Henry Robinson had six or seven dozen carp and goldfish delivered from France and deposited in his private ponds in Orange County, New York, adjacent to the Hudson River (DeKay, 1842). Within three years carp and goldfish escaped into the Hudson River when a freshlet broke the dam of one of his holding ponds. Robinson added additional fish to the Hudson each year establishing a reproducing population that was protected by the New York state legislature for a period of five years. During this time their numbers increased substantially and were taken frequently by fishermen after the protective law expired.

The story of Henry Robinson is generally accepted as the first introduction of carp into North America and was substantiated by several others (Cole, 1905), but there is still a question as to whether the original "carp" were indeed pure Cyprinus carpio. Baird (1879a) stated that it was doubtless that Captain Robinson brought over fish but there is no certainty that they were genuine carp. Baird was inclined to think that they were the Prussian (or crucian carp) of much inferior value. By the 1860's a form of Hudson River carp was commonly peddled in the streets of New York, but Baird (1879a) reported that he had not found a single fish among those sold as carp in New York which was any other than the common goldfish reverted to its original condition. He also found that the Hudson contained specimens with red and white coloration, a positive indication that fish other than carp were present. Baird added that he could not say whether genuine carp were transferred to the Hudson, but believed that the Prussian carp may have been the one introduced or possibly the hybrid progeny of this and the true carp may have gradually mixed with goldfish.

DeKay (1842), a naturalist, provided a description of a specimen from New York, presumably from the Hudson, that had two pairs of barbels at the corner of the mouth with a nape and back rising suddenly. My research shows that both carp and hybrids can have four barbels although the hybrid's are reduced in size; normally only the hybrid has an abruptly rising nape affirming Baird's belief that the fish were of hybrid origin. Goldfish and the crucian carp lack barbels. DeKay also stated that his fish had reached a length of no greater than 11 inches, much smaller than a pure carp would be expected to grow. From these observations, Robinson's, Dekay's, and Baird's, I must conclude that at least two and possibly three species of fish, *Carassius auratus*, *Cyprinus carpio*, *Carassius carassius*, or a hybrid combination of all three were introduced. So at least two different species of closely related fish were stocked in the Hudson prior to 1850. The common carp suggested by DeKay (1842) because of presence of barbels and the occurrence of the goldfish or the crucian carp in the Hudson by Baird (1879a) as suggested by their coloration. Even if pure forms were introduced hybridization was known to take place freely in European ponds (Baird, 1876) and the entire Hudson River's subsequent population of carp-like fish could have risen from hybridization of Henry Robinson's initial stock in 1831. Although Cole (1905) stated that we shall probably never know whether the fish brought over by Captain Robinson were true carp or hybrids, this thesis may partially resolve that long standing question.

The next reported introduction of carp into American waters occurred in 1872 when J.A. Poppe of Sonoma, California procured 83 fish from Hamburg, Germany (Poppe, 1880). These were shipped to New York, then to San Francisco with only five of the smallest fish surviving to be planted in Poppe's ponds near Sonoma. Within one year the fish had increased to 16 inches and produced an estimated 3000 young. The young were sold to farmers throughout California and adjacent states and some were distributed as far as Central America.

The purity of this line of carp was doubted also. Baird (1879a) concluded, upon examining some specimens sent to him by Poppe, that they were the scaled carp somewhat hybridized. It seems not unusual that early importations contained many hybrids considering a statement made by Baird (1879a) who described the greater part of fish sold in France and England to be hybrids of carp and goldfish or crucian carp.

It is debatable whether the 1831 and 1872 introductions had any substantial influence on the massive introductions undertaken by the U.S. Fish Commission shortly after its formation in 1871. The director of the commission, Baird (1876) stated that it would be safe to say that there was no other species that promised so great a return in limited waters as the carp. Baird (1874) felt that the carp had pre-eminent advantage over such fish as the black bass, trout, and grayling because it enumerated these good qualities:

1. Fecundity and adaptability to the processes of artificial propagation
2. Living largely on a vegetable diet
3. Hardiness in all stages of growth
4. Adaptability to conditions unfavorable to any equally palatable American fish and to varied climates
5. Rapid growth
6. Harmlessness in its relation to other fishes
7. Ability to populate waters to their greatest extent
8. Good table qualities

Baird (1879b) believed that within ten years this fish would be known throughout the country and esteemed in proportion. Baird's opinion was shared by others. Hessel (1878), and Henry (1877) felt that the carp would be of great economic value and would be a promising addition to the food resources of the nation. By 1879 the demand for carp was great and applications were being received from nearly every state in the Union, causing Baird (1883) to remark that the greatest success of the U.S. Fish Commission had been with the carp, for which the demand continued to a degree that was impossible to meet. Baird (1882) reported that it was safe to say that at least 15,000 ponds had been constructed in the U.S. specially for the purpose of receiving carp bred by the Commission. The expectations of the fish were so promising that the carp was considered to rank among the domesticated animals and to be as capable of profitable cultivation as poultry or pigs (Baird, 1882).

The Commission's expectations were not totally unfounded and in fact were supported by the successful cultivation of carp in Europe for several centuries (Baird, 1879a; Hessel, 1878; Balon, 1974). Most early American culturists shared the enthusiastic opinion of the Fishery Commission and intensive distributions continued until 1890 when public disapproval caused the Commission to cancel all further distribution. Even at this early date, when the U.S. Fish Commission stocking has ceased, the carp had already become so widely distributed that it was common in the flowing waters of nearly every state in the Union (Cole, 1905).

(1) Introgression and its Relationship with Cultivation

Few theories have been proposed to explain why a fish so highly esteemed as this food fish of Europe would be so universally distained in the U.S. by 1900. Environmental conditions and food habits have been the focus of most biologists and only recently Balon (1974) proposed a theory that compared the demise of the carp in America to that of the degenerate condition of domestic cattle under wild conditions.

Balon believed that the domestic carp gradually changes into a scaled, enlongate morph similar to the wild carp. This morph is easily recognized by a notch and hump posterior to the head, whereas in native wild carp, the transition between the top of the head and the back (nape) is slightly if at all marked. Balon described all differences between the ancestral wild carp and today's American carp to be a result of domestication*, but does not consider the possibility that hybridization could lessen the value of American carp as a food fish. The nape area description by Balon, slightly exaggerated, would describe that character of a naturally produced carp-goldfish hybrid. Through introgression, this character would be reduced to the less pronounced but sill noticeable rising nape described by Balon. I would further contest Balon's theory because he states the carp in North America have a limited ability to utilize natural food and poor growth. The American carp probably have a greater ability to utilize food resources than any other native fish. Its growth and reproductive potential is unfortunately attested to by hundreds of American biologists who vehemently described its proliferation and spent millions of dollars in unsuccessful efforts to exterminate it. Natural hybridiza-

*Balon believes that the wild carp of the Danube are the ancestors of all carp today, but he feels that the true wild carp is related to the domestic pond carp in a way similar to that of the ancestral auroch is to cattle. He believes that the inferiority of American carp is similar to that of domestic animals that are released into the wild and become feral.

tion between carp and goldfish is undoubtedly related to its domestication by man. Several approaches to explanation of this phenomenon are possible, but only with a detailed account of hybridization induced by man.

I feel that these theories do not fully explain the inferiority of the carp today. I maintain that the degeneration of pure European carp into its present day form in the United States is a result of genetic changes caused by introgression.

Early reviews by Cole (1905) and more recently McCrimmon (1968) stated that the original lot of fish obtained by Hessel from Germany consisted of 324 fish, 227 naked and mirror carp, and 118 common scale carp; but they all failed to mention that Hessel fully intended to import not only carp but also Carassius auratus, Carassius carassius, and Tinca tinca (Finsch, 1882). Baird, (1880) stated that of the fish Hessel obtained in 1877 (a total of 10 mirror carp, 90 leather carp, 80 scale carp, 40 golden orfe (Leuciscus idus), 50 king tench (Tinca tinca), 20 common tench, and 2 golden tench) were taken from the Druid Hill Pond, Maryland, and distributed to ponds in Washington, D.C. In addition, goldfish entered the pond at Druid Hill and hybridized with the carp present. The resulting hybrids were deemed worthless and reportedly destroyed. The origin of these goldfish is unknown but Smith (1924) stated that Rear Admiral Daniel Ammen U.S.N. presented Chinese goldfish to the United States Fish Commission in 1878 where they were bred extensively in Washington ponds. Another source of the goldfish could have been from those introduced by DeKay in the Hudson as reported by Baird (1879a). Goldfish were not a food fish but were commonly used as ornamentals in ponds.

Hessel (1878) warned that great care be taken that no goldfish get into any culture pond for these fish would soon mix with carp and tend to degenerate the breed. They were unfit for table use, did not grow larger than the goldfish and had as many bones. He emphasized that any hybrids be killed immediately because they could backcross and the breed would degenerate more.

Hessel warned against experimental cross breeding with any carp procured through the U.S. Fishery Commission. There are no reports of intentional crossing by the Fish Commission, but hybrids were produced (Smiley, 1884b). Hybrid progeny were reportedly destroyed but the ability of the early American culturists to distinguish between young hybrids and pure forms is doubted. To illustrate this Hessel (1878) said that the most common of all hybrids produced in Europe, Cyprinus carpio x Carassius carassius, called Carpio kollarii, resembled Cyprinus carpio so closely that it could be distinguished only with difficulty from pure carp. Hessel performed the following crosses to further resolve the problem of hybridization.

"In order to determine this question, I myself managed to bring about such crosses by placing (1) female common carp with male crucian carp, and (2) female crucian carp with male common carp, in small tanks, constructed with this end in view; (3) I also put together female Carpio kollarii with male common carp; this for the sole purpose of testing the capability of propagation of the C. kollarii, which had been doubted. In the two former cases I obtained forms analogous to the Carpio kollarii sometimes approaching in appearance the true carp, at others the crucian crop. In the third case, however, having placed ripe Carpio kollarii together with Cyprinus carpio, I obtained a product with difficulty to be distinguished from the genuine carp. I took the trouble to feed them for three years, in order to try their fitness for the table, but their flesh was exceedingly poor and very bony and could not be compared by any means to that of the common carp."

Hessel established that Cyprinus kollarii was actually a hybrid that could backcross with Cyprinus carpio and produce fish externally indistinguishable from carp. Hessel added that he expected that hybrids were carelessly cultivated for three to ten generations and unknowingly sold as carp in Europe. Many of the original fish procured by the Fish Commission for propagation and distribution could have been of this type.

One of the first attempts of the Fish Commission to secure carp from Germany did result in an 1875 shipment of Cyprinus carpio x Cyprinus carassius hybrids because pure carp in Europe were so rare they were unobtainable (Finsch, 1882). These fish must have died in shipment since there was no record of them reaching the United States alive.

Early distributions of goldfish further enhanced chances for hybridization. The Fish Commission has no early records of goldfish distribution, but from Smiley (1884a) there is evidence that carp and goldfish were both distributed under the name of "carp" to anyone applying for fish. One recipient of Commission "carp" in 1880 was successfully rearing and selling carp and three varieties of goldfish within three years. With the Commission distributing carp for propagation and the goldfish being raised as ornamentals, hybridization would be inevitable. The eventual impact was enormous because from the initial brood of Commission fish stemmed the massive distribution of fry to over 2700 persons in 25 states and territories within a 2 year period.

These arguments do not conclusively prove that the original introduction of fish by the Commission was not pure carp but lends considerable credence to the theory that some type of hybrid was imported to or produced in those first ponds by Washington, D.C.

Although these hybrid fish were morphologically similar, their palatability distinguished them as being inferior as seen from Hessel (1878). At this time in Europe when carp were highly esteemed as a food fish, the idea that some carp were inferior first arose (Baird, 1879a). He maintained that some European carp were of superior value, those of inferior status arising from hybrid origin. The pure carp of Germany were indeed fine fish as testimony from Baird (1874) proved when he described the pure carp's table qualities equal to that of the European trout. Early accounts from fish culturists who received carp fry from the U.S. Fish Commission vary widely in their opinion over the table qualities of their fish, possibly indicating that some received inferior hybrid fish. Smiley (1883) reported the results of 242 questionnaires sent to 23 states in 1883. A total of 175 complimented carp with praise ranging from moderate to emphatic expressions of approval, while only 29 disapproved of overall table qualities. The majority of fish culturists conveyed opinions such as these: "Equal to any fish, nicest fish ever tasted, finest fish in the country, never ate anything more delicious, better than sunfish, equal to any native fishes." Indeed the carp was accepted at this time as one of the finest food fishes available except for a few peculiar isolated cases like this one from Smiley (1883):

"Tough and unfit to eat -- Our country is getting pretty thoroughly stocked with German carp, and there is hardly a paper in the land but teems with praises of their wonderful growth, loveliness, adaptability to the shallow ponds, etc., all of which we most unqualifiedly indorse;

but how seldom do we hear one word as to their eating qualities. Although our esteemed senator from this district, who introduced them here three years ago, tells us they sell "side by side" with the best fish in the Washington, D.C. market, still that does not keep other Kentuckians, at least, from having their tastes. From fifteen to twenty families around here have tried them at different times during the past year, and except two persons who could eat them but didn't relish them, they unhesitatingly pronounced them unfit to eat. They have tried them boiled, baked, and fried, and discards them every way. One lady says, they are well named leather carp, for we would prefer leather served in any style to them; and, right here, might not our fish commissioners have mistaken their use, and ought we not to send a few to the tannery? Another person says he intends to keep praising them, for he knows they will prove valuable for soap grease. Such are some of their indorsements here, and we would like for others throughout the country not to think so much of them as we did, having them nearly three years before trying them, but to try them as soon as possible, and report the results."

Such discrepancies in opinion cannot be easily resolved if all parties concerned were dealing with the same item of interest, the common carp; however if two different but closely related forms were concerned a plausible explanation involving hybridization can be formulated.

Earlier I stated that hybridization in the government holding ponds occurred accidentally and that Hessel obtained crosses of the common carp and Carassius carassius. These produced an inferior food fish only distinguishable from the common carp with difficulty. Hessel stated that these fish were exceedingly poor and could not be compared by any means to that of the common carp. If some of these inferior hybrid fish were distributed along with a larger number of common carp by the Fish Commission for pond propagation, a favorable explanation now appears for the few isolated adverse reports of the carp's table qualities. Gradual introgression between these inferior hybrid fish and the common carp is suggested to be responsible for the decreased food value and skepticism against the carp beginning in the late 1880's and continuing today. Doubtless, other factors are involved but a massive genetic change must have occurred during those early days.

(2) Summary

1. The first reported introductions of carp-like fish into North America occurred in 1842, 1872, and 1876.
2. Evidence suggests that these introductions in part, contained introgressed fish of the type *Cyprinus carpio* x *Carassius carassius* or *Cyprinus carpio* x *Carassius auratus*.
3. There is further evidence that hybridization took place in government holding ponds in the original stock of nearly 400 fish procured by the U.S. Fish Commission from Europe.
4. From this original stock stemmed the massive distributions to 25 states and territories all within a 2 year period.
5. Hybrids accidentally produced in the Fish Commission's ponds were reportedly destroyed, but backcrosses of the carp x crucian carp were said to be nearly indistinguishable from carp.
6. Some of the earliest areas of distribution of Commission fish to Ohio coincide with areas where large proportions of hybrids are reported today.
7. Hybrids of the carp x crucian carp and backcrosses were known to provide an inferior food fish that was at least externally nearly indistinguishable from the carp.
8. Wide discrepancies in the public acceptance of the carp as a food fish could be easily explained if two types of fish were involved (1) the common carp known to be an excellent food fish and (2) a hybrid with inferior table qualities.

D. Study Area Selection

Western Lake Erie was selected as the focal point for this study because massive hybridization was first reported in this area. Hybrids have more recently been reported in several of Ohio's larger streams but never in great numbers (Trautman, 1957). Museum specimens from 1939 to present show that hybrids were found in the Ohio River, Maumee River, Muskingum River, East Branch Portage River, Auglaize River, and several smaller tributaries in the Lake Erie drainage of Ohio (Ohio State University Museum of Zoology). I have found hybrids in the Olentangy River in central Ohio but they never amounted to more than 3% of the goldfish-carp catch. Fish collections were concentrated in the Maumee Bay and Bass Islands area while additional collections were taken from other accessible areas. The table below lists specific localities, dates, methods, and the corresponding collection sites are plotted on the maps following the table (Figure 5).

Bass Islands Area

Site Number	Fishery Bay Area
2	15 April, 1975, Oak Point, trapnets
1	20 June, 1975, Alligator Bar on west shore of Gibralter Island, gill nets variable mesh
3	30 June, 1975, Marsh adjacent to Perry's Monument, 8 ft. seine
2	1 July, 1975, Alligator Bar on west shore of Gibralter Island, gill nets variable mesh
4	10 July, 1975, Terwilligers Pond, 8 ft. seine

North Bass Area

Site Number	
5	21 June, 1975, Marsh along southeast point of North Bass, 30 ft. bag seine
6	6 July, 1975, Manila Bay along west shore of North Bass, 30 ft. bag seine

Rattlesnake Island Area

7	7 July, 1975, between South Bass and Rattlesnake Island, 30 ft. trawl

Maumee Bay Area

8	30 October, 1975, 15 November, 1975. Several reefs in Maumee Bay to several miles upstream into the Maumee River, variable mesh gill nets

Other Locations

9	18 July, 1975, Carp pond on Kelleys Island near State Park, 30 foot bag seine
10	31 October, 1975, several areas in East Harbor State Park, 30 foot bag seine

At the following localities carp, goldfish, and crosses were taken for experimental crossing and observations of spawning behavior were made.

12	(1) Wightmans Grove and three miles east in South Creek, Riley Twp., Sandusky Co., Ohio
11	(2) Little Portage River Wildlife Area, Salem Twp., Ottawa Co., Ohio
14	(3) Marshes surrounding the mouth of Touissant Creek, Carroll Twp., Ottawa Co., Ohio
15	(4) Overflow ditch adjacent to Rte. 2 at roadside rest between Crane Creek State Park and Locust Point, Ohio
13	(5) Magee Marsh in Crane Creek State Park, Carroll Twp., Ottawa Co., Ohio

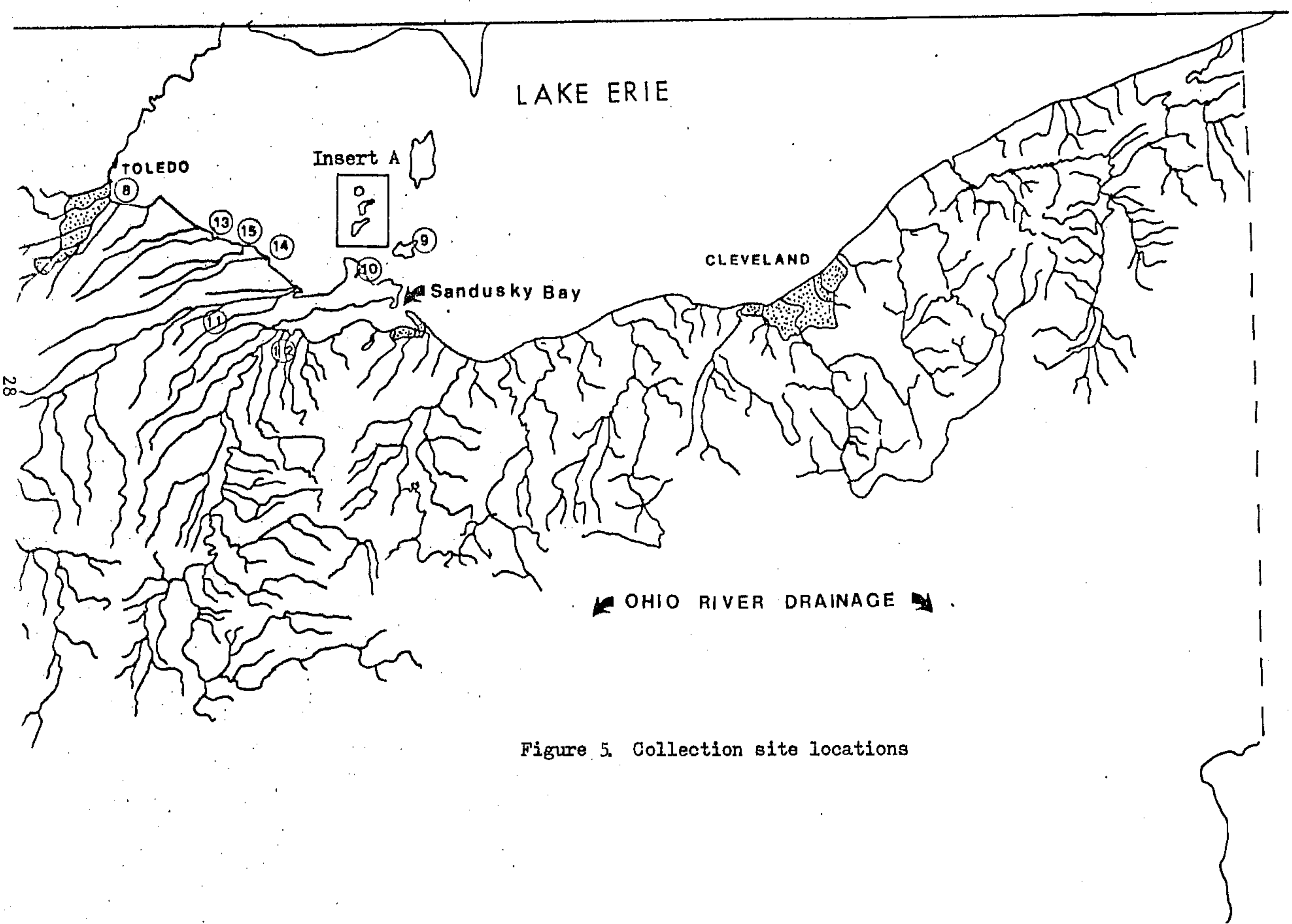
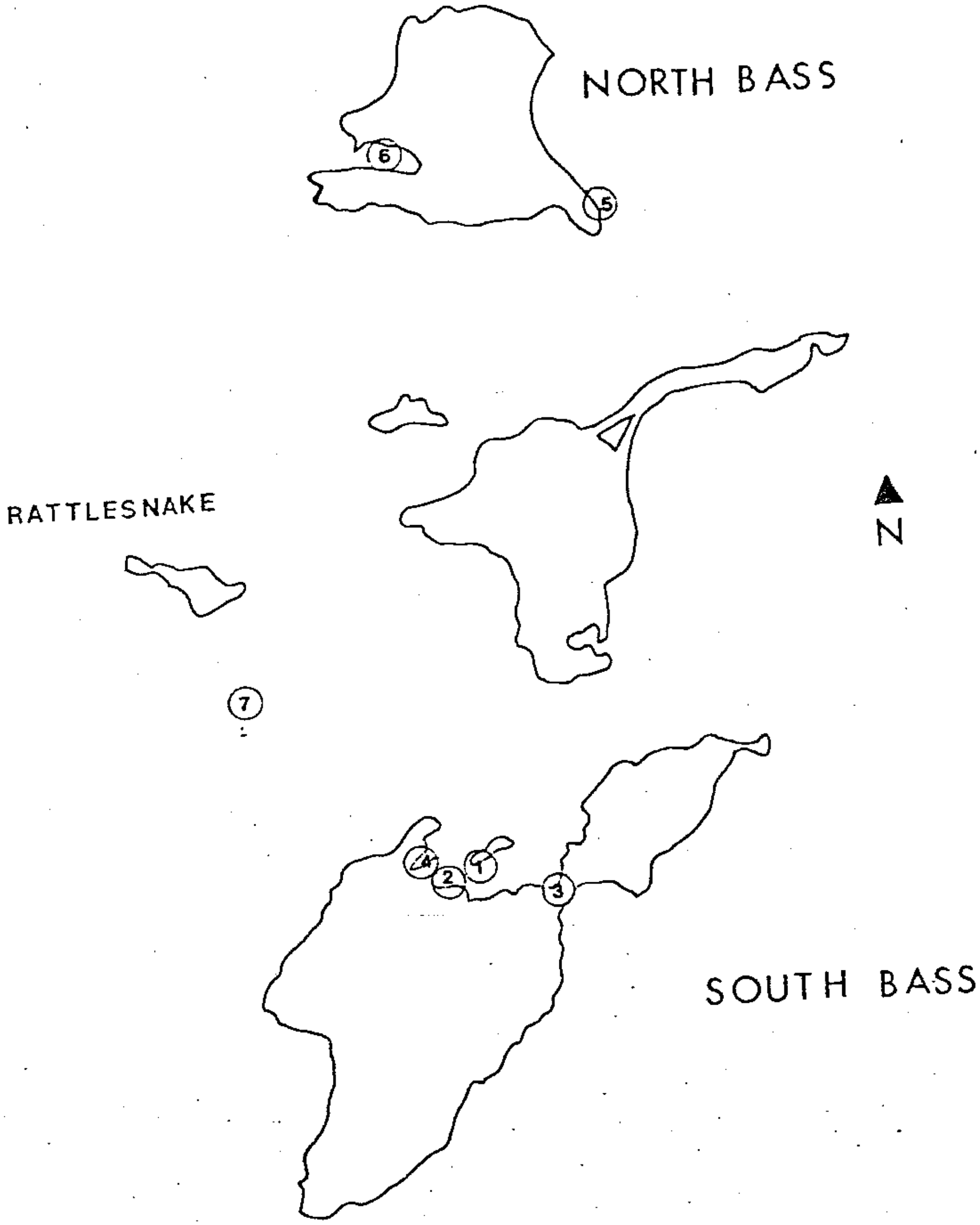


Figure 5. Collection site locations



Insert A

Figure 5.

PART II: A MORPHOLOGICAL ANALYSIS OF INTROGRESSION

A. Methods and Justification

The identification of hybrid specimens is largely dependent upon meristic characters. Other characteristics such as karyology, muscle proteins and behavior supplement identification. Hubbs (1955) stated that F_1 hybrids are generally intermediate between parentals while more recently several instances of non-intermediacy of inheritance have been reported. Greenfield et al. (1972) reported that F_1 hybrids between *Gila orcutti* and *Hesperoleucus symmetricus* exhibited a mosaic of both *Gila* and *Hesperoleucus* characters in addition to characters which were strictly intermediate. Berry and Low (1970) found that hybrids of the Chinese carp *Ctenopharyngodon idella* and *Aristichthys nobilis* were intermediate in most morphological and anatomical features although some features were more similar to one parent. Measurements that exceed either of the parentals are often attributed to complimentary gene action (Hocutt and Hambrick, 1973). This points toward a possible genetic dominance of particular morphological species-specific expressions. Ojima (1973) showed that the inheritance of characters in laboratory produced hybrids of *Cyprinus carpio* and *Carassius auratus* varied with reciprocal crosses with most characters intermediate. Makeyeva and Vergin (1974) stated that hybrid characters of *Cyprinus carpio* and *Ctenopharyngodon idella* deviated slightly toward the maternal parent. All these instances of non-intermediacy or slight deviation toward one of the parents involve only one or two characters. Intermediacy is the rule rather than the exception. More typically, measurements of F_1 hybrids will appear nearly intermediate and clustered between parental measurements (Nelson, 1966; Smith, 1973; Keenleyside et al., 1973).

If measurements of F_1 hybrids are intermediate and tightly clustered between the parentals then introgression would reveal itself by the dispersion of the cluster and a blending with the parentals. Measurements of two species with suspected introgression would not be separable into clusters and would appear to be one homogeneous population. Testing the hypothesis that the carp, goldfish, and suspected introgressed hybrids are represented by one homogeneous population involves the selection and measurement of a number of parameters. The sequence to be followed in answering this question is presented below:

1. Materials examined
2. Selection of differentiating characteristics
3. Multivariate analysis of selected characteristics as a numerical taxonomic technique
4. Conventional analysis by hybrid indices
5. Comparison of Lake Erie crosses with known F_1 hybrids
6. Descriptive analysis of selected morphological characteristics

(1) Material Examined

Samples for the following morphological and descriptive analyses were collected from two general areas: the Bass Islands area, Lake Erie collection sites 1-7, 9, 10 (n=58); the Maumee River and Bay area, Lake Erie collection site 8 (n=46). Representative specimens were preserved in formalin and skeletal preparations were made and cataloged into The Ohio State University Museum of Zoology. Catalog numbers O.S.U.M.

36878-36888. In addition comparative material from the Olentangy River was used in the descriptive analysis.

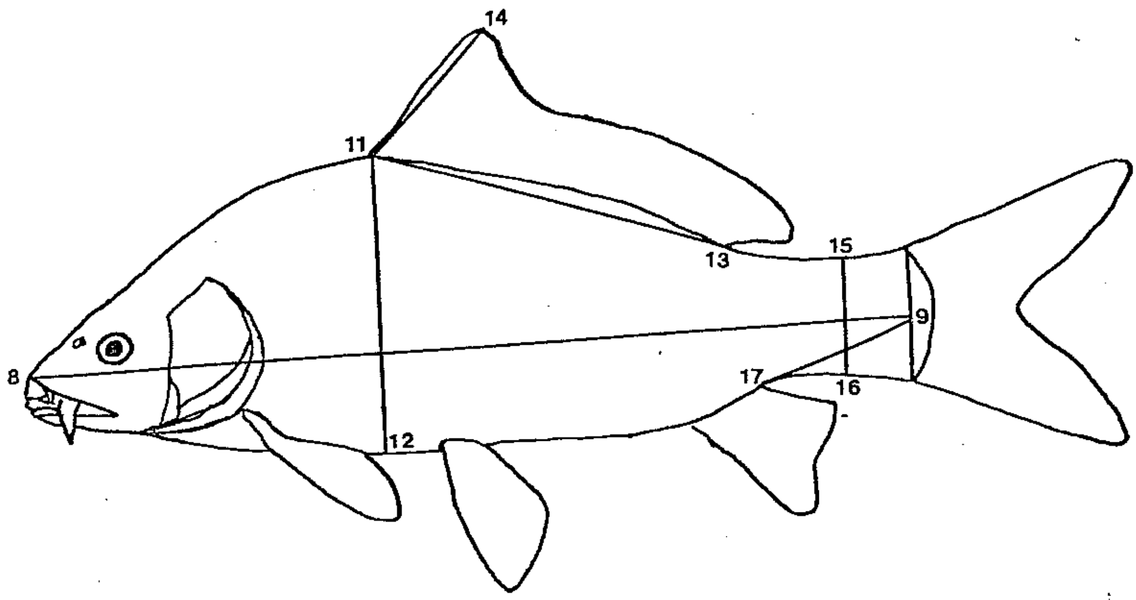
(2) Selection of Differentiating Characteristics

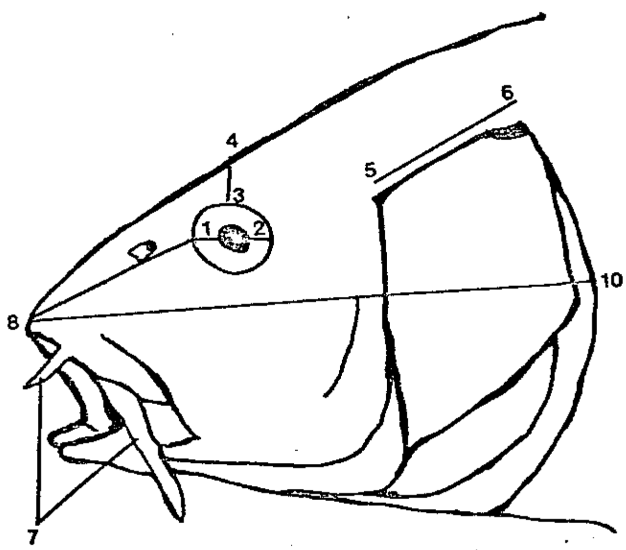
Twenty one characters were chosen for their discriminating ability and measurements or counts were made on a sample of 109 fish, 47 carp, 33 goldfish, and 29 suspected crosses. Fish possessing intermediate characters were tentatively identified as hybrid forms. The method of measurement of characters 1-13 are listed and diagrammed in Figure 6. The description of the remaining characters (14-21) are considered individually. Character 14 is the orbit width. Characters 15 and 16 are the length and width of the lacrimal bone of the head. Character 17 is the presence or absence of pigmentation at the scale base. Characters 18 to 21 are counts of the vertebrae, pharyngeal teeth, number of gill rakers, and lateral line scales.

(3) Principal Component Analysis

Principal component analysis is a relatively new method used in numerical taxonomy. Its usefulness in analyzing hybrid populations of *Etheostoma nigrum* and *Etheostoma nigrum olmstedi* was shown by McAllister (1972) and its usefulness in detecting introgression between *Rhinichthys cataractae* and *R. osculus* was discussed by Smith (1973).

For this study I considered a sample of 109 carp, goldfish, and suspected hybrids with 15 parameters for each fish (Table 1). If the 109 fish represent two different species, a PCA will display 109 points in two separate clusters on a graph. If only F_1 hybrids and pure





1. Dorsal border opercle concave (5-6)
2. Barbels-number and length (7)
3. Standard length (8-9)
4. Head length (8-10)
5. Snout length (8-1)
6. Body depth (11-12)
7. Caudal peduncle length (17-9)
8. Caudal peduncle depth (15-16)
9. Longest dorsal ray length (11-14)
10. Length dorsal fin base (11-13)
11. Dorsal width opercle (5-6)
12. Eye diameter (1-2)
13. Interorbit width (3-4)
14. Orbit width
15. Lacrimal length
16. Lacrimal width
17. Pigmentation of scales
18. Number of vertebrae
19. Pharyngeal teeth formula
20. Number gill rakers-first arch
21. Lateral line scales

Figure 6. Measurements made on a sample of 109 carp, goldfish, and suspected crosses. (Method of measurement in parenthesis)

1. DS=dark spots on scale base, presence of absence

2. DBC=dorsal border of opercle concave, yes, no, or intermediate condition

3. LL=number of lateral line scales

4. B=number of barbels

5. A=head length/total barbel length

6. C=standard length/head length

7. D=head length/snout length

8. E=standard length/body depth

9. F=caudal peduncle length/caudal peduncle depth

10. G=standard length/longest dorsal ray

11. H=standard length/length dorsal base

12. I=head length/dorsal width opercle

13. J=head length/eye diameter

14. K=head length/interorbit width

15. L=lacrimal length/lacrimal width

Table 1. Fifteen parameters scaled for use in the principal component analysis.

parentals are present then a third distinct cluster would appear intermediate between the other two clusters. If the population is introgressed then a homogeneous cluster of 109 points would appear indicating extensive backcrossing.

The PCA derives the points of the 2 dimensional graph from a representation of 109 points by a 15 dimensional character space that uses a 15 x 109 matrix table. A line is generated which is positioned to minimize the squared distances of all 109 points and is called the first principal component. A second line is generated orthogonal to the first, and an additional line can be generated, but little additional information is derived. This technique has the distinct advantage of allowing several different characters to be used simultaneously in the identification of a hybrid specimen. Morphometric measurements, counts, pigment characters and osteological features can all be given equal weight by this method.

(4) Conventional Analysis by Hybrid Indices

The conventional method of hybrid analysis most often involves the use of hybrid indices. Numerous authors have utilized these simple indices in describing the intermediate inheritance of hybrid characters. Several of these have been shown to be as efficient as more sophisticated discriminating procedures (Goodman, 1967). Most of the indices are similar with the index of Hubbs and Kuronuma (1942) being the most commonly used.

The Hubbs index gives the hybrid suspect an index value of:

$$Vh = \frac{1}{M} \sum_{i=1}^{M} (Xi-W1i/W2i-W1i)$$

Where Xi is the value of the hybrid character i and W1i and W2i are the means for character i in the parental population and M is the number of characters selected. The value of the hybrid index Vh is expected to approximate .5 for F_1 hybrid specimens when W1i and W2i are scaled to 0 and 1. This index is somewhat biased since characters selected for use must be known to be intermediate apriori, otherwise aberrant hybrid index values will result.

The Hubbs index was used in this analysis with calculations made by IBM 360 computer. The characters were similar to those listed in Figure 6 but were scaled differently for use in the Hubbs index.

(5) Comparison of Lake Erie Crosses With Known F_1 Hybrids

Statistical comparison of Lake Erie crosses with known F_1 hybrids would provide conclusive evidence for introgression. However, direct comparison of crosses with known F_1 hybrids produced from potentially introgressing populations would be invalid, therefore, the use of known F_1 hybrids produced from Lake Erie parentals would also be invalid. Correct analysis would require a comparison of suspected Lake Erie crosses with known F_1 hybrids produced from naturally non-hybridizing sympatric populations of carp and goldfish. Such populations are not believed to exist in North America. Only East Asian crosses would be acceptable for direct comparison assuming divergence of American and Asian fish is negligible. Ojima (1973) has produced such hybrids from Japan, artificially raised them to 6 months, and published a series of 26 morphological meristics. Out of Ojima's 26 meristics, 7 corresponded to those made earlier in this study and were selected for further extensive study. These were the lateral line scales, the gill rakers, and 5 scaled parameters, head length/snout length, standard length/body depth, caudal peduncle length/caudal peduncle depth, head length/eye diameter, and standard length/head length.

(6) Descriptive Analysis

Several characters that proved useful in the identification or in understanding the ecology of carp-goldfish crosses were selected for further study. The most useful are described and summarized for overall comparison of the three forms.

B. Results and Discussion

(1) Character Selection and Evaluation

The ability of each of the 15 parameters to discriminate between carp and goldfish (neglecting intermediates) is found in Tables 2A-O. The parameters in order of discriminating ability are A 100%, B 100%, DBC 100%, LL 100%, F 97%, DS 96%, D 95%, E 95%, L 91%, K 84%, I 75%, J 73%, C 69%, H 60%. Although four variables had perfect discriminating power between the carp and goldfish, A, B, DBC, and LL, none could completely separate the hybrid suspects from the parentals. Twenty-eight percent of the hybrid (A) values overlapped with either the carp or goldfish preventing complete separation from the parentals on that character alone. Twenty-nine percent of the (B) values of the hybrids overlapped, 19% of the DBC values, and 76% of the LL values overlapped. The remaining parameters listed in Table 1 had even less hybrid discriminating power and complete overlap existed in several parameters indicating that no one parameter would correctly separate a hybrid from either parent. This necessitated the use of a multivariate statistical technique that evaluates more than one or two parameters simultaneously. Principal component analysis (PCA) equally weights N parameters and is useful where more simple parametric techniques fail.

Tables 2A-O. Frequency distributions for the 15 parameters used in the principal component analysis. Hybrid suspects are included.

Table 2A Parameter DS

DS=dark spots on scale base, presence or absence

	Yes	No	
Goldfish	0	33	
Hybrid	6	23	96% separation
Carp	41	3	

Table 2B Parameter DBC

DBC=dorsal border of opercle concave, yes, no, or intermediate

	Yes	I	No	
Goldfish	0	0	33	
Hybrid	17	9	3	100% separation
Carp	44	0	0	

Table 2C Parameter LL

LL=number of lateral line scales

	27	28	29	30	31	32	33	34	35	36	37	38	39	
Goldfish	10	13	8	1	1									
Hybrid					1	6	16	4	1	1				
Carp							1		2	6	23	9	3	100% separation

Table 20
Parameter B

B=number of barbels

	0	1	2	3	4	
Goldfish	33					
Hybrid		1	18	2	8	100% separation
Carp					44	

Table 2E
Parameter A

A=head length/total barbel length

	0	2	8	16	24	32	40	48	56	64	
Goldfish	33										
Hybrid		2	10	7	6	2	1		1		100% separation
Carp		43	1								

Table 2F
Parameter C

C=standard length/head length

	3.21	3.32	3.43	3.54	3.65	3.76	3.87	3.98	4.12	
Goldfish	5	6	4	11	1	5		1		
Hybrid		1	3	5	8	7	3	2		69% separation
Carp	1	1	4	10	8	8	9	3		

Table 2G
Parameter D

D=head length/snout length

	2.4		2.6		2.8		3.0		3.2		3.4		3.6		3.8		4.0	
Goldfish								2		12		9		6		4*		
Hybrid				3		7		10		9								92% separation
Carp		2		13		17		8		2				2				

Table 2H
Parameter E

E=standard length/body depth

	1.88		2.09		2.30		2.51		2.72		2.93		3.14		3.35		3.59	
Goldfish		1		8		13		7		3		1						
Hybrid								4		15		8		1		1		95% separation
Carp								1		11		18		10		4		

Table 2I
Parameter F

F=caudal peduncle length/caudal peduncle depth

	.92		1.04		1.16		1.28		1.4		1.52		1.64		1.76		1.88	
Goldfish		5		20		8												
Hybrid				4		8		14		3								97% separation
Carp				1		1		9		15		14		2		2		

G=standard length/longest dorsal ray

		4.69		5.08		5.47		5.86		6.25		6.64		7.03		7.42		7.81	
Table 2J Parameter G	Goldfish		6		11		11		3		2								
	Hybrid		2		3		12		10		2								92% separation
	Carp						1		11		10		18		2		2		

H=standard length/length dorsal base

		2.17		2.31		2.45		2.59		2.73		2.87		3.01		3.15		3.29	
Table 2K Parameter H	Goldfish		2		7		13		8		2						1		
	Hybrid				9		10		6		2		1		1				60% separation
	Carp				60		14		12		10		1				1		

I=head length/dorsal width opercle

		3.02		3.22		3.42		3.62		3.82		4.02		4.22		4.44		4.62		4.82	
Table 2L Parameter I	Goldfish		1		3		3		60		13		7								
	Hybrid		1		5		6		6		7		2		2						75% separation
	Carp		1		21		8		8		4		2								

J=head length/eye diameter

Table 2M Parameter J		3.87	4.44	5.01	5.58	6.15	6.72	7.29	7.86	8.43	
	Goldfish		4	16	5	6	2				
	Hybrid	2	3	5	4	12	3				73% separation
	Carp		6	1	6	17	8	3	2		

K=head length/interorbit width

Table 2N Parameter K		2.13	2.28	2.43	2.58	2.73	2.88	3.03	3.18	3.33	
	Goldfish	4	11	9	6	3					
	Hybrid	2	8	10	5	1	1	1	1		84% separation
	Carp			3	13	13	8	6	1		

L=lacrimal length/lacrimal width

Table 2O Parameter L		.89	1.22	1.55	1.88	2.21	2.54	2.87	3.20	3.53	
	Goldfish	18	10	5							
	Hybrid	2	9	10	5	3					91% separation
	Carp		2	4	22	7	5	3	1		

(2) Principal Component Analysis

Characters for the 109 fish were measured and scaled as previously described and the fish were tentatively identified as carp, goldfish, or hybrid suspects. Calculations were made by IBM computer, prepackaged PCA programs by SPSS. Hybrid suspects were removed from the first PCA run and the carp and goldfish data were used to test the collective discriminating power of all 15 variables. Figure 7 shows two distinct clusters of points representing 2 species affirming the discriminating power of the 15 parameters chosen. The addition of known F_1 hybrids with several intermediate characteristics would be projected as a third distinct cluster into a median position between the two parental clusters. The second PCA run included the hybrid suspects and projected a nearly homogeneous elongate distribution unlike the pattern suspected if known F_1 hybrids were included (Figure 8). This supports a hypothesis of introgression rather than simple hybridization. The overlap of the carp and goldfish by the intermediates indicates that backcrossing is common in these populations.

This test provides supportive evidence for introgression but has several limitations. The appearance of two distinct clusters in the first PCA run indicates that the parentals are currently intact but it has little predictive value in assessing future changes. The parentals do not appear to be lost by introgression but changes may have occurred that could not be detected unless the Lake Erie population was compared with sympatric populations that did not hybridize. Inferences about the relative numbers of crossbreeds can not be made from Figures 7 or 8 because they do not represent the true proportions found in Lake Erie.

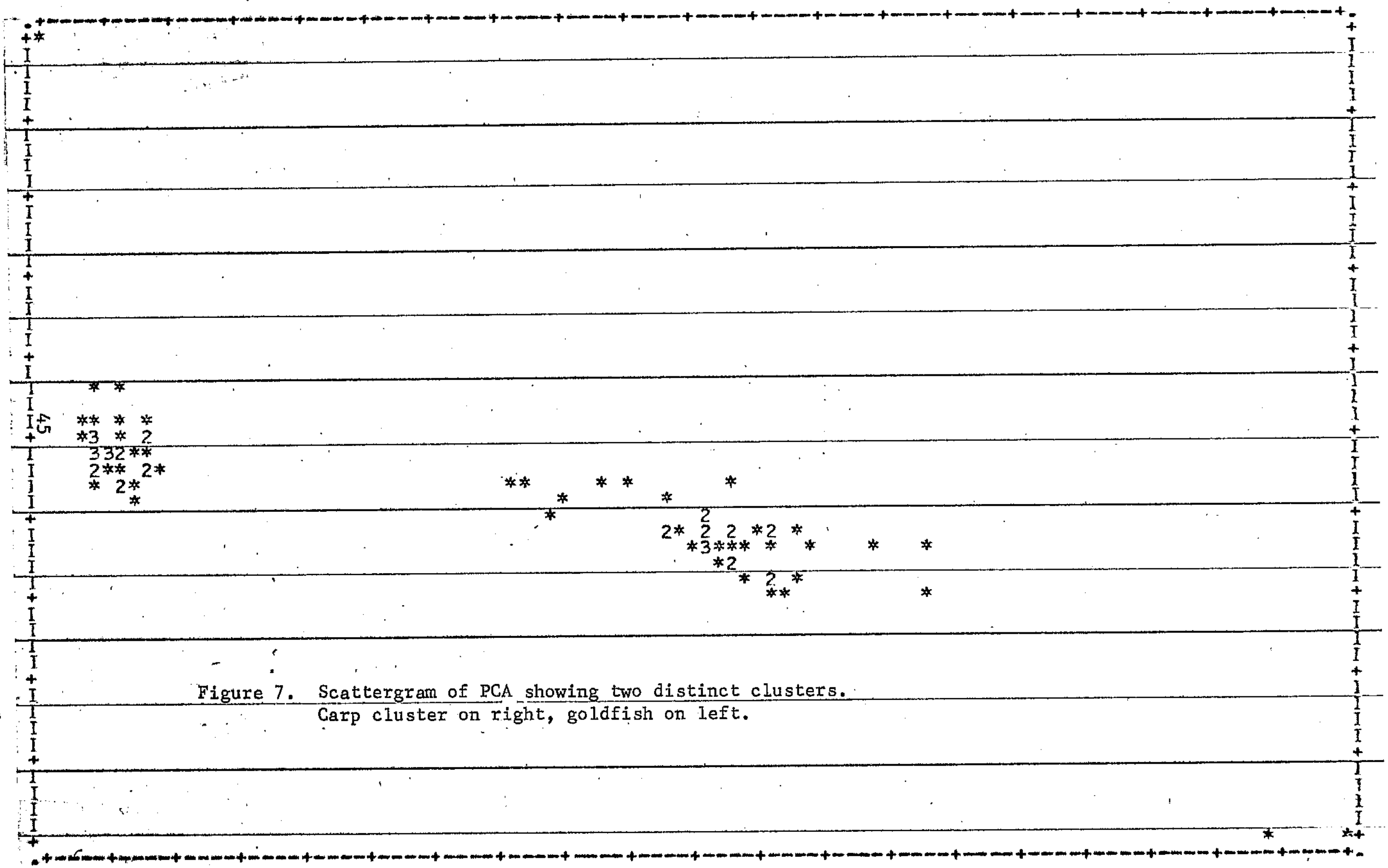


Figure 7. Scattergram of PCA showing two distinct clusters. Carp cluster on right, goldfish on left.

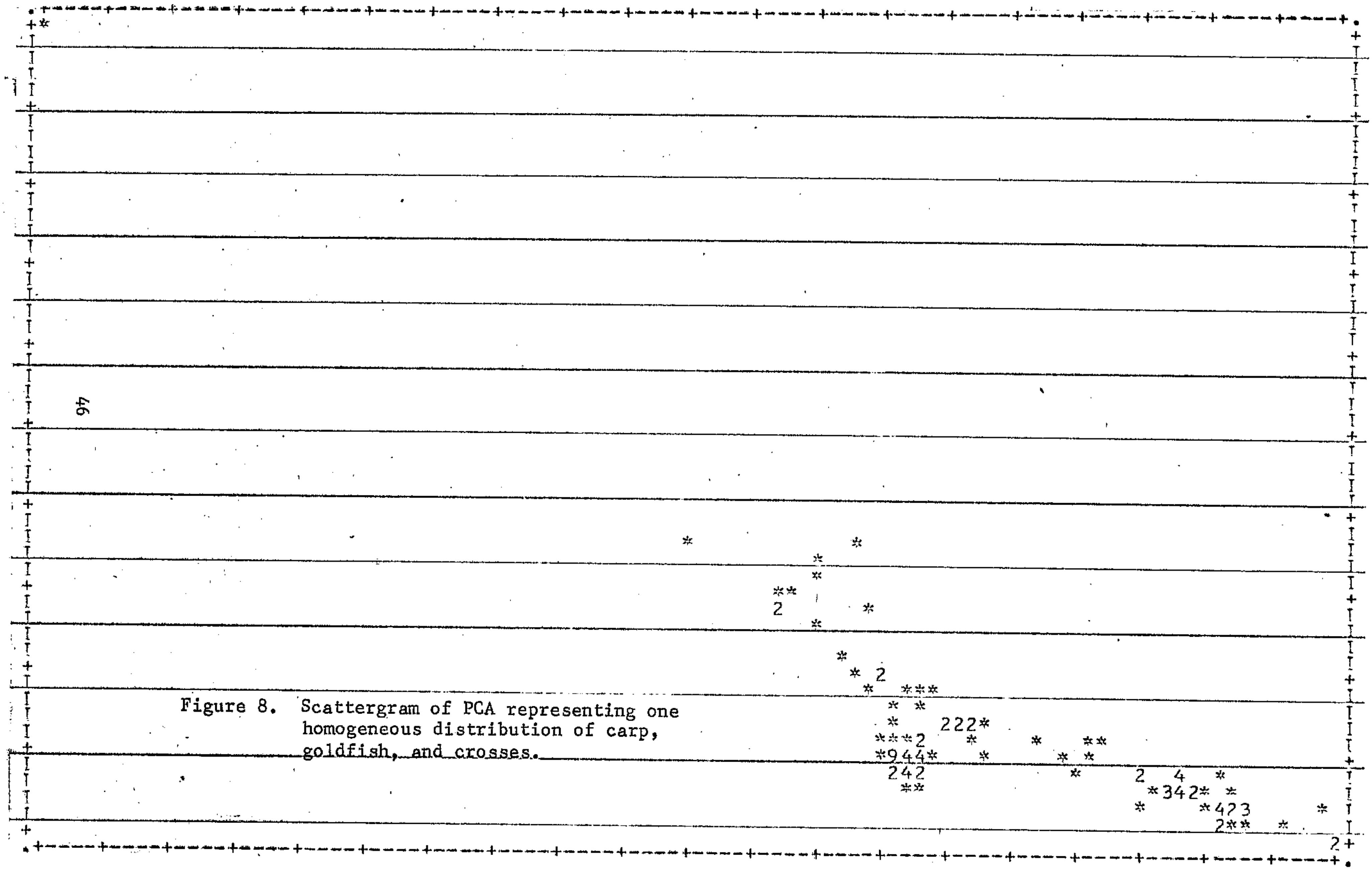


Figure 8. Scattergram of PCA representing one homogeneous distribution of carp, goldfish, and crosses.

The percentage composition varies with locality and estimates must be based on extensive sampling rather than the selective sampling done here.

(3) Conventional Analysis by Hybrid Indices

Table 3 summarizes the range and means of 13 parameters and the range and mean hybrid index values for each parameter.

Average values were intermediate for all parameters except C, H, and I. The range of hybrid index values for all parameters were from 0 to 22 with values greater than 0 and less than 1 representing an intermediate condition. Values less than 0 or greater than 1 usually indicate little difference between the average values of the hybrids and parents or that the hybrid parameters exceed either parental. Values that do exceed either parental can often be attributed to complimentary gene action described previously or may be purely an effect of the arithmetic scaling technique used. Those hybrid index values less than .5 are skewed toward carp as parentals while those greater than .5 more nearly approximate the goldfish as parentals.

The mean hybrid index values for each parameter range from .27 to 10.31 with 10 of the 13 parameters representing the intermediate condition between 0 and 1. Parameter C, (head length/standard length) was nearly identical for all 3 types of fish resulting in an aberrant hybrid index of 10.31. Parameter I (dorsal width opercle/head length) also resulted in an aberrant index value of 1.7 because the average parameters for the 3 types of fish closely approximated one another. Parameter H (length dorsal base/standard length), hybrid index value 1.99, was deviant for the same reasons. Seven of the 10 parameter index values more nearly approximate the goldfish while the remaining 3 more nearly approximate the carp.

The overall average of hybrid index values for all 29 suspected

Table 3. Hubbs hybrid index values for 13 parameters.

G=goldfish H=hybrid C=carp

PARAMETER	Fish	Range	Average	Range of hybrid index values for each parameter	mean index value
A*	G	0	0		
	H	.01-.23	.057	.02-1.04	.73
	C	.01-.35	.22		
C*	G	.13-.30	.27		
	H	.24-.31	.27	.09-22	10.31
	C	.24-.30	.27		
E*	G	.33-.53	.41		
	H	.29-.37	.35	.0-.76	.30
	C	.25-.38	.33		
F*	G	.82-1.0	.91		
	H	.66-.91	.78	.11-1.02	.46
	C	.54-.87	.63		
G*	G	.15-.21	.19		
	H	.15-.20	.17	.11-1.37	.56
	C	.12-.17	.15		
H*	G	.35-.46	.39		
	H	.32-.42	.39	.17-5.1	1.99
	C	.30-.42	.38		
I*	G	.23-.56	.27		
	H	.23-.30	.27	.03-5.8	1.7
	C	.21-.32	.28		
J*	G	.14-.24	.19		
	H	.12-.25	.27	.05-2.9	.77
	C	.11-.22	.16		
K*	G	.16-.47	.41		
	H	.18-.42	.39	.03-3.0	.92
	C	.30-.39	.36		
L*	G	.55-.96	.81		
	H	.46-.81	.61	.04-1.1	.44
	C	.29-.59	.46		
DS	G	0	3		
	H	1-3	2.65	0-1.0	.82
	C	1-3	1		

(Cont'd)

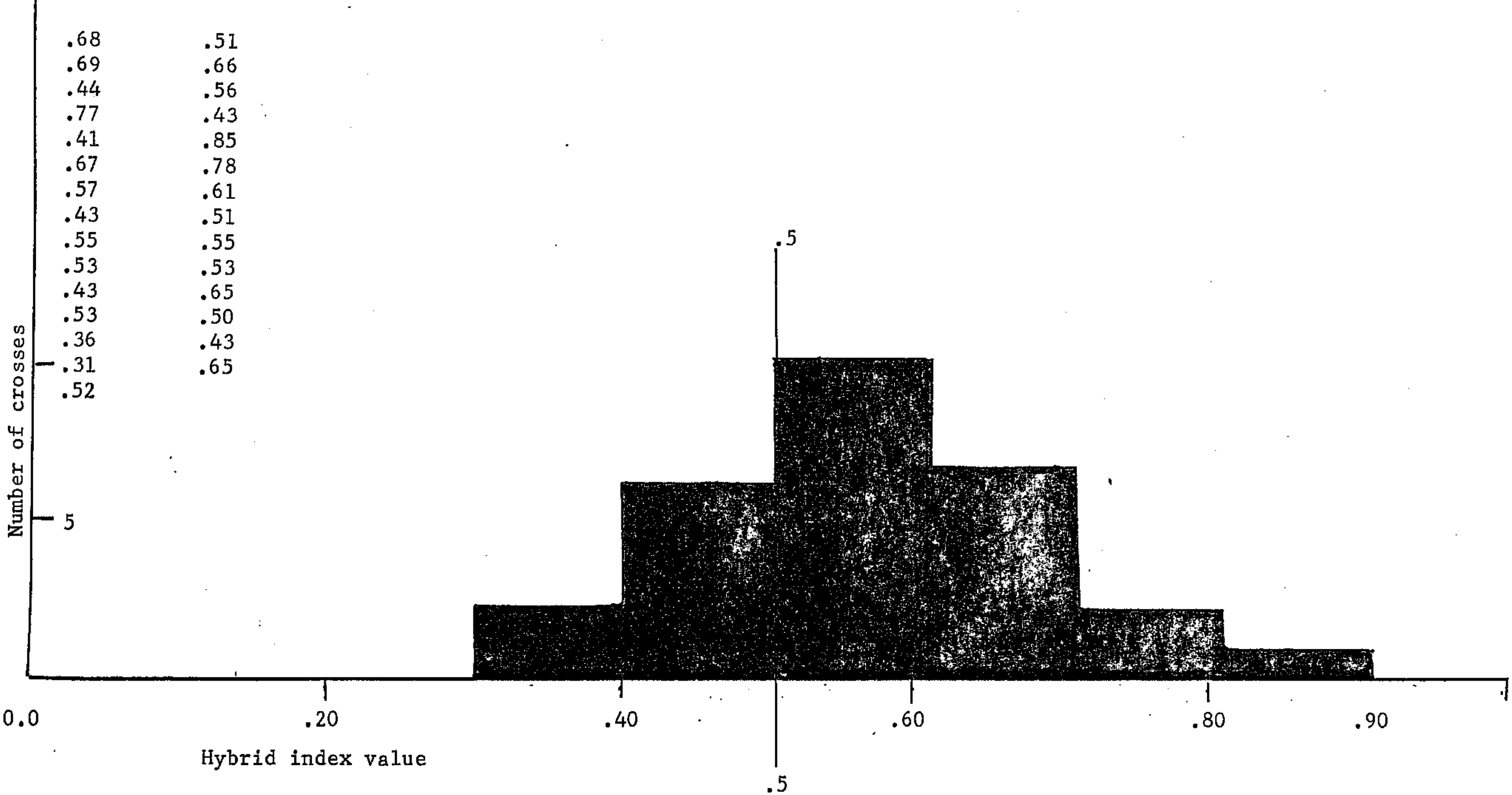
Table 3. Hubbs hybrid index values for 13 parameters.

G=goldfish H=hybrid C=carp

PARAMETER	Fish	Range	Average	Range of hybrid index values for each parameter	mean index value
B	G	0	0		
	H	1-4	2.4	.5-1.0	.37
	C	4	4		
LL	G	27-31	28		
	H	31-36	33.9	.34-.79	.43
	C	33-39	36.8		

*Parameters are the reciprocal of those listed in Table 1.

Figure 9. Hybrid index values for each cross with histogram of values.



crosses using the 10 parameters (neglecting aberrant values) is .59 (a conservative estimate) compared to a value of .50 expected for perfect intermediacy. The hybrid index for each cross is listed in Figure 9. The question raised is whether or not .59 differs significantly from .5. Testing the null hypothesis that $Ho:Vh=.5$ versus $Ha:Vh \neq .5$ reveals that Ho is rejected (Fisher Sign Test .05 probability, n=29). The results of this test present a dilemma because two possible inferences can be drawn; (1) the sample from Lake Erie represents only F_1 hybrids whose inheritance of characters is not intermediate; (2) the sample represents an introgressed population that is skewed slightly toward goldfish as parentals. Considering the evidence already presented, the second inference becomes more plausible. Therefore, a hypothesis of widespread introgression can be further affirmed.

With intergeneric introgression becoming more apparent, the word "hybrid" will be deleted and replaced by the word "cross" to better define the intermediate individuals. Undoubtedly, F_1 hybrids are still produced, although they are probably outnumbered by backcrossed individuals. If only F_1 hybrids and F_1 or F_2 backcrosses existed, the ratio of hybrids to backcrosses could be estimated easily with the Hubbs hybrid index. Those fish with index values that approached 0 would more likely represent backcrosses with the carp and those that approached 1.0 would more likely represent backcrosses with the goldfish. F_1 hybrids would be represented by values approaching .5. However, this situation does not exist and the fish have probably backcrossed for many generations. Therefore, highly introgressed fish would be expected to approach the median position of F_1 hybrids after many generations of backcrossing. This prevents an accurate estimation of the ratio of backcrosses to hybrids.

(4) Comparison of Lake Erie Crosses with Known F_1 Hybrids

Comparisons were made between data published by Ojima (1973) and suspected crosses from Lake Erie. Ojima's data were taken exclusively from 6 month old hybrids while the data from Lake Erie crosses were taken on fish ranging from .5-8 years. In order to establish the validity of comparing proportional measurements for a wide range of fish sizes, a linear regression for each of the five parameters against standard length was made (Table 4). If a linear relationship exists between each of the parameters and body length, the comparison of a wide size range of fish can be made.

Parameter F was eliminated since the ratio of C.P.L./C.P.D. is not linearly related with size, therefore, comparison between large and small fish cannot be made. This lack of linearity is largely due to the opposite signs of the regression slopes of the parentals (Table 4). Parameters D and E are only marginally significant (.26 and .18 respectively) for the same reasons, but can be included in an analysis with reservations. The remaining two parameters, C and J, were highly linearly significant thus allowing direct comparison between a wide range of fish sizes. Counts such as gill rakers and scales are generally constant with size after postlarval development.

Two tailed t-tests were performed testing the hypothesis that Ho: $X_1=X_2$ versus Ha: $X_1 \neq X_2$. Accepting Ho would imply that the Lake Erie population was not different from the known F_1 hybrids, thus discounting any hypothesis of introgression. If Ho is rejected it would imply that the Lake Erie crosses differed significantly from the control group of known F_1 hybrids and therefore substantiating any hypothesis

Table 4. Testing 5 parameters for linearity. Slope is included

Parameter		Significance of linearity	Slope
C=standard length / head length	G	.00777*	.00165
	H	.00051*	-.00737
	C	.00001*	.00150
D=head length / snout length	G	.02291*	.00549
	H	.26127	-.00124
	C	.00175*	-.00062
E=standard length / body depth	G	.07312	-.00157
	H	.18272	-.00064
	C	.00001*	.00173
F=C.P.L. / C.P.D.	G	.22088	-.00047
	H	.81334	-.00047
	C	.12966	-.00037
J=head length / eye diameter	G	.00001*	.01339
	H	.04144*	.00843
	C	.00001*	.00761

*highly significant for linearity .05 or less.

of introgression.

The results show that Ho is rejected in all cases at the .001 significance level, therefore proving that the Lake Erie crosses are indeed largely introgressed and not simply hybridized (Table 5).

Table 5. Two tailed t-tests with significance levels for 6 characters

Parameter	Average values		Standard deviation		Test Statistic Z value	.001 significance
	Ojima (1973) n=138	Lake Erie n=29	Ojima (1973)	Lake Erie		
$C=\frac{SL}{HL}$	3.05	3.7	.1	.17	5.85	*
$D=\frac{HL}{\text{snout L.}}$	3.65	3.07	.3	.56	5.38	*
$E=\frac{SL}{BD}$	2.65	2.89	.1	.17	7.27	*
$J=\frac{HL}{ED}$	3.45	5.92	.3	1.57	5.85	*
lateral line scales	30.63	33	1.9	.97	9.75	*
gill rakers first arch	38.5	30	3.7	1.59	22	*

(5) Summary: Statictical Analysis

Character Selection and Principal Component Analysis

1. Several characters exist that will separate the carp from the goldfish, however, none of them will perfectly separate crosses from both parentals.
2. Several characters considered simultaneously will account for natural variation and better describe the intermediacy of crosses.
3. Principal component analysis used as a numerical taxonomic technique handles a large number of variables simultaneously and represents species by projecting clusters of points onto a two dimensional graph.
4. The principal component analysis of the sample of 109 carp, goldfish, and crosses reveals one homogeneous distribution, supporting the hypothesis of the presence of an extensively introgressed population in Lake Erie.
5. The parentals are intact and do not appear to be lost by introgression, but pure reference samples from sympatric non-crossing populations of carp and goldfish would be necessary for more conclusive results.

Conventional Analysis by Hybrid Indices

1. The conventional hybrid index of Hubbs describes 10 of the 13 parameters as intermediate.
2. Seven of the ten parameters more nearly approximate the goldfish, three more nearly approximate the carp.

3. The overall hybrid index Vh of all parameters = .59 more nearly approximates the goldfish than the carp.

4. A two-tailed statistical test reveals that $Vh \neq .50$, further affirming the hypothesis of widespread introgression in Lake Erie.

Comparison of Lake Erie Crosses With Known F_1 Hybrids

1. Lake Erie crosses differ significantly (5 characters, .001 significance level) from known F_1 hybrids providing conclusive evidence for introgression.

(6) Descriptive Analysis of Selected Morphological Characteristics

A comparative description of carp, goldfish and crosses is summarized in Table 6. Detailed comparisons follow.

(a) Size

Mature hybrids are nearly intermediate between the adult size of goldfish and carp. Adult carp average from 381-457 mm. and adult goldfish average about 127-254 mm. The largest hybrid observed was 310 mm., average about 250 mm. The hybrid body is robust and intermediate between that of the laterally compressed goldfish and the more elongate body of the carp. Figure 10 shows an overall comparison of the three fish.

Table 6. Morphological comparison of the goldfish, crosses, and carp from Lake Erie samples

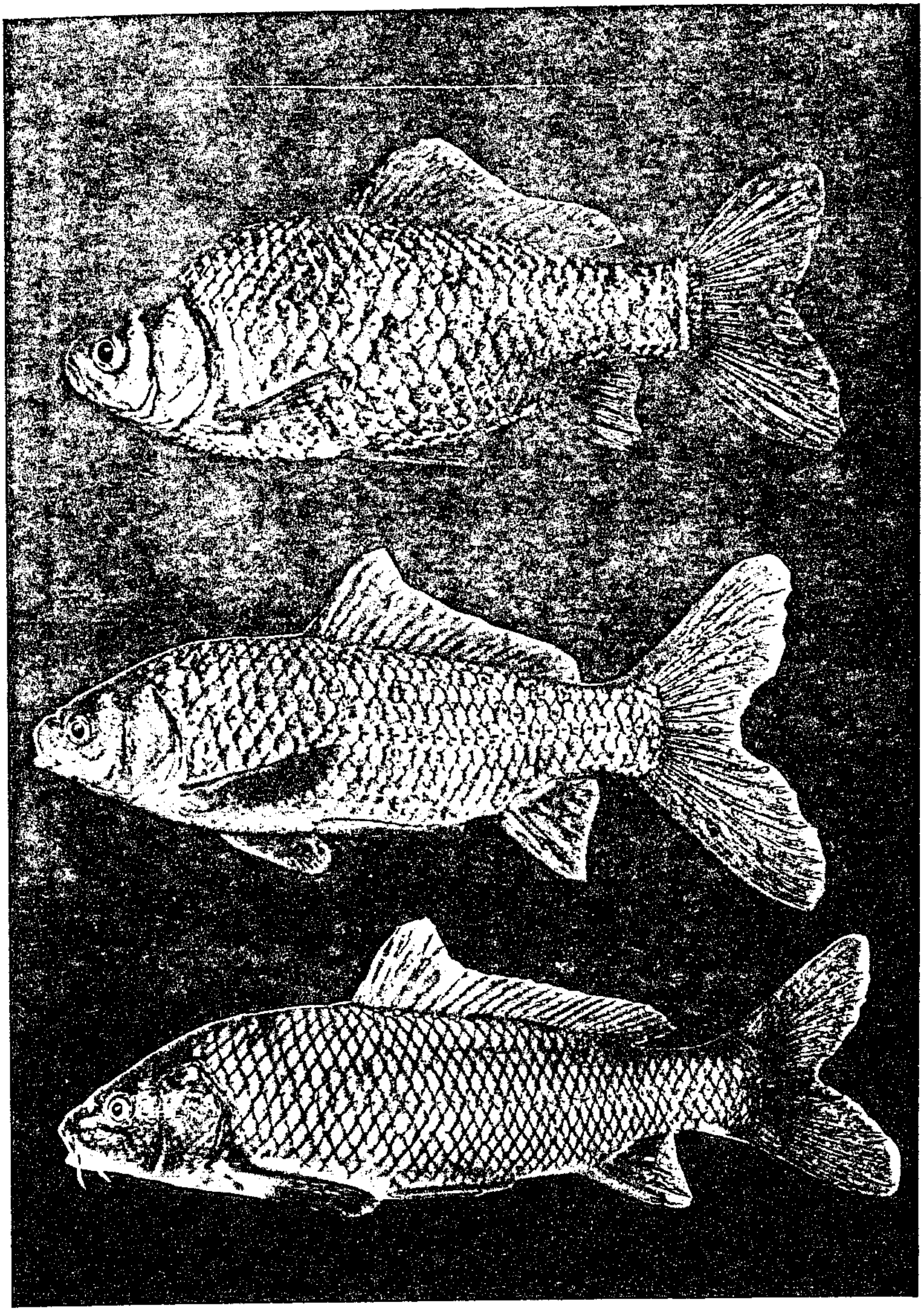
	body depth	head length	eye diameter	snout	interorbit width	mouth	barbels
goldfish	33-53% of total length	13-30% of total length	14-24% of head length	25-33% of head length	16-47% of head length	oblique protrusible	none
crosses	29-37% of total length	24-31% of total length	12-25% of head length	29-36% of head length	18-42% of head length	angle intermediate protrusible	1-4, 2 or 4 most common total length of all barbels an average 6% of head length
carp	25-38% of total length	24-30% of total length	11-22% of head length	27-40% of head length	30-39% of head length	nearly horizontal very protrusible	4, total length of all 4 about 22% of head length

(Cont'd)

Table 6. Morphological comparison of the goldfish, crosses, and carp

	teeth	gill rakers	dorsal fin	caudal	scales	vertebrae	tubercles	color
goldfish	4-4	37-43	not emarginate	bilobed-exaggerated in ornamentals	27-31	29-31	fine and scattered on nape	variable, olive green to gold, orange, and yellow
crosses	variable, often 1,4-4,1	27-33	slightly emarginate	bilobed often enlarged	31-36	28-38	fine and scattered on head	silvery grey, no colors
carp	1,1, 3-3, 1,1	21-28	emarginate to falcate	bilobed	33-39	34-38	fine and scatterd on head	olive green with yellowish abdomen, males with lower half of caudal and anal fins bright orange

Figure 10. Goldfish (top), cross, and carp (not to scale SL=160 mm., 220 mm., 240 mm., respectively).

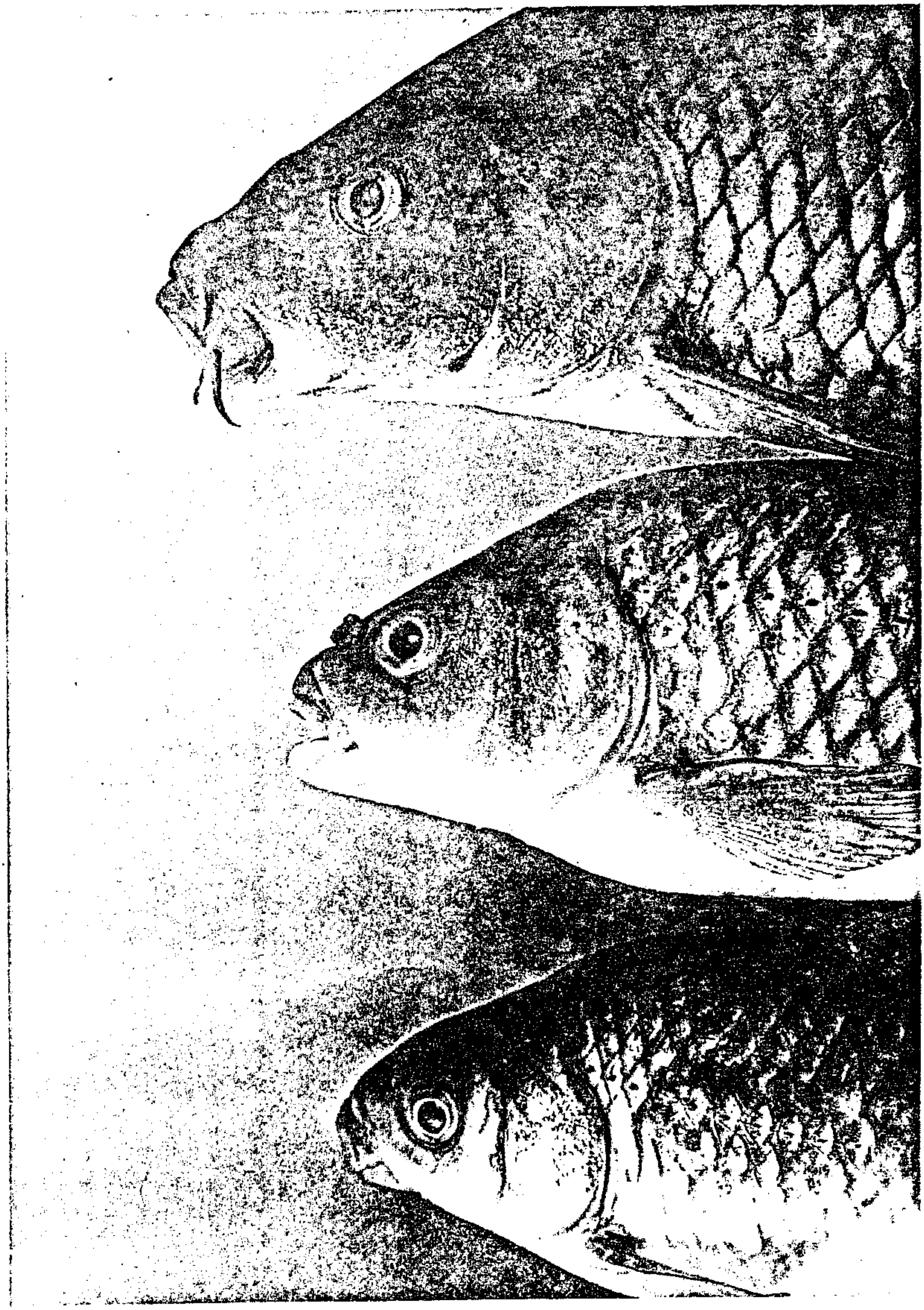


(b) Barbels

Carp have two pair of fleshy barbels protruding from the upper portion of the jaw (Figure 11). Barbel growth begins at approximately the 20 mm. stage and continues with an increase in the total length of the fish. The posterior pair are always longer (1.2-2.8 times) the anterior pair depending on the size of the fish. The total length of all four barbels is an average of 22% of the head length. Goldfish have no barbels (Figure 11). The blending of the parentals in the hybrids is seen in this character. The number and length of barbels in hybrids is highly variable, although at least one minute barbel is always present. One hybrid had 1 barbel, 62% had 2, 2 fish had 3, and 27% had 4 barbels, n=29. The barbel length is always shorter than the carp's in similar size fish, with the posterior pair equal or up to five times the length of the anterior (upper) pair (Figure 11).

Crosses with four barbels might be expected to have a closer relationship with the carp and may represent F_1, F_2, F_3, etc., backcrossing with pure carp. Those with two barbels may be more closely related to the goldfish in the likewise manner. Crosses with minute barbels (1.5 mm. on a 268 mm. hybrid) may have resulted in several generations of backcrosses exclusively with goldfish. The appearance of an odd number of barbels (1 or 3) is possibly a result from injury, however it could be a result of the lack of development of bilateral appendages. The knowledge of the inheritance of the size and number of barbels in crosses alone could help clarify the extent of introgression. However, this is not feasible since it would require at least 10 to 20 years of controlled breeding to map the genetic inheritance.

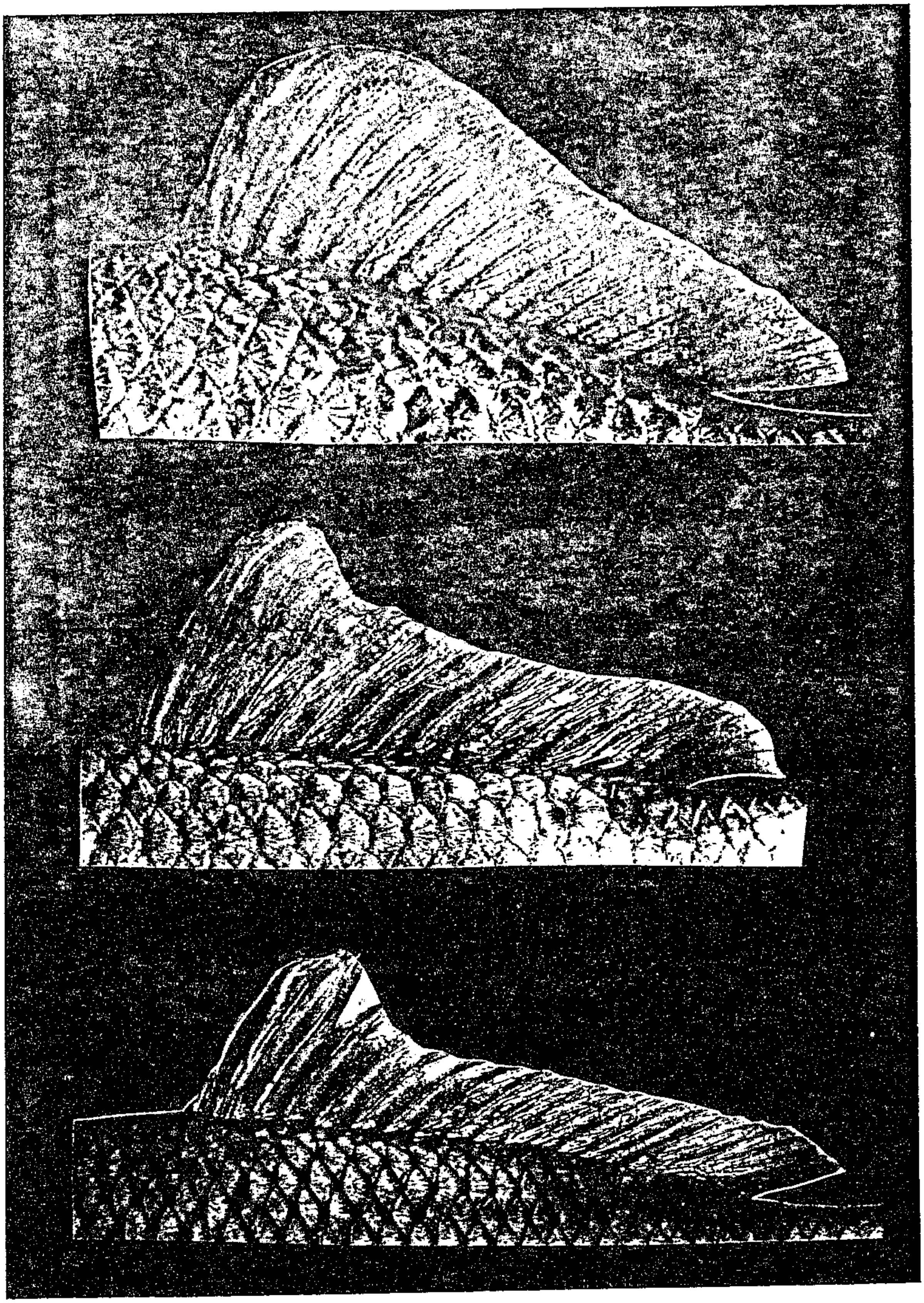
Figure 11. Carp (top), cross, and goldfish (to scale, carp HL=70 mm.)



(c) Dorsal Fin

The shape of the dorsal fin is an excellent field character used to identify crosses. The dorsal fin of the carp is composed of 18-20 soft rays and 1 toothed spine. Note the outline of the dorsal trailing edge of the fin (Figure 12). A slightly concave margin originates at the apex of the soft rays and gradually diminishes posteriorly, expressing the falcate condition. The goldfish dorsal fin consists of 15-18 soft rays and 1 stout toothed spine. The outline shows a more linear trailing edge lacking concavity (Figure 12). The dorsal fin of the hybrid exhibits the intermediate condition with a slightly concave margin.

Figure 12. Dorsal fin: goldfish (top), cross, and carp (same scale as Figure 10)

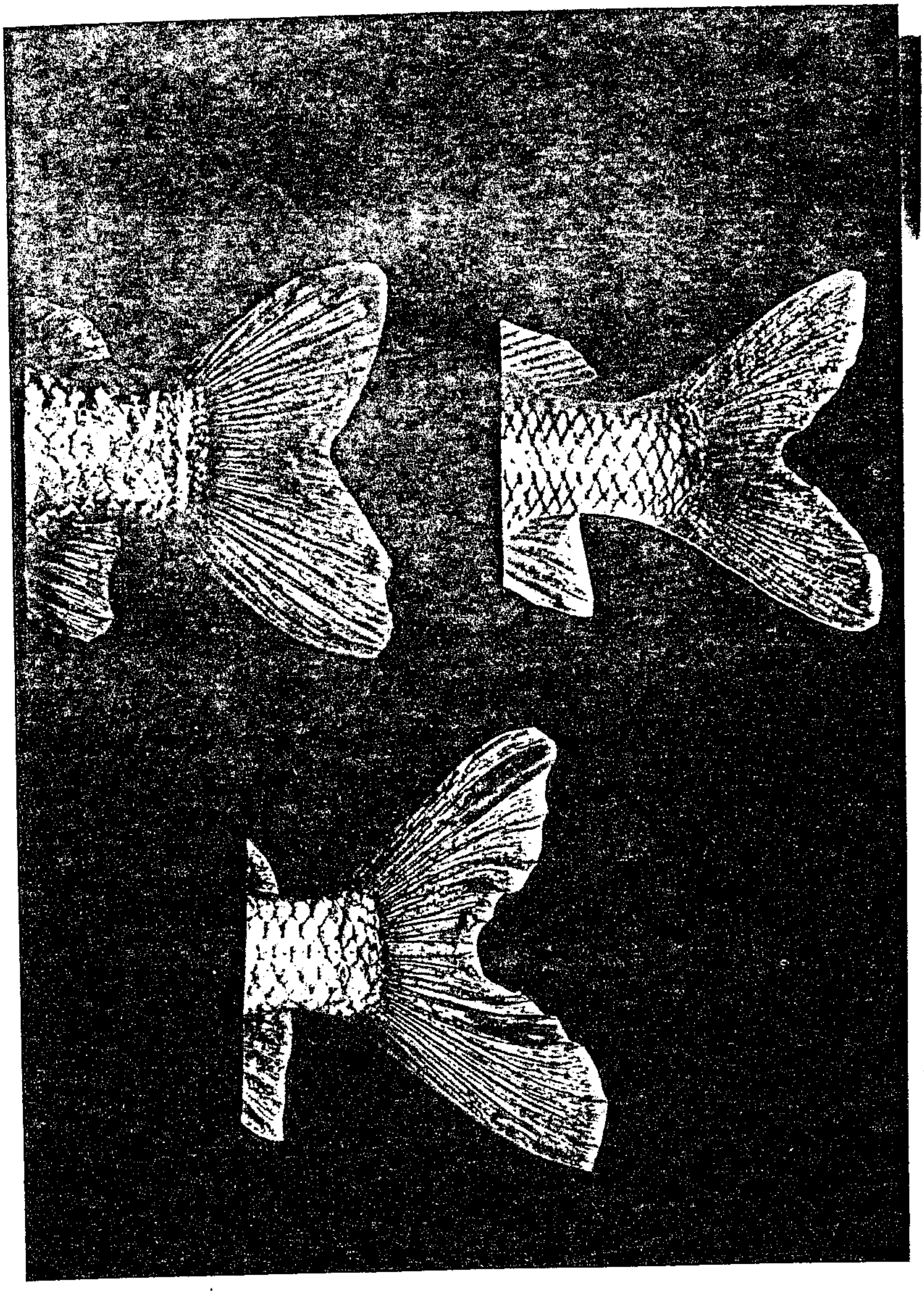


(d) Caudal Fin

The shape of the caudal fin is more highly variable than any other hybrid character except barbel length and number. This is largely a result of interbreeding with several varieties of ornamental goldfish such as the comet or tripletail. Hybrids resulting from these crosses have enlarged caudal fins. The enlarged caudal lobes shown in Figure 13 were undoubtedly influenced by interbreeding with an ornamental, probably a comet variety. Although rare and never verified, tripletail hybrids have been reported by some commercial fishermen.

The caudal fins of carp and goldfish are also presented in Figure 13 for comparison.

Figure 13. Caudal fin: goldfish, carp, and cross (top left, top right, and bottom respectively)



(e) Color

Color is an excellent field character. The majority of goldfish are drab olive green with a white yellowish abdomen. Less common are the gold, orange, yellow, brown, black, and albino varieties. The more brightly colored ornamentals are reported to revert to the natural drab coloration when released into the wild. Several wild goldfish held in indoor tanks reverted from their drab olive color to orange and white within 60 days indicating that color can be environmentally controlled. Selective pressure of predation probably acts against brightly colored fish but this may not be substantial since goldfish do not develop bright coloration until after their first year when they reach a length of several centimeters. No difference in the coloration of males or females was detected.

Carp are more uniform in color but with dichromatism. Males are normally dark olive green dorsally with a light colored abdomen. The lower half of their caudal and anal fin are a yellowish orange throughout the year and intensified during the breeding season. Generally the females are similar except lighter in overall coloration. They also lack much of the orange coloration in the lower caudal and anal fin.

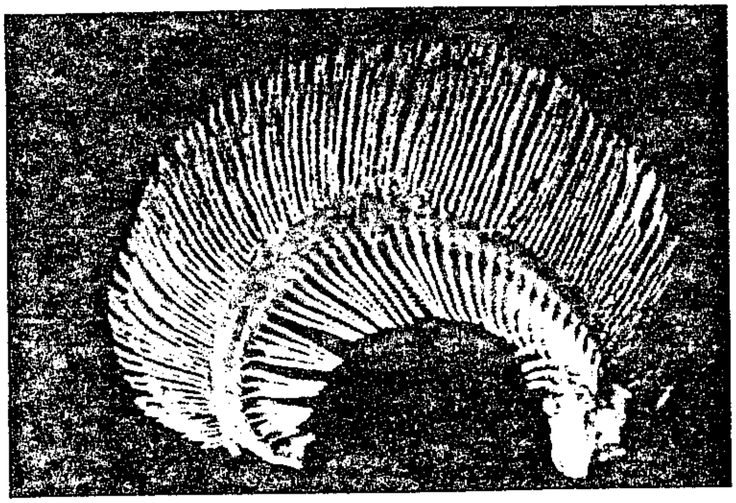
The hybrids are uniform in coloration with an overall silvery appearance when alive. This condition appears to dominate any of the brighter colors that might be inherited from the goldfish. I have on several occasions observed small flecks of gold pigment scattered widely, but they were always obscured by the silvery pigment. Although calico goldfish are often mistaken for hybrids there is no verified evidence of hybrids exhibiting a mosaic of colors. This may eventually change since brightly colored *Cyprinus carpio*, much like that of the goldfish, called koi, are now commonly cultured as ornamentals in the United States. It is only a matter of time before these escape in the river systems and hybridize.

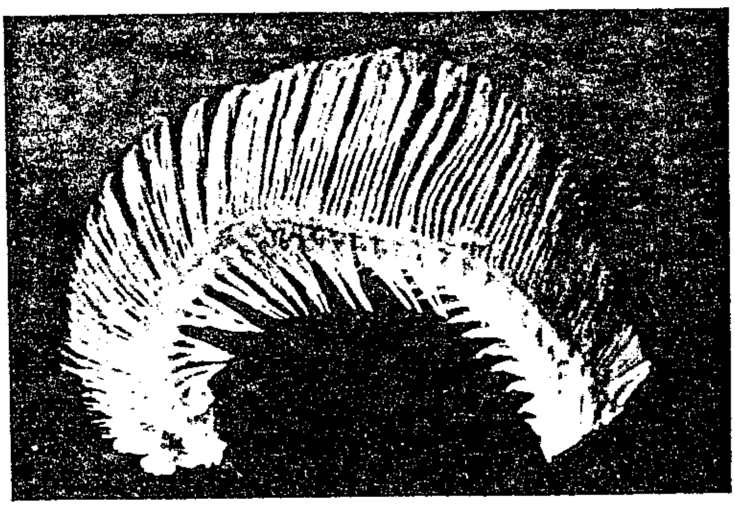
(f) Gill Rakers

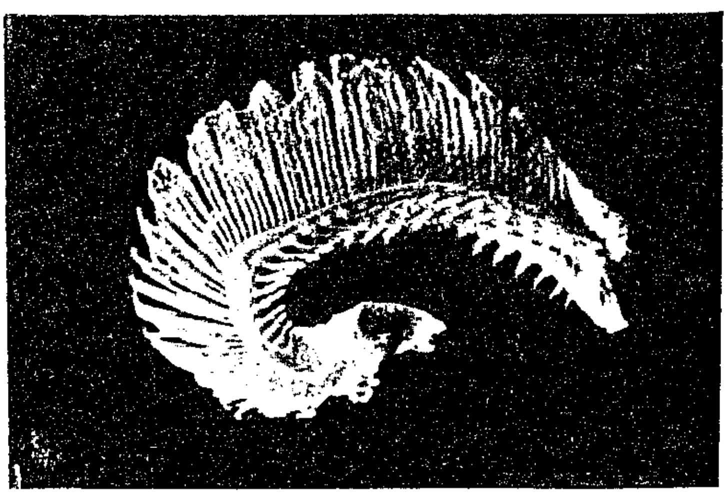
The gill rakers are tapered and biserially arranged along the margin of each branchial arch (Figure 14). The individual lengths increase from both apexes to a maximum in the center of the arch with the outer series generally two times the length of the inner series. The number and shape of the first row gill rakers of carp and goldfish on the first arch differs substantially. Figure 15 statistically shows a median relationship in the number of gill rakers in the hybrids. The gill rakers are triangular in shape with the longitudinal trailing edge composed of a smooth, stout supporting material. The skeletal portion is covered with a flexible mucus extension of equal size anteriorly. The angle of insertion varies with the position on the arch, those median possess a more perpendicular insertion and those terminal possess an acute angle of insertion causing the gill rakers to lie close to the margin rather than protruding from it.

Carp have the stoutest gill rakers, their width 41% of their length (Figure 16). The basal portion is broad, nearly equilateral rather than laterally flattened, tapering to a point. The first and second rows of the gill rakers on the first arch are of the same size and shape and lie in the same plane (Figure 17). Water passes from the buccal cavity parallel to the plane of gill rakers over the pliable portion that is biserially toothed and then across the adjacent, smooth supporting portion. The gill rakers are closely spaced but with only the toothed sticky mucus portions overlapping. This enables much of the water to be strained free from suspended particles and passed over the gill filaments. Retained particles are sorted, expelled back out the mouth

Figure 14. Gill rakers, first arch: goldfish (top), cross, and carp. Taken from similar size fish.







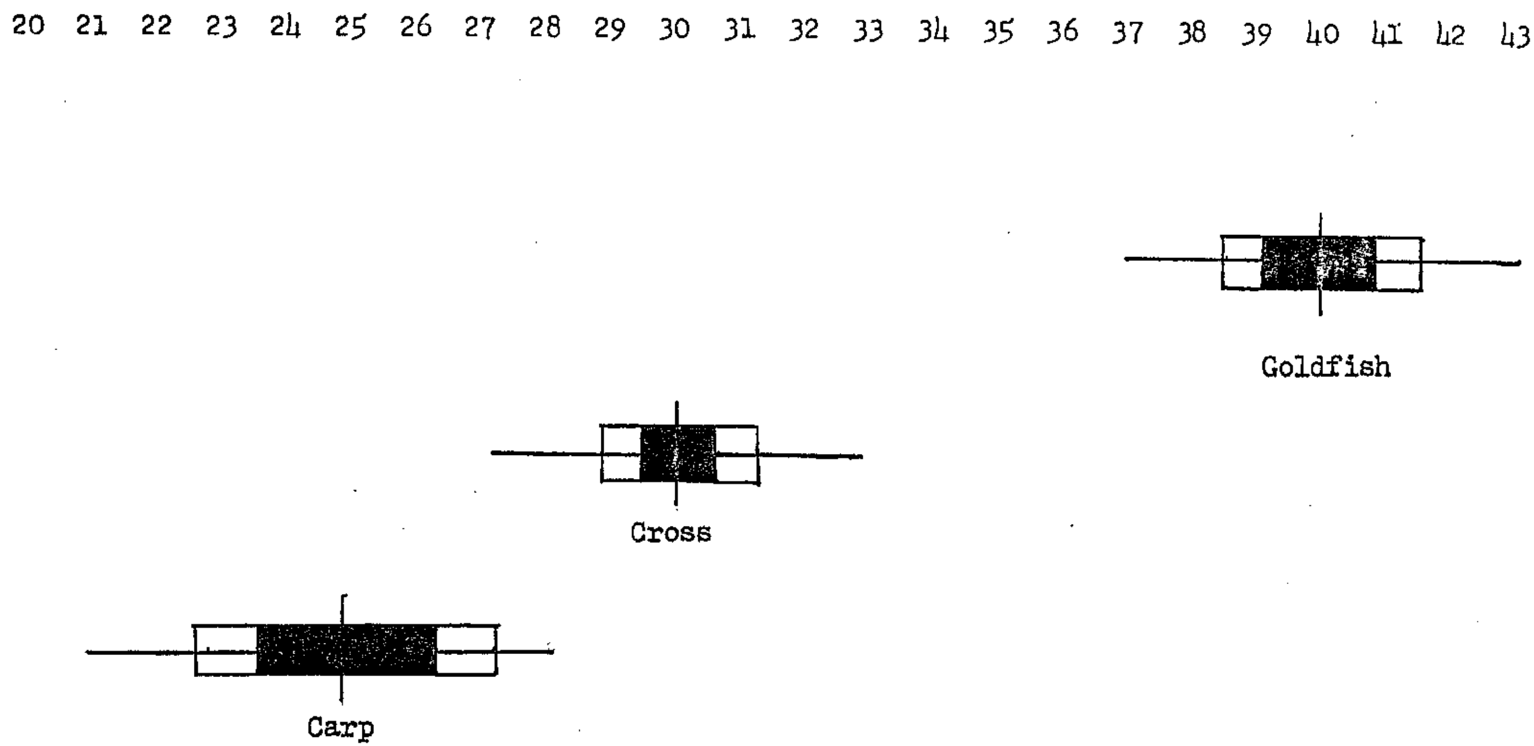
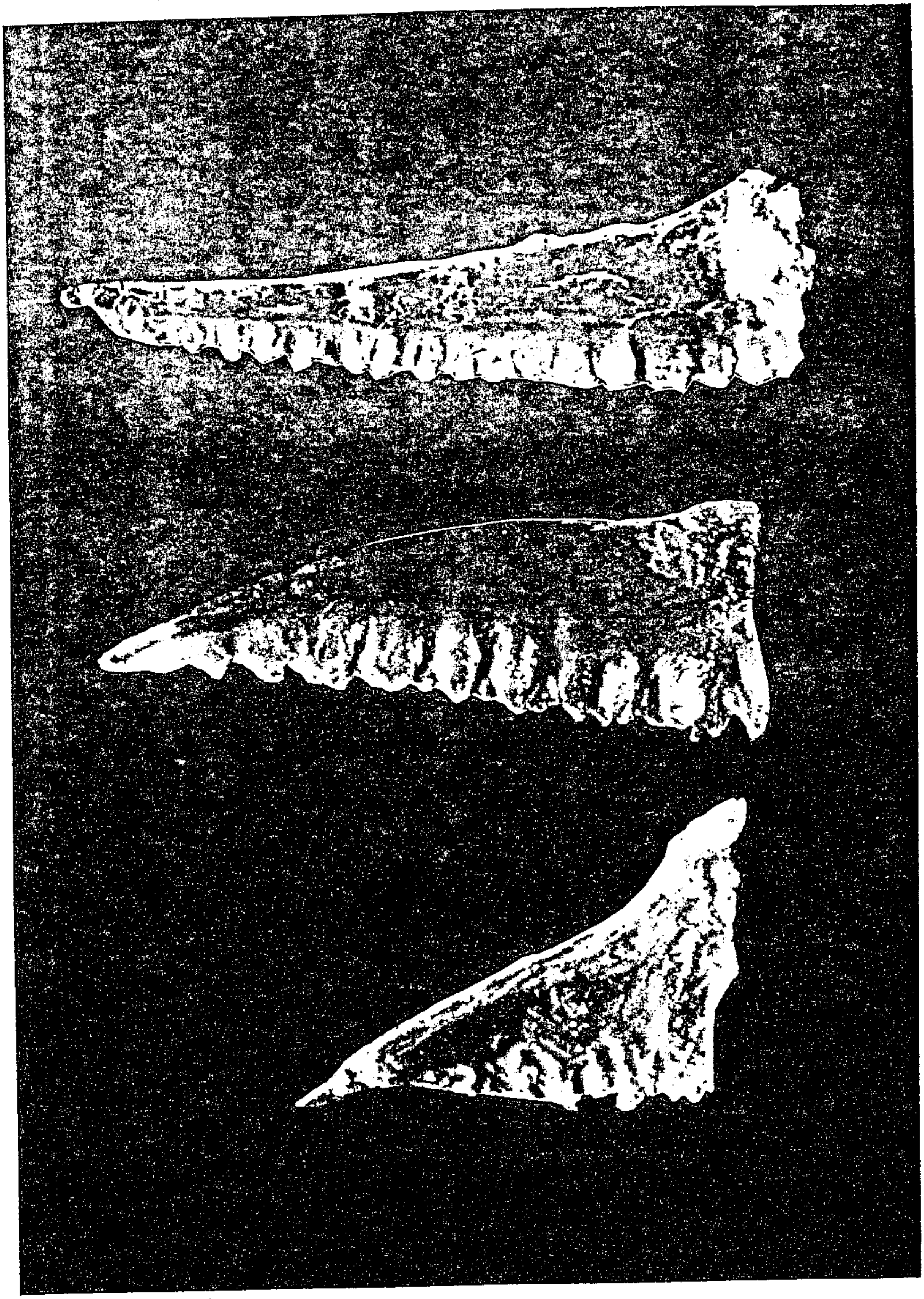
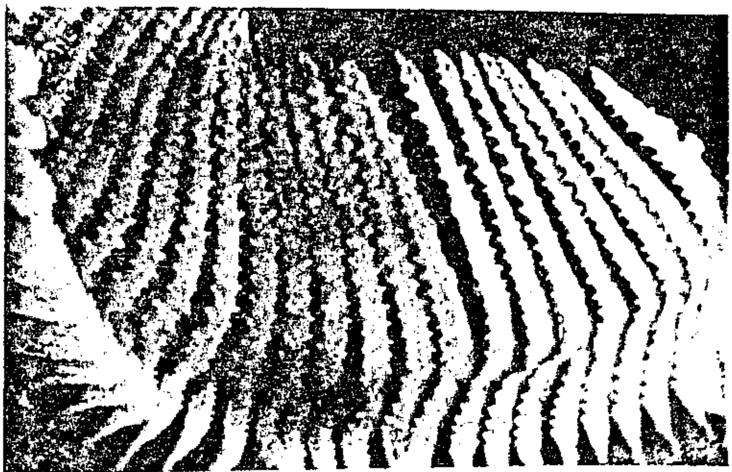


Figure 15. Statistical relationship among the number of first arch first row gill rakers in carp, crosses, and goldfish. Horizontal and vertical lines represent mean and range. Light and dark areas represent standard deviation and two times the standard error on either side of the mean.

Figure 16. Single gill rakers, first arch: goldfish (6 mm.), cross, and carp, top to bottom (to scale).

Figure 17. Closeup of gill rakers showing biserrate edge: goldfish (top), cross, and carp, top to bottom.





opening, or passed as food through the pharynx.

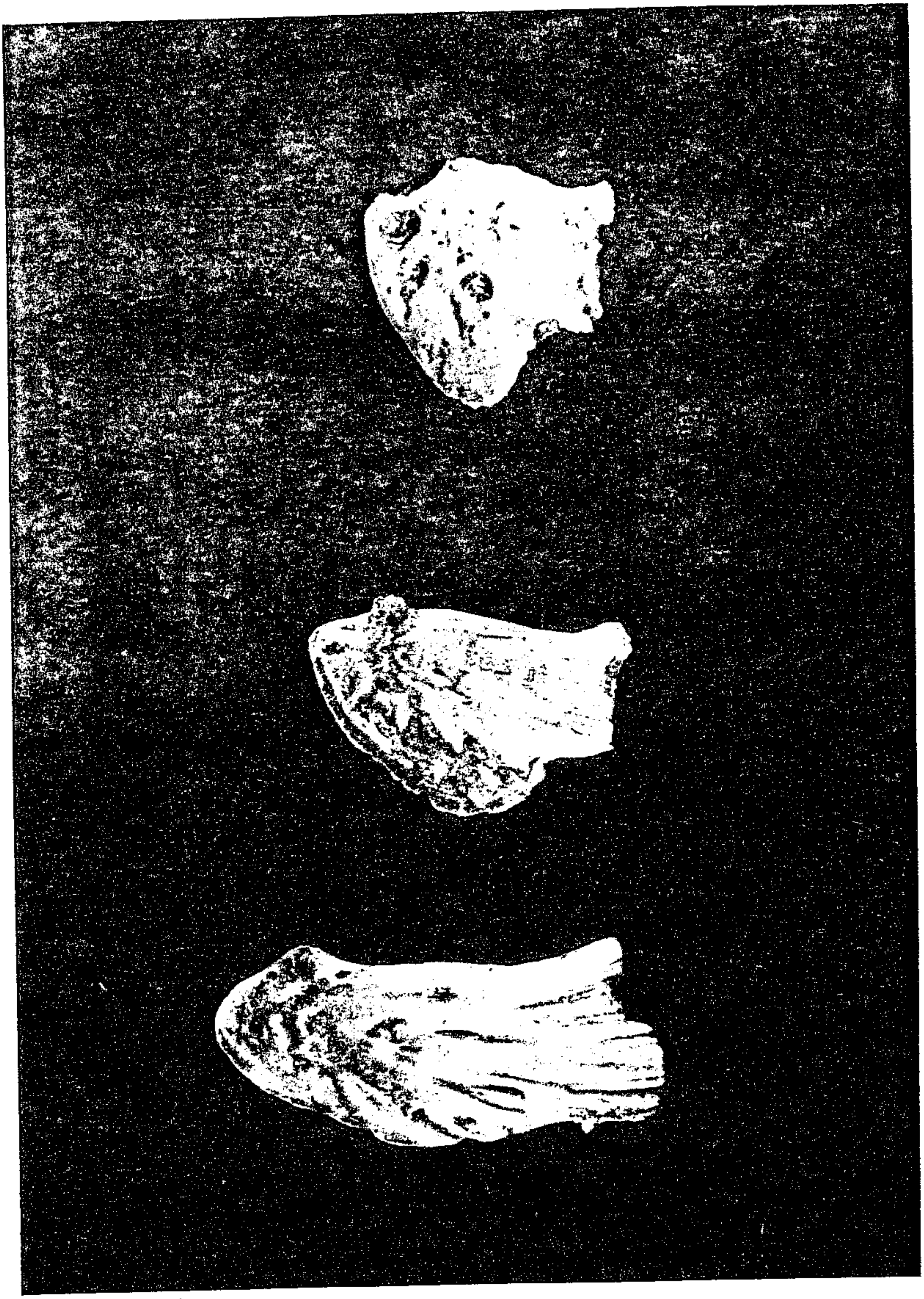
The first and second row of goldfish gill rakers on the first arch, unlike the carp, are dissimilar in size. The first row gill rakers of the goldfish are triangular, but slender with their width only 16% of their length (Figure 16). The length is about twice that of the carp, while their width is about .5 that of the carp's. The second row first arch gill rakers are much like those of the carp. The angle of insertion is more nearly perpendicular through the length of the arch causing the first row of rakers to extend into the buccal cavity. The gill rakers are equally divided into two longitudinal portions as in the carp with the rigid skeletal material supporting the pliable portion. The series of biserrate soft teeth are smaller (.1-.2 mm. in width) but more uniformly arranged than in the carp. The numerous and elongate gill rakers provide more than twice the surface area for straining food particles in the goldfish (Figure 17). This results in the goldfish being better adapted but not limited to a planktonic food supply. They are omnivorous like the carp but can handle the smaller particles with greater efficiency.

The first row gill rakers of the crosses are intermediate, longer, thinner, and more laterally compressed than the carp's but shorter and wider than the goldfish (Figure 16). They often appear slightly irregular and unevenly spaced with an insertion upon the arch varying close to 90 degrees (Figure 17). The posterior skeletal portion of the gill raker is much like that of the goldfish or carp while the anterior portion is highly variable. The soft trailing edge lacks the placement of biserrate teeth. Often the pliable portion has slight to moderate reticulations with little overlapping surface area for particle entrapment, a condition much less efficient for planktonic existence (Figure 17). The carp and more often the goldfish are known to exist on a purely planktonic diet while the hybrid may be restricted to another type of subsistence. This may indicate that the hybrids are utilizing a third habitat and competing with another sector of the fish community. Food habits of the hybrids have never been analyzed and would be required in order to fully resolve this question.

(g) Lacrimal

The most anterior of the suborbitals, the lacrimal, proved to be an excellent discriminating character. Length to width ratios listed in Table 2 show that the lacrimal of the goldfish is relatively short and triangular, that of the carp longer and more rectangular, and intermediate in the crosses. See Figure 18.

Figure 18. Lacrimal bone: goldfish (8.9 mm.), cross, and carp, top to bottom (to scale).



(h) Opercular Series

The opercles of the carp and goldfish show two distinct differences. The anterodorsal edge of the goldfish opercle forms a concave depression with a short opercular arm in the anterodorsal corner. The carp opercle lacks the depression and has a reduced opercular arm. The hybrid opercle expresses an intermediate condition approaching both extremes (Figure 19). The opercle of larger fish is ornamented with radiating striae originating from the anterodorsal edge.

The number of pores in the preopercle of the three types of fish is a good discriminating character if prepared skulls are available. The number of pores found in the preopercle of the carp is (10-13) mode 12, the goldfish (8-9) mode 9, and the crosses (8-13) mode 10. Again, an overlap exists characterizing introgression.

Figure 19. Opercle, note dorsal border: goldfish (dorsal border width 16.5 mm.), cross, and carp, top to bottom (to scale).

(i) Pharyngeal Teeth

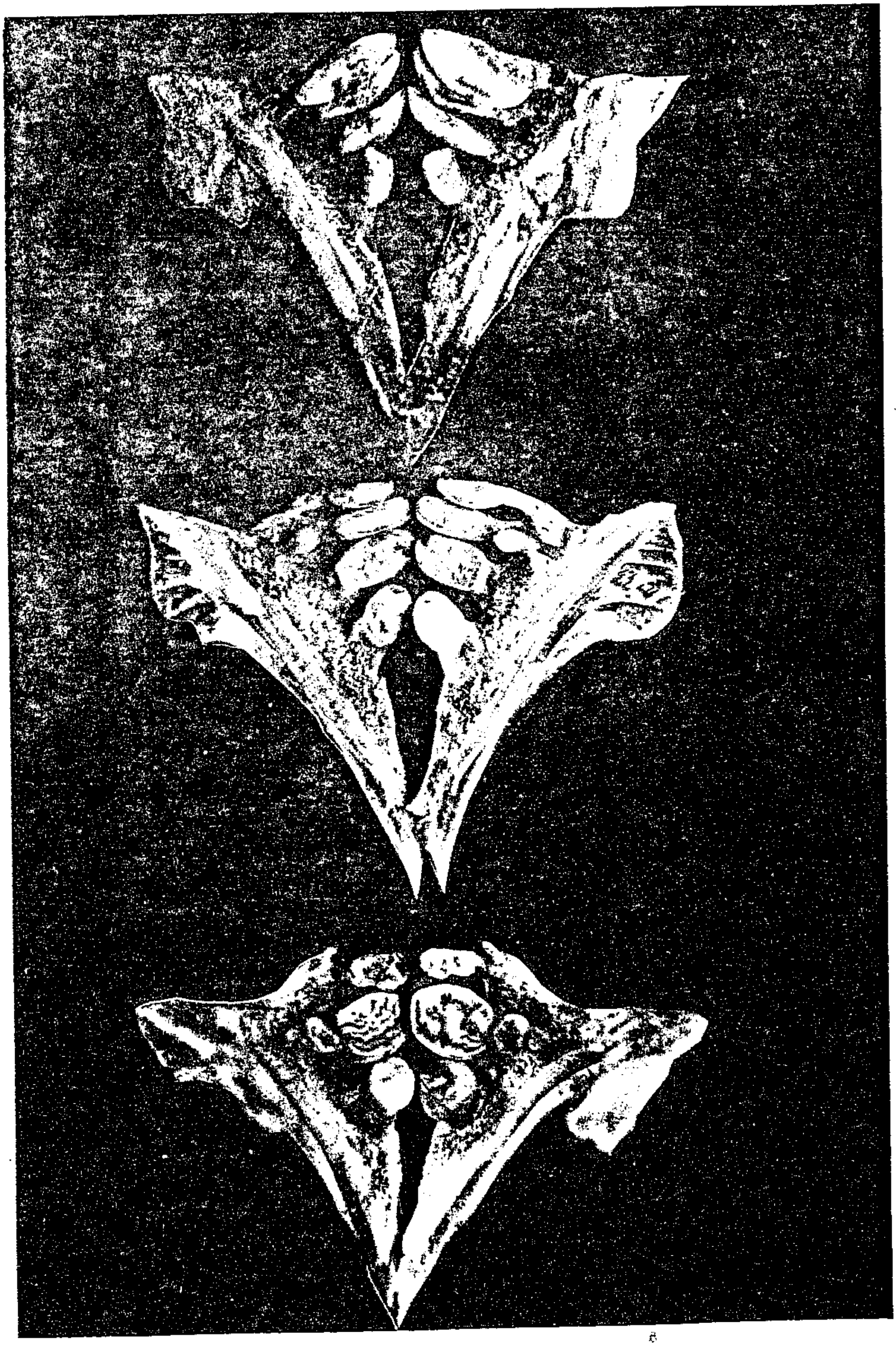
The fifth pharyngeal arch is modified to bear teeth and is one of the more frequently used characters in the identification of hybrids. The pharyngeal teeth of the carp and goldfish are reported as 1,1,3-3,1,1 (5.8% variation) and 0,4-4,0 (0% variation) (Eastman and Underhill, 1973). They reported such variants as 1,2,3- / 0,1,3- / 1,1,1,3- / 1,1,2,3- in the carp while no variation was found in the 4-4 configuration of the goldfish. Forty percent variation in tooth formulae from Lake Erie specimens compared to a 5.8% variation in other populations may indicate an overall genetic change in the parental carp population due to introgression; however, the sample is much too small to demonstrate statistical shifts (Table 7). The aberrant pharyngeal tooth formulae of the carp in this case was largely due to the loss or addition of one or two of the thin and delicate marginal teeth (Figure 20). Goldfish teeth were consistently 4-4 (Figure 20). The hybrid formula varies with 1,4-4,1 occurring most frequently (75%) (Figure 20).

The size and shape of the teeth differ significantly. Carp teeth are of the crushing molar form and expose nearly twice the grinding surface found in the goldfish. The occluding surface is ornamented with shallow crenulations that engage the basioccipital process during mastication. Goldfish teeth are thinner, more rectangular, and better adapted to cutting. The length to width ratio of the grinding surface of the hybrids is intermediate and can be used to distinguish crosses with 95% success. The statistical relationship of the width to length ratio, the range, standard deviation, and standard errors of the three types of fish is graphed in Figure 21.

Table 7. Pharyngeal tooth formulae and variants for a sample of 45 fish

	usual number	% variation	variant formula	N
carp n=17	1,1,3-3,1,1	41	1,1,3-3,1	1
			1,1,3-3,2	1
			2,3-3,2	1
			2,1,3-3	1
			1,1,1,3-1,1	3
cross n=17	1,4-4,1	24	1,1,3-4,1	1
			1,2,1-4,2	1
			1,4-3,1	1
			1,1,4-4,1	1
goldfish n=11	0,4-4,0	0		

Figure 20. Pharyngeal arches and teeth of goldfish (top), cross, and carp



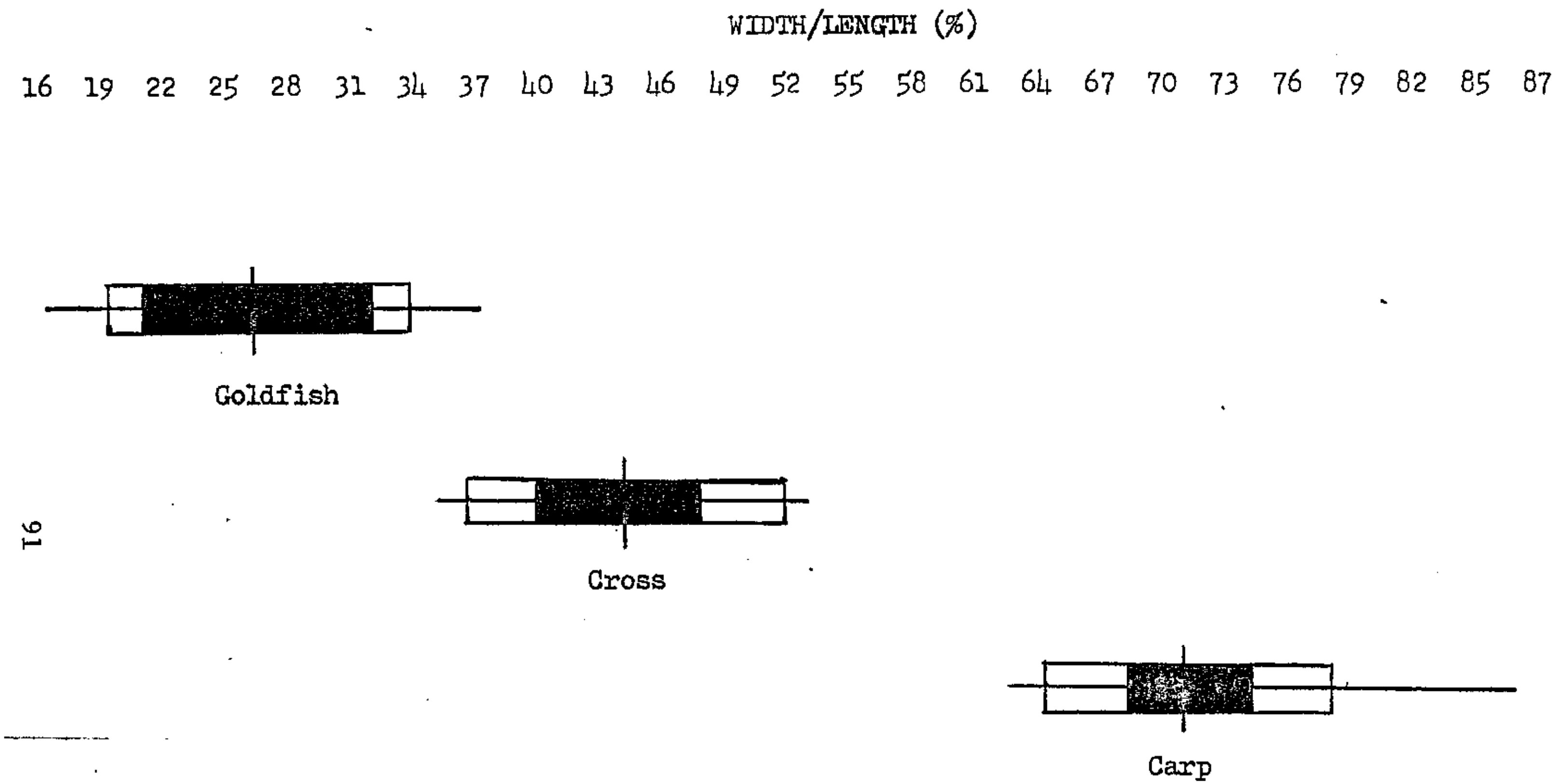


Figure 21. Statistical relationship of width to length pharyngeal tooth ratios, goldfish, crosses and carp. Statistics same as Figure 15. The large center tooth was measured in the carp and the largest of the two center teeth in the crosses and goldfish.

(j) Vertebrae

Vertebral counts made on a sample of 40 skeletal preparations are listed in Table 8. The number of vertebrae from Lake Erie carp ranged from (36-39)* model 38, goldfish (28-31) mode 30, and hybrids (28-36) mode 34. The number of vertebrae in hybrids is variable and overlapped considerably with both extremes, again indicating introgression rather than simple hybridization.

---

* Weberian complex and urostyle included

Table 8. Frequency table of vertebral counts

	28	29	30	31	32	33	34	35	36	37	38	39
carp									2	3	10	3
crosses	1	1	1				9	2	2			
goldfish	1	2	4	3								

PART III--AN ANALYSIS OF ISOLATING MECHANISMS: THEIR ROLE IN INTROGRESSION

Hybridization is the result of a breakdown in the isolating mechanism. A breakdown may be initiated by natural cuases or result from human manipulations. Isolating mechanisms described after Mayr (1963) can be defined as prezygotic and postzygotic factors which prevent the interbreeding of populations that are potentially sympatric. The mechanisms of importance and the ones to be considered in detail are:

1. Premating Mechanisms
   a. seasonal and habitat isolation
   b. behavioral isolation
   c. mechanical isolation
2. Postmating Mechanisms
   a. gamete mortality
   b. zygote mortality
   c. F_1 hybrid sterility

The fundamental difference between the two types of isolating mechanisms is that premating mechanisms prevent the wastage of gametes while postmating factors prevent the formation of sexually reproducing individuals. Hybridization results in a breakdown of one or more of these mechanisms. Each of these will be evaluated as contributing factors to hybridization and introgression in the following sections.

A. Postmating Mechanisms

1. Methods and Justification

(a) Gamete Mortality

If gametes are capable of fertilization over long periods of time then sperm driftage could account for the production of F_1 hybrids (Childers, 1967). Thus, if fish spawn in close proximity accidental fertilization could occur. Gamete mortality acting as an isolating mechanism apparently reduces the chance of hybrids being produced by limiting the lifespan of gametes swept downstream (Hubbs, 1961). Greenfield et al. (1973) attributed a breakdown in this mechanism as the probable cause of crosses between Notropis cornutus and Phoxinus erythrogaster. Hubbs (1957) found that Percina caprodes, a fish with frequent hybrids had a longer functional sperm lifespan than Etheostoma spectabile, a fish with fewer hybrids. He concluded that sperm viability may affect natural hybrid frequency. Childers (1967) found that the eggs of Lepomis gulosus remained viable for as long as three hours after contact with water. If this were true those eggs spawned but unfertilized, about 50% in carp (Swee and McCrimmon, 1966) could conceivably be accidentally fertilized by a different species utilizing the same spawning habitat some time later. Since the spawning season and habitat of the carp and goldfish largely coincide, extended egg viability may result in the chance production of hybrids.

Two species that are sympatric are believed to develop isolating mechanisms that reduce the chance of hybridization, but once separated geographically this function is lost. Hybridization is often of this type, resulting from two previously allopatric populations extending their ranges until overlap occurs. Greenfield (1972) attributes the cross of Gila orcutti and Hesperoleucus symmetricus to be a result of this type of hybridization. Hubbs (1955) and Woodruff (1973) have suggested that this is the case with the carp and goldfish in Lake Erie, but since sufficient evidence for this theory is lacking, this approach is doubted as the sole cause of hybridization.

Measurements of gamete lifespan are of particular biological significance because they act as isolative mechanisms. The functional lifespan of carp and goldfish gametes was unknown before this study. This information is essential in determining whether active spawning must take place between the carp and goldfish or whether hybridization is just a consequence of using identical spawning habitats with fertilization resulting from sperm draftage. A number of experimental crosses were made in order to delineate this question.

Carp and goldfish were collected by seine and dipnet as they spawned from Lake Erie drainage collection sites 11, 12, and 15. They were transported to the laboratory in 30 gallon metal tanks in well aerated water. All crosses were made within 12 hours of collection to insure that the fish were ripe and maintained in good condition.

A stripping technique similar to Baylock (1971) proved satisfactory. Eggs and milt were stripped into a 500 ml. beaker with 200 cc. of a .6% solution of NaCl in aged tap water with constant swirling. All water was charcoal filtered and aerated at least three days before use. The salt solution prevented the eggs from clumping before they were poured into a series of 20 x 15 cm. plastic petri dishes that contained a depth of 1 cm. of aged tap water. The eggs instantly adhered to the bottom of the petri dishes and were not disturbed for 30 minutes. The salt solution was poured off and fresh water added and then changed twice daily. The eggs were incubated at room temperature (20-23 degrees C.) for three to four days until hatching occurred.

Counting of eggs was simplified by the use of the clear plastic dishes over a dark graph paper background. Counts were generally made at 13 and 48 hours to determine if any embryos had died in development.

The fry were removed as they hatched, counted and moved to larger containers.

Test I: male carp x female carp--aging of gametes

This test was performed to determine if the functional lifespan of sperm and eggs could be a contributing factor causing hybridization. Approximately 1000 carp eggs were stripped into a beaker as described above and poured into five petri dishes without fertilization. At the same time several drops of carp milt were diluted in 60 ml. of aged tap water and aliquoted in 10 ml. portions at the time intervals of .5, 1, 2, 4, and 8 minutes into the dishes of unfertilized eggs. They were left undisturbed for 30 minutes, drained and incubated. The number of normally appearing fry that hatched within 4-5 days were counted and percentage of hatch success calculated. The data are found in Table 9.

Test II: male goldfish x female goldfish--aging of gametes

The procedure was similar to the previous test cross except that goldfish gametes were aged. The data are listed in Table 10.

Test III: male carp x female goldfish--aging of eggs only

Eggs were stripped into a beaker and poured into petri dishes and aged. Fresh milt was then added to the eggs at intervals of .5, 1, 5, 10, 30, and 60 minutes, incubated until hatched and counted. The data are listed in Table 11.

(b) Zygote Mortality

The second postmating mechanism of importance is zygote mortality. Zygote mortality as an isolating mechanism is obviously not operating fully since hybrids are present, but the full extent of its role in limiting hybrid production was unknown before this study. In order to examine the influence of zygote mortality a number of reciprocal crosses were made.

Male and female fish were obtained and handled as before and then stripped simultaneously into a 500 ml. beaker. Fertilization was immediate and eggs were poured into petri dishes. Four different crosses were made: male carp x female goldfish, male goldfish x female carp, male goldfish x female goldfish, male carp x female carp. The normal appearing fry that hatched within 4-5 days were counted with results tabulated. Four or five replicates were performed for each cross with results combined.

(c) Hybrid Sterility

Sterility in hybrids is the rule rather than the exception. This is partially true in the case of F_1 carp-goldfish hybrids. Makino et al. (1958) reported that male hybrids were completely sterile while females were fertile. The extensive crossing experiments of Ojima et al. (1975) since 1945 support this contention. They both found that meiosis in the male hybrid was greatly disturbed being arrested at early states of meiotic prophase. Introgression could occur if the female hybrids spawned with either parental but would be much more widespread if both sexes of the cross were involved.

To determine if male crosses in Lake Erie were fertile, a number of specimens, collected with the aid of commercial beach seiners, were examined in June, 1976 (Lake Erie site no. 14).

(2) Results and Discussion

(a) Gamete Mortality

Carp and goldfish gametes are characterized by a short functional lifespan in water (Tables 9-11). All capacity for fertilization was lost within one minute exposure to water. This conforms with other investigations. Childers (1967) found that the functional lifespan of 3 species of sunfish was less than 1.1 minutes. Hubbs (1961) found that sperm function decreased linearly with time and ceased to function in as little as 25 seconds in *Etheostoma lepidum*, 15 seconds in *Etheostoma spectabile* and 21 seconds in *Percina caprodes*.

The functional lifespan of carp and goldfish eggs in water was limited also, less than 1.5 minutes. There is much more variation in the length of egg viability than sperm viability in other fishes. Hubbs (1957) stated that fertilization was reduced in *Etheostoma spectabile* after 180 seconds, while Childers (1967) found eggs viable in *Lepomis gulosus* for as long as 3 hours. Buss (1966) stated that the lifespan of eggs was approximately equivalent to the time needed for the eggs to water harden and swell to maximum size. The rate of water hardening is extremely rapid in carp and goldfish, normally less than two minutes, thus accounting for their short lifespan.

It is apparent that the functional lifespan of eggs and sperm in carp and goldfish acts as an isolating mechanism described in other fishes. This mechanism is not foolproof and often results in the accidental production of a few hybrids between two fish using the same spawning habitat. Carp and goldfish swarm into similar spawning

9. male carp x female carp

Age of eggs (minutes)	Age of sperm	# eggs	% hatch
.5	.5	210	18
1	1	240	0
2	2	220	0
4	4	260	0
8	8	220	0

10. male goldfish x female goldfish

.5	.5	281	40
1	1	160	0
2	2	151	0
4	4	150	0
8	8	189	0

11. male carp x female goldfish

1	0	224	26
1.5	0	200+	0
5	0	200+	0
10	0	200+	0
30	0	200+	0
60	0	200+	0

Tables 9-11. Tests I-III Duration of fertility of eggs and sperm as postmating mechanisms.

grounds and under favorable weather conditions spawn under crowded conditions accounting of a few chance unions of egg and sperm, but massive hybridization has never been attributed to chance occurrences, but rather to more drastic changes in isolating mechanisms. Even though two fish may spawn simultaneously over the nest of a third species an extraordinary low number of hybrids, less than 1%, are accidentally produced by sperm driftage. Certainly 1% of the carp-goldfish hybrids could be produced accidentally in a similar manner but the large numbers observed could not be accounted for. In conclusion, sperm driftage and the short functional lifespan of eggs is successfully acting as an isolating mechanism and is not solely responsible for the large number of hybrids in Lake Erie.

(b) Zygote Mortality

The first reciprocal cross of male carp x female goldfish resulted in a 90% hatch (Table 12). The 10% mortality can be broken down into (1) early mortality; those eggs which failed to be fertilized or those that died within 24 hours after fertilization, (2) late mortality; those eggs that were fertilized but died during the time interval designated as that period between 1 day after fertilization and 1 day after hatching, about 3-4 days. Only 16% represented zygote mortality 1 day after fertilization with the remaining 84% representing the lack of fertilization or an interference with early cleavage.

The second cross, male goldfish x female carp resulted in a 94% hatch (Table 13). The 6% mortality can be divided into 30% early mortality with the remaining 70% occurring 1 day after fertilization.

12. male carp x female goldfish

	# eggs	mortality early	mortality late	% hatch
replicates 1	170	11	3	93
2	240	16	5	91
3	220	14	4	92
4	310	21	9	91
5	180	22	7	84
Total	1120	112		90

13. male goldfish x female carp

	# eggs	mortality early	mortality late	% hatch
replicates 1	174	7	2	95
2	187	3	2	98
3	136	7	1	95
4	178	10	8	90
5	137	5	4	94
Total	812	49		94

14. male goldfish x female goldfish

	# eggs	mortality early	mortality late	% hatch
replicates 1	220	6	8	94
2	170	16	11	94
3	250	12	3	85
4	160	4	2	94
5	280	10	5	95
Total	1080	77		91

15. male carp x female carp

	# eggs	mortality early	mortality late	% hatch
replicates 1	319	11	8	94
2	240	16	18	86
3	262	2	5	97
4	275	10	15	91
Total	1096	86		93

Tables 12-15. Reciprocal crosses and controls in relation to zygote mortality as a postmating mechanism.

The third cross, a control, male goldfish x female goldfish resulted in a 91% hatch (Table 14). The 9% mortality is divided into 37% early and 63% late. The fourth cross, also a control, male carp x female carp, resulted in a 93% hatch with 45% early mortality and 55% late (Table 15).

The data show a relatively high rate of hatching for the reciprocal crosses as well as for the controls. Neglecting early mortality which is partly a result of the two gametes failure to meet, hatching success was in excess of 95% overall. Since zygote mortality is low, less than 10% for all crosses, zygote mortality is not acting as an isolating mechanism. Thus, this mechanism has been broken down or possibly never existed and could be an important factor contributing to the production of hybrids if the appropriate gametes should meet under natural conditions.

(c) Hybrid Sterility

Out of 30 males examined, 2 extruded milt after applying pressure to the abdomen. These fish were returned to the lab and proved to be the first reported spermatocyte producing hybrid males. Microscopic examintion at 1200 x oil immersion revealed actively swimming sperm. The duration of active swimming of the sperm was about two minutes in water, but could be extended to ten minutes when no water was added to the slide mount. Fribourgh (1966) stated that motility is unquestioned as evidence that sperm is living. On this basis the spermatocytes were believed to be viable, but no artificial crosses were attempted. Gross structure of the spermatocytes appeared normal and did not differ from the descriptions of the spermatocytes of the goldfish and carp by Fribourgh (1970). The sperm head was spherical about three microns in diameter with an axial filament of about sixty microns (Figure 22). Descriptions of ultrastructure were not attempted.

Examination of the testes of the fertile male revealed no external abnormalities, however intersexuality has been reported in related hybrids (Makino, et al., 1958) and observed in several instances in hybrids taken from Lake Erie. Makino associated this intersexuality with sterility, however, monoecious carp have also been reported and self fertilization demonstrated experimentally (Gupta and Meske, 1976). The testes of the fertile male hybrid were sectioned at six microns and stained with Heidenhain's Iron Hematoxylin. Figure 23 shows mature sperm in ducts. Spermatogenesis in the anterior one third of the testes had apparently slowed or stopped because few developmental stages were observed. The hybrids had been held at room temperature in tanks for two months before sectioning and spermatocyte production may have ceased, however, male carp and goldfish held under the same conditions continued to produce sperm. Since F_1 male hybrids and early backcrosses have all been reported as sterile by previous researchers, the occurrence of a fertile male provides evidence for longstanding introgression, perhaps since their introduction into Lake Erie in the 1880's. This provides additional evidence that the first introductions in Lake Erie may have contained crosses.

The appearance of male hybrid fertility signifies that another isolating mechanism is broken. The full extent of fertility is unknown but, with full hybrid sterility as an isolating mechanism removed,

Figure 22. Photomicrograph of spermatocytes from male hybrid, a suspected backcross (scale--3.3 mm. app.=1 micron)

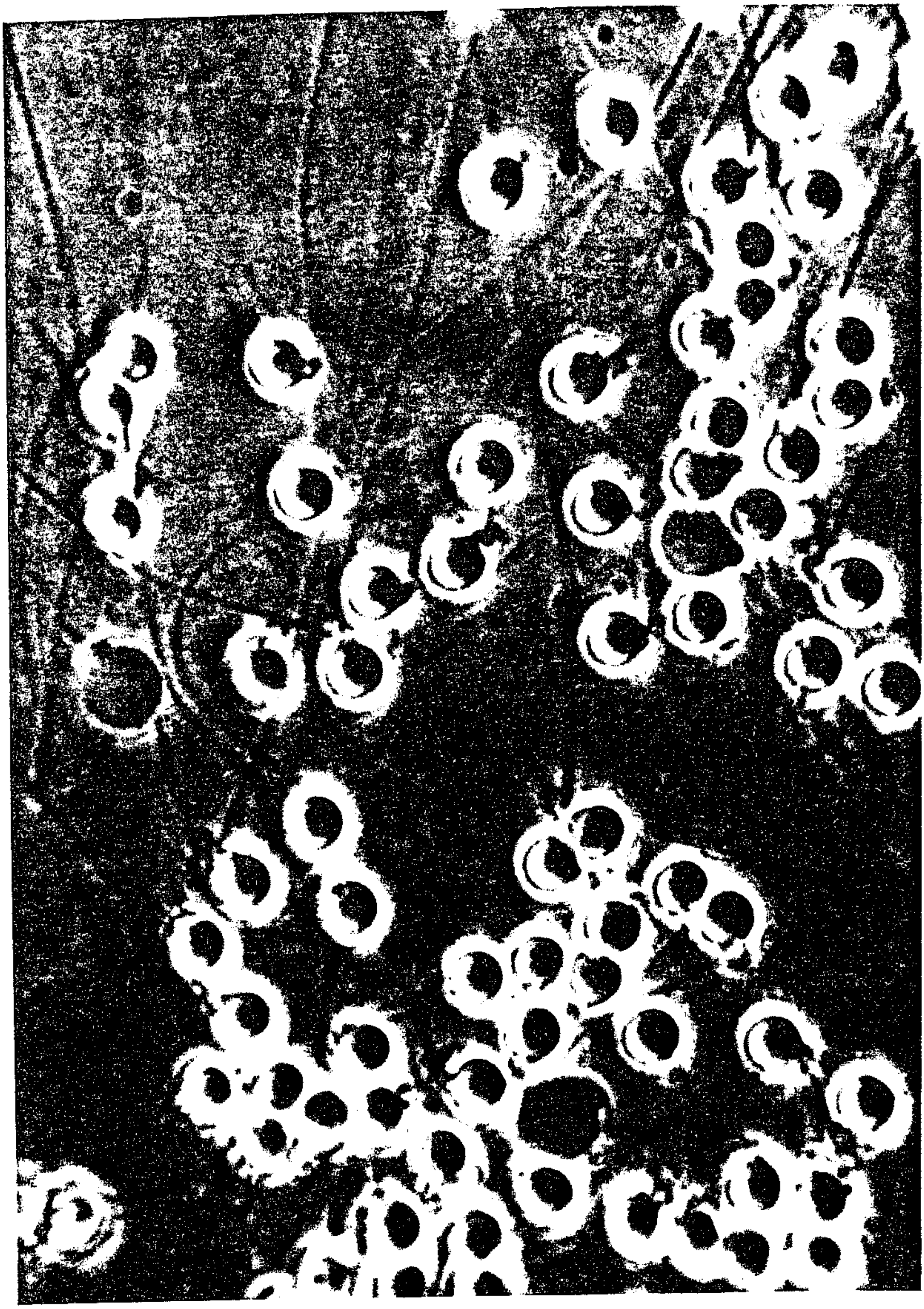
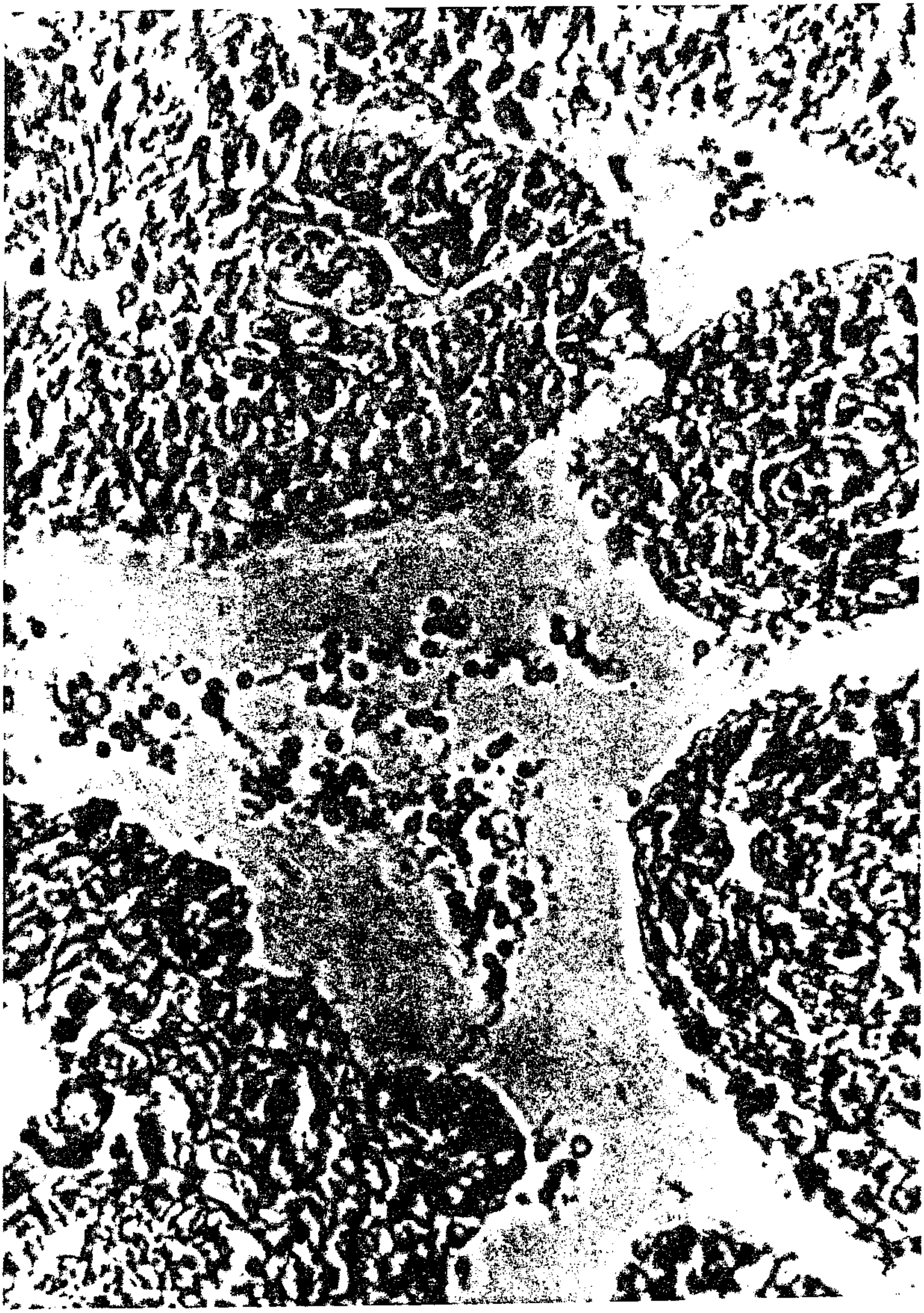


Figure 23. Photomicrograph of a 6 micron testes section showing spermatocytes in ducts (scale 1.2 mm. app.=1 micron)



massive integration is possible. Only a small percentage of the male crosses are believed to be fertile at this time, however, if introgression continues their numbers will increase.

(3) Summary

1. The three postmating mechanisms that normally act preventing hybridization have been analyzed.

2. Gamete mortality is the only mechanism that is still operative, thus ruling out the possibility that the large number of crosses in Lake Erie were produced accidentally.

3. Hybrid zygote mortality is inoperative as an isolating mechanism. Reciprocal crosses as well as controls resulted in greater than 90% hatching success.

4. Fertile male hybrids of carp and goldfish have never to my knowledge been reported previously, however they were found during this investigation indicating that hybrid sterility as an isolating mechanism is inoperative.

5. The inoperative mechanisms are important factors contributing to the production of hybrids if the appropriate gametes should meet under intentional and not accidental circumstances.

B. Premating Mechanisms

(1) Methods and Justification

Premating mechanisms are economical of gametes and are more efficient than postmating mechanisms (Littlejohn, 1969). Subsequently, a breakdown in premating mechanisms creates a greater potential conducive to massive hybridization. Previously it was established that two of the three postmating mechanisms were inoperative in respect to their activities of maintaining genetic integrity and were potential causes of hybridization, but without intentional simultaneous spawning few hybrids would be produced. This indicates that postmating mechanisms alone could not account for massive hybridization in some areas of Lake Erie today and that other factors must be involved. Addressing this question entails a detailed analysis of the three major premating mechanisms: (a) seasonal or habitat isolation, (b) behavioral isolation, (c) mechanical isolation. The following observations were based upon field studies conducted during the spawning seasons of 1975-76.

(2) Results and Discussion

(a) Seasonal Isolation

Two species of closely related fish may reduce the chance of hybridization if their reproductive seasons are isolated temporarily. Even if they share common spawning seasons they may segregate into entirely different spawning habitats. A review of the spawning characteristics of carp and goldfish reveals many similarities. Both species are characterized by an extended intermittent spawning season. Goldfish spawning beginning in April and continuing as late as August (Battle, 1940) overlaps with the spawning season of carp in the Bass Islands area of Lake Erie. Swee and McCrimmon (1966) in a study of the reproductive biology of the carp reported that spawning occurred between 16 May and 1 August in Lake St. Lawrence, Ontario. They suggested that female carp may spawn more than once a season on the basis of a 20% retention of green eggs in fish collected immediately after spawning. Other authors are in general agreement. Shikhshabekov (1972) stated that wild carp in Arakum Lake, Soviet Union, had a protracted spawning period of three months with females producing two batches of eggs per season under favorable conditions. Ivanov (1971) found in Lake Balkhash, Soviet Union, that carp four to six years old spawning for the first time most frequently laid two batches of eggs per season; fish six to twelve years laid three batches of eggs per season, and fish thirteen to nineteen years laid one or two batches of eggs per season. Fedotova (1971) reported that each batch of eggs was laid at intervals of seven to twenty days dependent largely on meteorological conditions. Swee and McCrimmon (1966) found as many as 18% of the carp collected in September of 1965 from Lake St. Lawrence contained nearly ripe ova although the spawning act was not observed later than August 1. These fish probably represent young females, age two to three, that had not previously spawned. These late maturing females probably reabsorb the eggs and regenerate them the following spring at normal spawning times.

Fish from this study were found to retain as much as 20% of their egg mass even with artificial stripping again indicating that further spawning is possible. All females collected from spawning habitats in April and May were fully ripe or nearly so and extruded eggs upon handling whereas females collected offshore showed ovaries in a much earlier state of development indicating differential rates of maturity and an extended spawning season.

Males normally remain in breeding condition longer than females. Male carp generally are found to be ripe from early Spring until September (Skikhshabekov, 1972; Fedotova, 1971). In this study male carp were found to be ripe in all months collected, March through November, whereas male goldfish were ripe from March through September.

These data indicate that carp and goldfish produce several batches of eggs per season and are stimulated to spawn over a period of at least 90 days. So in conclusion, the spawning seasons of these two fish are not separated temporally, thus seasonal isolation is non-functional and not reducing the chance for hybridization.

Habitat Isolation

The spawning habitats of carp and goldfish do not substantially differ either. The spawning habitat of carp and goldfish is in shallow water, usually less than one meter and preferably in less than 30 centimeters of water. Stacey and Liley (1974) found that goldfish enter floating vegetation to release eggs while carp eggs were spawned over a variety of vascular plants, aquatic and terrestrial, as well as algae. Swee and McCrimmon (1966) reported that carp spawned over rushes, reeds, and willow grasses, while Fedotova (1971) described the spawning habitat simply as meadow vegetation. In fact, the requirements for spawning were so general that it prompted Yamazak (from Stacey and Liley, 1974) to state that the only requirements for successful spawning of female goldfish was green plants and males. However, green plants are not essential and any artificial substrate capable of having adhesive eggs attached to it is suitable.

I found in this study that the spawning habitat of carp and goldfish could be best described as any locally abundant vegetation. Although all spawning was observed in shallow water there is some question as to whether carp and goldfish can spawn in beds of floating algae overlying deep water. Carp have previously been observed spawning in beds of floating _Myriophyllum_ in small lakes in Ohio and some goldfish were observed spawning in beds of dense _Cladophora_ along shorelines of small pools. Goldfish have also been observed to spawn along _Cladophora_ covered breakwalls in deep water. The recent appearance of great masses of floating algae, especially bluegreens, offer a suitable substrate for egg attachment and probably could support the breeding activities of a sizeable portion of the carp and goldfish in Lake Erie. This habitat is probably not utilized in the Western Basin of Lake Erie because of the presence of the extensive marshes along the southern shore of Lake Erie between Toledo and Sandusky.

In conclusion, the spawning habitat of the carp and goldfish largely coincide, thus there is no habitat isolation acting as an isolative mechanism preventing two potential mates from meeting. Hybridization is the culmination of the failure of several isolating mechanisms. Of those already discussed only one, gamete mortality, is still functional. The remainder, zygote mortality, hybrid sterility, and seasonal and habitat isolation, although non-functional and contributing to hybrid production are of little importance or consequence unless the last of the mechanisms to be considered, behavioral isolation, is also non-functional. Many fish spawn simultaneously over identical habitats and even in the same nest and produce few if any hybrids. These fish prevent hybridization by maintaining rigid premating behavioral cues that allow only species specific pairing. When behavioral cues reproductively stimulate another closely related coexisting species on spawning grounds, interspecific or intergeneric pairing can take place resulting in the production of large numbers of hybrids. With several mechanisms already discounted this leaves only one mechanism accountable for hybridization.

(b) Behavioral Isolating Mechanisms

Although the breakdown of behavioral isolating mechanisms is believed to be the leading cause of hybridization it is the most difficult mechanism to analyze. This is particularly true in fish because observations and manipulations under natural conditions are compounded by a living medium that is not adaptable to the experimental techniques of terrestrial ethologists. Although recent advances have added much to our knowledge of the behavior of a few fish species there are no broad generalizations that can be applied to the study of other species. Even in the goldfish which has been domesticated and observed as long as any aquatic animal, very little is known about reproductive regulatory mechanisms. Only recently Whiteside and Richan (1969) correlated a chemical substance released into the water of closed ponds with inhibiting the maturation of gonads under crowded conditions. Similar chemical substances have been postulated, but never isolated, as controlling the growth rates of some pond cultured fish. Even less is known about pheromones released into the water that may control premating reproductive behavior. Field observations as I have made provide more useful information than any other technique available but results at best lead only to speculation at causal relations. Much more research in this area is needed and will likely lead to advances not only in animal behavior but also in applied fields of aquaculture.

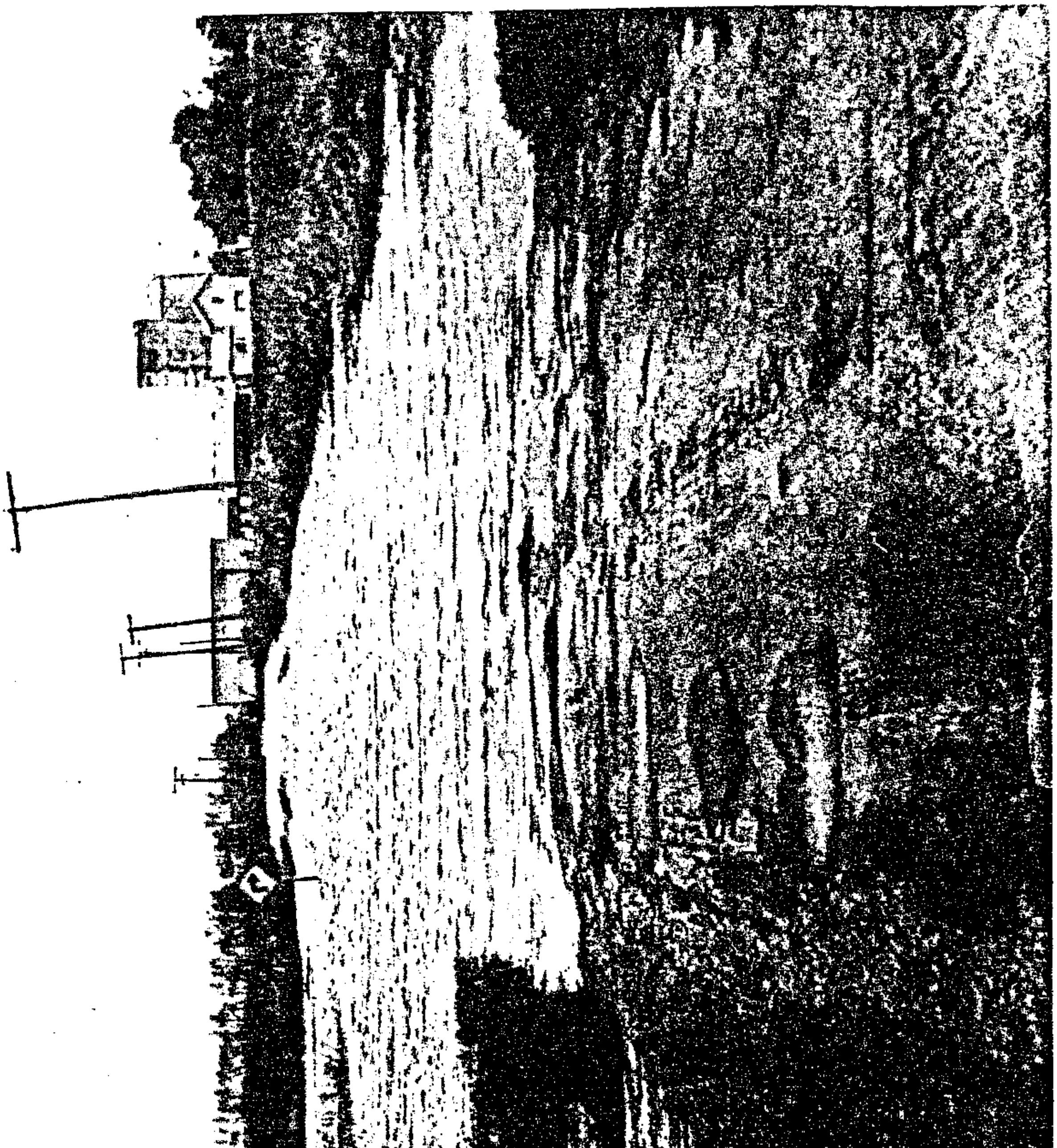
The analysis of behavioral mechanisms begins with the stimulus that drives potential mates to the spawning grounds. Temperature has previously been regarded as initiating and controlling the movement of carp and goldfish on the spawning grounds (Swee and McCrimmon, 1966). They reported that spawning was not observed at water temperatures below 17 degrees C and reached an optimum at 19 to 23 degrees C, decreased at temperatures above 26 degrees C and ceased at 28 degrees C. The preferred spawning temperatures reported by other authors approximate this: Shikhshabekov (1969) 17-25 degrees C, Nikolskii 18-20 degrees C, and Beamish 24-25 degrees C from Swee and McCrimmon (1966).

In all cases spawning was not observed at temperatures less than 17 degrees C. A much different situation was observed in the Western Basin of Lake Erie. The most intense spawning activity of the year occurred on April 24 at a water temperature of only 10 degrees C, substantially lower than any temperature previously reported. The difference is significant because it reveals a primary stimulus that initiates spawning which had been previously obscured by temperature. The existence of flooded land, not only temperature, stimulated the massive movement and spawning of carp and goldfish 7 degrees C lower than previously reported.

The periodic flooding that stimulates spawning results from seiche activity in the Western Basin of Lake Erie. Every spring much of the low lying area between Toledo and Sandusky, Ohio is flooded creating ideal spawning habitat. A study site from this area was selected two miles east of Whightmans Grove, Sandusky Co., Ohio (Lake Erie site no. 11).

On April 24, 1976, water levels in southwestern Lake Erie were rapidly rising due to a 30-40 mph wind out of the northeast. Water levels rose and reversed the flow of streams emptying into Sandusky Bay. On Arpil 24 at 4 p.m. the reversed tributaries reached flood level inundating low lying areas. Within a matter of minutes the rising waters had flooded the county road running east out of Whightman's Grove (Figure 24). Large goldfish and carp moved with the advancing water up the reversed tributaries of South Creek and onto

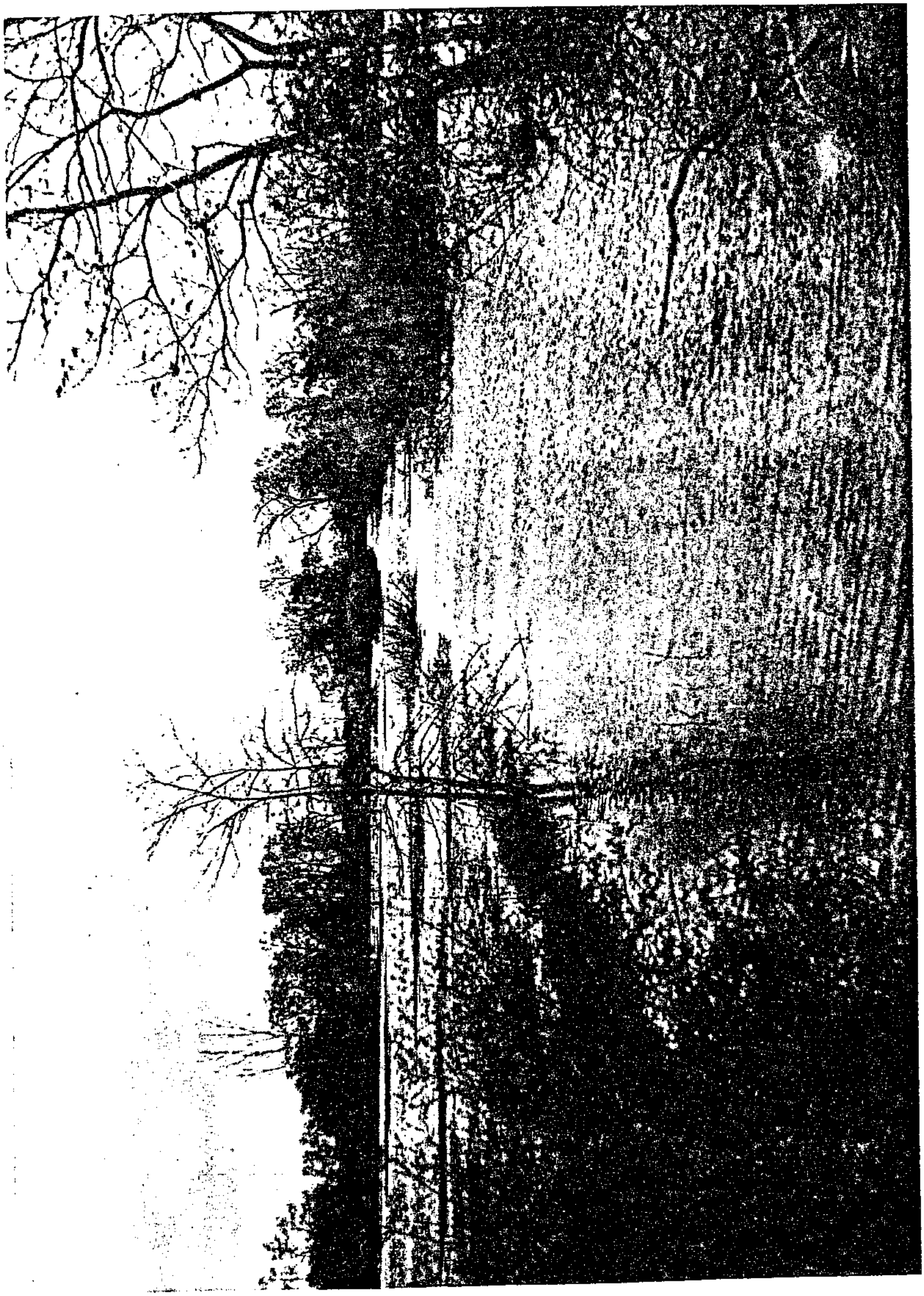
Figure 24. The rapid advance of water quickly flooded much of the low lying area adjacent to South Creek, a tributary of Sandusky Bay.



the flood plain even though the water was under 20 centimeters in depth. Spawning had not yet commenced but fully ripe male and female fish extruded gametes upon handling. Waters continued to rise for another 24 hours flooding the area with .3 to .5 meters of water. Massive spawning commenced the following morning and continued throughout the day. Literally millions of carp invaded the shallows shown in Figure 25. Spawning densities were estimated at 5-10 fish per square meter over several hundred hectares. Spawning occurred in aggregations with typically one or two large females surrounded by one to ten males of equal or smaller size. Limited sampling of these aggregations revealed only carp, no goldfish, and a few hybrids. Direct spawning was observed by Miles Coburn (Ohio State University) between a goldfish and either a carp or a hybrid during the same seiche at Mentor march. Observations of spawning behavior were impossible at this location due to high turbidities and more detailed observations were made on a later date. Spawning continued as long as the water was rising or stable, but was abruptly halted as the fish immediately returned to feeder streams when the water began receding. Seiche activity is highly variable but, within four days the water had returned to normal levels (Figure 26). Only a few isolated pools remained in the formerly flooded area and remarkably few carp were stranded considering the shallow waters they had spawned in. Goldfish, however, preferring a slightly different habitat, often were trapped in isolated pools. The goldfish preference for the slightly quieter waters of marginal pools with no current often led to their entrapment during the spawning season. Exact mortality figures were not estimated but a sizeable number of goldfish are

Figure 25. Widespread flooding created optimum habitat adjacent to this ditched tributary of South Creek.

Figure 26. Four days after the initial seiche, water levels had returned to normal levels. The same area as Figure 25.



stranded each year. Isolated pools that remain for several days after each major seiche almost invariably contain many goldfish. Some may escape back to deeper water if another seiche inundates their small refuge, but most die as those shown in Figure 27. This pool had been covered with 1-2 meters of water 5 days previously, but was completely dry about 6 hours after this picture was taken.

Much more drastic than this is the massive mortality that takes place when the spawning grounds are drained before the spawned eggs hatch. The adaptive significance of carp and goldfish laying eggs in extremely shallow water resulting in high mortality from water level fluctuations appears to be poor strategy, yet upon closer examination reveals a highly successful method of fry production. The spawning sequence begins with the fish stimulated by rising waters to move to freshly inundated vegetation, much of it in less than 10 centimeters of water. Many of the fertilized eggs perish as the water recedes but some in low depressions near the water's edge hatch and survive. Their isolation in pools provides them with ideal habitat for the first few weeks (Figure 28). They are supplied with an abundance of plankton from the decay of land vegetation, and protection from fish and insect predators, thus greatly increasing their chance of survival in the first few weeks of critical postlarval development. These pools often are reflooded by periodic seiche activity releasing the fry into permanent waters when they are larger and less vulnerable. Often, if the spawning grounds are flooded for several days the eggs will hatch and the fry will return immediately with the receding water. Maximum success of this type of spawning strategy could be achieved if the length

Figure 27. Most of the spawning carp return immediately to deep water as the seiche recedes, however, goldfish are often trapped and perish in the small isolated pools as shown.

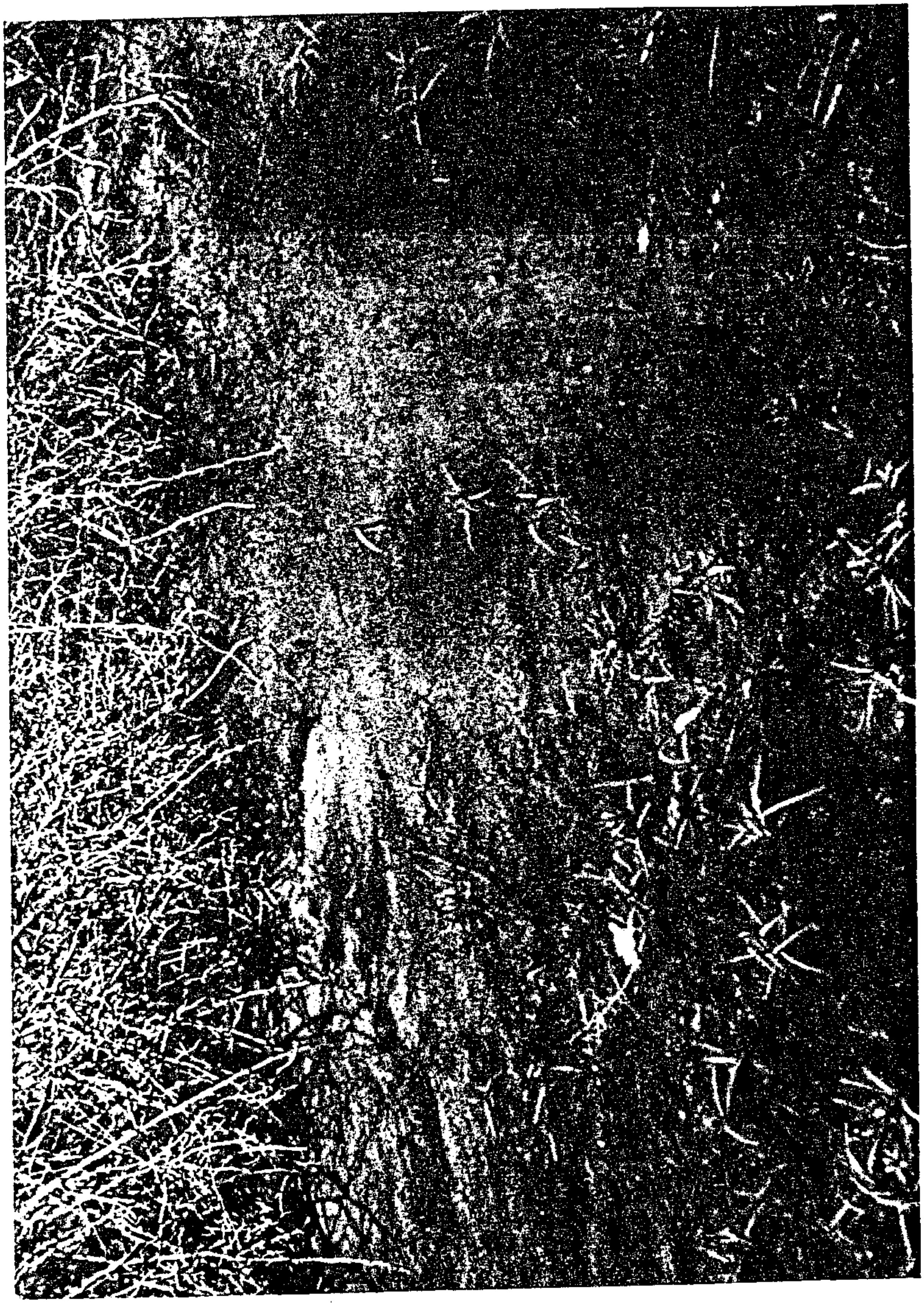
Figure 28. Many of the fry hatch in the protected areas of isolated pools as shown and move to deeper water (shown in background) when water levels fluctuate.

of time required for the eggs to develop was timed to the duration of the seiche. Battle (1940) found that goldfish eggs hatched in about 50 hours at 29 degrees C and Swee and McCrimmon (1966) found that most carp eggs hatched within 3-6 days at temperatures 11-23 degrees C. This indicates that the optimum period of flooding, allowing most of the eggs to hatch, would be at least five days.

More detailed observations of spawning behavior were made during another period of high water on May 14, 1976, in the Crane Creek St. Park (Lake Erie site #13 p. 28). Flooding was less extensive, thus spawning was not as intense as the activity on April 24. Spawning aggregations were smaller and often consisted of only two fish. The mechanisms initiating and perpetuating the spawning act involve visual and more importantly tactile responses. The male possesses tubercles on the head region that contact the female during the spawning act but, due to their small size, any direct tactile stimulus by the tubercles alone is doubted. The female responds to contact by lifting her body slightly, lashing her tail violently, propelling herself forward, releasing hundreds of sticky eggs. Simultaneously the male with similar violent movements releases milt and fertilizes the eggs (Figure 29). The water where spawning takes place is often so shallow that the fish have to swim on their sides. Observations are extremely difficult because the splashing quickly stirs up bottom sediments and obscures all view (Figure 30). As groups became exhausted the intense spawning was characterized by short periods of rest. Often exhausted females would head for cover in vegetation with fresh unspawned males in hot pursuit. These females were often so thoroughly exhausted that they

Figure 29. Spawning activity in carp showing the approach of a male from the rear.

Figure 30. The spawning fish (backs exposed) move to shallow water, often stirring up bottom sediments.



could be approached and lifted from the water by hand. After resting for several minutes to hours they would return and again commence spawning. This sequence would be repeated until all ripe eggs were spent.

The spawning of the goldfish was similar to that of the carp. Other than the reduced intensity of spawning due to the smaller size of the goldfish there were few differences observed. Spawning was initiated by visual contact. Males were able to detect females at a distance of several feet in clear water. Unlike the carp there are no color differences between the males and females so visual sex recognition might involve the sighting of the females extended abdomen. Once a female was sighted, one male, quickly followed by other males, would actively pursue a female who would flee upon sighting the males. The chase would continue a short distance (3-5 meters) until the female would bury herself in a bed of dense vegetation. One or more of the males would make contact in the same manner as the carp with the female responding by rapidly vibrating her tail and releasing eggs. The female would remain hidden in the vegetation for several minutes while the males would return to open water.

Due to the extremely turbid water caused by the spawning activity, I was unable to observe direct spawning between carp and goldfish. The intergeneric fertilization that occurs could result from direct pairing between a single goldfish and carp. Under confined conditions the male of each species can initiate spawning in the other species, but under natural conditions males probably enter swarms where spawning activity has already been induced. Once spawning is initiated in a pure swarm of carp smaller males often join and participate. Once spawning has commenced it allows the entrance and joint spawning of male goldfish with the female carp in the swarm. In this manner the goldfish need not initiate spawning by displaying appropriate stimuli, but must still exhibit correct stimuli to prevent the breakup of the spawning swarm. The threshold of stimuli necessary once spawning has commenced is probably much less than that required to initiate it so crossing would be easily accomplished in this manner. Likewise smaller male carp stimulated by similar reproductive behavior could enter spawning swarms of goldfish, display the appropriate mechanisms to prevent the breakup of the spawning swarm, and fertilize eggs.

Both reciprocal crosses would be produced by this method. Since there is much overlap in the mature size of the carp and goldfish both reciprocals should be produced in equal numbers.

Major differences in the reproductive behavior of the carp and goldfish of Lake Erie were not detected. However, without the direct observation of sympatric populations of carp and goldfish that do not hybridize, it is impossible to detect the exact changes in reproductive behavior that led to a breakdown of behavioral isolation. For reasons outlined earlier especially through confinement under domestication, the carp and goldfish in Lake Erie lost the behavioral mechanism that prevents crossbreeding. Ethological isolation is based upon the reception of stimuli by sex partners and is a result of an interaction between external stimuli and internal drives. Under confined conditions when an appropriate sex partner may not be available internal drives continue to build until mating can be induced by an inappropriate stimuli of another species. If there is a genetic basis for this behavior it will be inherited like any other character and may appear intermediate. An intermediate inheritance of reproductive behavior will be transmitted down through subsequent generations further enforcing the lack of isolation between these two fish. This compounds itself through introgression and both species can gradually mix until a third species is formed and the parentals are lost. Assuming the large numbers of introgressed fish in Lake Erie, this thesis could be the documentation of the formation of a new species. If so, it raises a number of significant questions relevant not only to biologists but also to sportsmen, commercial fishermen, and governmental agencies. Are the parentals being lost? Have the introgressed fish been isolated reproductively from their parentals? What are the ecological ramifications of the production of a fish with intermediate feeding habits? The carp and goldfish are the most visible fish in Lake Erie and may soon be replaced by a fish that shares common attributes of both. Although natural hybridization in the carp and goldfish was known as early as the 1920's much more research is needed to fully answer these questions.

(c) Mechanical Isolation

Mechanical isolation is not a factor influencing hybrid production. Mechanical isolation in fish is insignificant because they nearly all exhibit external fertilization. Although this mechanism is inoperative, it has probably been compensated by a further development of other isolative mechanisms.

Overall size differences could isolate two fish, but it does not in the carp and goldfish since the larger male and female goldfish overlap in size with the smaller mature carp. Carp as small as 250 mm. which extruded milt upon handling, were commonly taken spawning with carp two to four times their own length. Thus, mechanical isolation appears to be of little importance in this problem.

(3) Summary

1. Since postmating mechanisms alone could not account for the massive hybridization in some areas of Lake Erie today simultaneous intentional spawning must occur.
2. Mechanical isolation is not a factor influencing hybrid production.
3. Carp and goldfish are characterized by extended, intermittent, and overlapping spawning seasons, thus there is no seasonal isolation.
4. The spawning habitats of the carp and goldfish largely coincide, thus there is no habitat isolation preventing two potential mates from meeting.
5. Intentional simultaneous spawning between the carp and goldfish is necessary for the production of hybrids.
6. The intergeneric fertilization could result in the direct pairing of a goldfish and a carp, but more likely results in the entrance of a male carp into a spawning swarm of goldfish or the entrance of a male goldfish into a spawning swarm of carp.
7. The breakdown of behavioral isolation is believed to be the leading cause of hybridization.
8. Reproductive isolation was lost when each species accepted inappropriate premating stimuli and spawned under the confined conditions of domestication. The resultant hybrids may possess an intermediate reproductive behavior further reinforcing the lack of isolation between these two fish.
9. Without direct observation of sympatric populations of carp and goldfish that do not hybridize it is impossible to detect the exact changes in reproductive behavior that led to the breakdown

of behavioral isolation.

10. The existence of flooded land, not only temperature, as previously believed, is the primary stimulus controlling the movement of carp and goldfish into the spawning grounds.

11. As long as the preferred spawning habitat, the marshy areas of Lake Erie are available, the major reproductive activities of carp and goldfish are likely to occur in these shallow waters. However, as more marshland is inevitably drained for commercial activities, the beds of algae overlying deep water may play a greater role in perpetuation of carp and goldfish in Lake Erie.

THE RESULTING HYPOTHESIS

The question of introgression and its conceptual framework was the focus of this research. Hybridization and especially introgression is one of the most difficult concepts to explain if the conventional "species concept" is adhered to. Despite much speculation in this area, it has led to many accepted principles that allow conceptual models of hybridization to be produced. The explanation of why natural carp-goldfish hybrids exist is dependent upon whether introgression is possible. It has been suggested but never proven before this study that backcrossing is possible between both sexes of crosses. With this established the hypothesis to be presented would not be easily discounted.

I have discussed several contributing factors that could account for the relative ease of hybridization between carp and goldfish. I have also presented an argument that hybridization today is related to introgression of *Cyprinus carpio* and *Carassius auratus* or *Carassius carassius* confined in ponds during domestication prior to or at the time of the introduction of carp into North America. I have evaluated how the evolution and reproductive barriers could result in introgression. Considering these observations I have proposed a central hypothesis integrating these arguments and thus accounting for the naturally occurring carp-goldfish crosses in Lake Erie. It is based upon the facts:

1. Pure carp and goldfish under longstanding sympatric conditions do not hybridize.

2. Pure European carp were rare with the majority in that region being at least partly introgressed with the crucian carp during pond cultivation in the 1800's.

3. The crucian carp is known to hybridize in Europe with the common carp and subsequent backcrosses with the carp are difficult to distinguish.

4a. Partially introgressed individuals were introduced in the United States in 1831 and 1877 and most subsequent American carp arose from these

or

4b. Pure carp were introduced in 1877, they hybridized in government ponds with goldfish and most American carp arose from these.

In either case (4a or 4b) a fish is produced that is partially introgressed with inferior table qualities explaining why the American carp is an inferior food fish and also accounting for the relative ease of which hybridization can occur today. A hypothesis incorporating this information is presented in Figure 31. This diagram depicts a relationship whereby reproductive isolating barriers between the carp and goldfish are broken by introgression with a closer relative of the carp, Carassius carassius. Thus, Carassius carassius bridged and broke down the isolating mechanisms separating the two species allowing the carp and goldfish to freely hybridize.

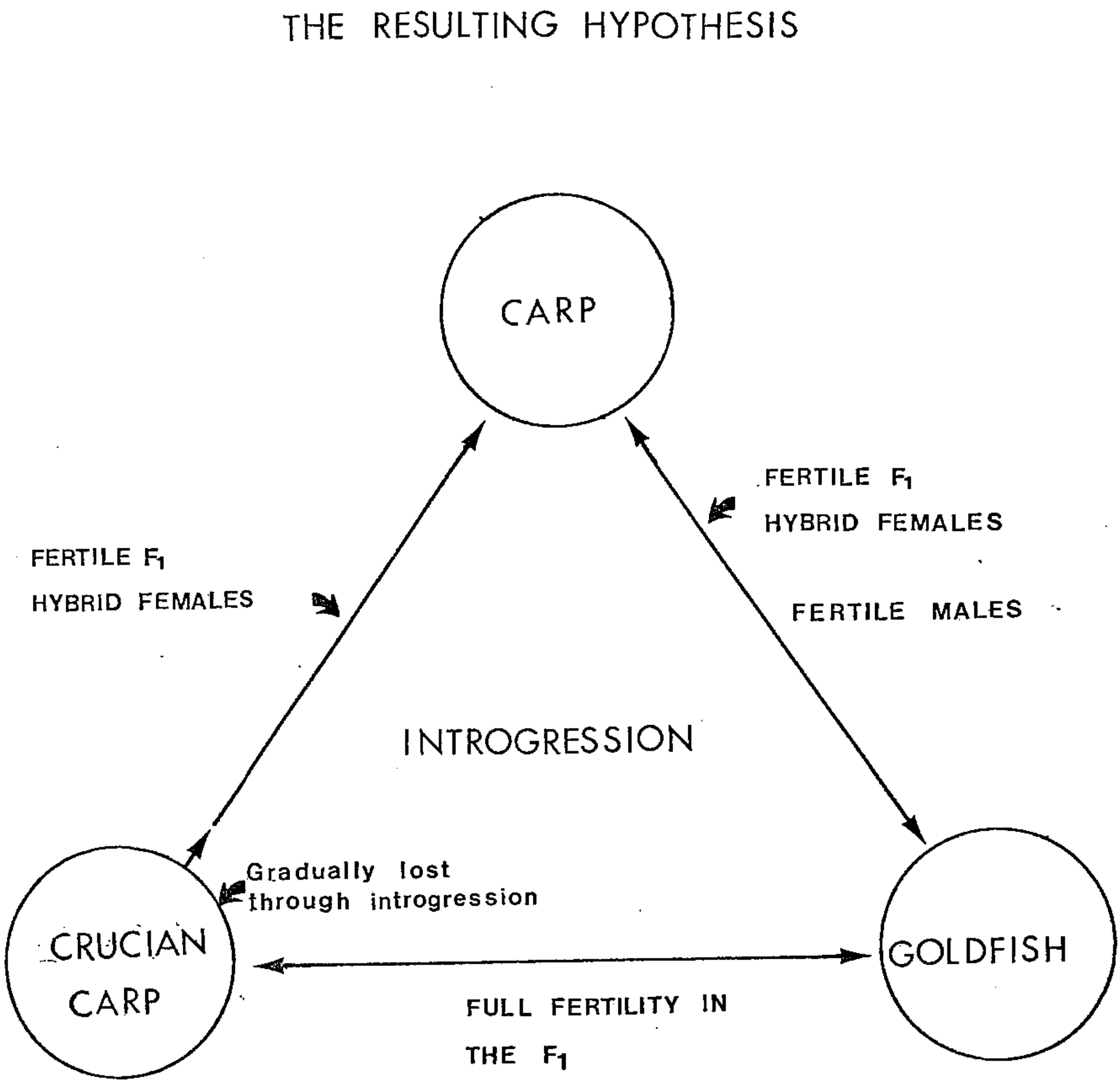


Figure 31. A proposed hypothesis explaining how introgression was initiated by the confinement associated with domestication and early cultivation of the carp in the presence of the crucian carp (Carassius carassius) and or the goldfish (Carassius auratus).

CONCLUSION

A comprehensive analysis of the introgressive hybridization between the carp and goldfish has been completed. The reader is referred to the summaries at the end of each section for major points brought out by this study (pp. 9, 25, 58, 110, 136). The model presented in Figure 4 and the hypothesis presented in Figure 31 have been evaluated. They adequately answer many of the questions generated here. Historical evidence supports my belief that the original stock of carp-like fish brought to the U.S. from which all North American carp were derived contained introgressed fish of the carp x crucian carp or carp x goldfish type. Hybridization was initiated even in longstanding sympatric populations of carp and goldfish through the confinement of domestication. Under confined conditions when an appropriate sex partner was not available, internal drives continued to build until mating was induced by an inappropriate stimuli of another species. The resultant hybrids if fertile transmitted an intermediate reproductive behavior capable of initiating spawning behavior in either of the parentals. An intermediate inheritance of reproductive behavior was transmitted down through subsequent generations further enforcing the lack of isolation between the two species. The initial breakdown of isolation between the carp and goldfish may have been attributed to a closer relative of the carp, Carassius carassius. Thus Carassius carassius bridged the isolative barriers and broke down the isolating mechanisms, separating the two species, allowing the carp and goldfish to freely hybridize. Perhaps we shall never know for sure if the crucian carp was involved since it was probably lost through introgression with the carp within

20 years of its introduction. Nearly all vestiges of the crucian carp would be expected to have long been obscured. However, the malice directed toward the North American carp due to its poor table qualities may have had its origins in an early introgression with *Carassius carassius*.

A thorough analysis of postmating mechanisms has revealed that only gamete mortality was operative. The lack of zygote mortality and hybrid sterility were relevant only if intentional spawning occurred. This implied that the lack of behavioral isolation is the leading cause of introgression in western Lake Erie. Another explanation such as spawning habitat destruction could be easily discounted since more carp and goldfish habitat is present now than ever as shown by their rapid increase in distribution during the last 20 years. Hybridization as a result of the preponderance of one species was also be discounted since both are mutually temporally on spawning sites.

Statistical analysis of morphological measurements further confirmed my hypothesis of introgression. Principal component analysis using 15 characters revealed that the carp, goldfish, and crosses were represented by 1 single homogeneous population. The parentals appeared intact, however, direct comparison with naturally nonhybridizing populations would be necessary for more conclusive results.

Accurate estimations of the ratio of F_1 hybrids to backcrosses are unavailable. Only many years of carefully controlled selection and backcrossing would allow selection of characters that would discriminate the hybrids from the backcrosses. This situation arises because highly introgressed fish might be expected also to approach the median F_1

condition, assuming nonpreferential backcrossing, thus preventing an accurate estimation.

Simple hybrid indices further support introgression. The overall hybrid index of .58 for all Lake Erie crosses differed significantly from an expected index of .5 indicating that crosses are not F_1 and are more similar to the goldfish. This may indicate preferential backcrossing with goldfish or simply that the inheritance of goldfish characters is slightly dominant.

Comparison of known F_1 hybrids from naturally nonhybridizing parentals with Lake Erie crosses provided conclusive evidence for introgression. Highly significant statistical differences existed for each of six characters analyzed indicating that Lake Erie crosses are introgressed.

Descriptive analysis of selected characters generally revealed an intermediate condition with a blending of characteristics. A gradation was seen in several characters that approached and overlapped both parental extremes. Two characters, gill raker counts, and length to width ratios of pharyngeal tooth ratios could be used to separate the crosses from the parentals with 95% confidence.

Since to my knowledge this was the first comprehensive analysis of goldfish-carp introgression in North America, an accurate prediction of the future status of the crosses can not be made. Introgression may continue until the parentals are lost or the parentals may gradually reestablish isolating mechanisms. The actual rate of introgression is not generally agreed upon. Opinions of commerical fishermen in the Lake Erie area are equally divided between those that feel the crosses are on the increase and those that feel they are on the decrease. My estimation of the percentage of crosses in southwestern Lake Erie averages between 10 and 20%. This is not to be construed as an accurate estimate since the extent of my sampling efforts does not by any means represent a quantitative sample. Accurate estimation of the number of hybrids is recommended as a future research problem. Crosses are produced elsewhere in Ohio, but in substantially lower numbers, generally less than 10%. Less than 3% of the carp and goldfish in the Olentangy River are crossed, possibly indicating a more recent introduction of goldfish. The goldfish were likely recent "fishbowl" introductions of pure aquarium varieties, thus reducing the chance for crossing. The higher rates of introgression more closely correspond to many of the sites of early Fish Commission introductions.

Biological implications of full fertility between two different genera may necessitate a revision of the taxonomic relation between the carp and goldfish. The results of this study may stimulate further research on the relationship of these two genera.

LITERATURE CITED

Baird, S.F. 1874. Report of the Commissioner. U.S. Commission of Fish and Fisheries for 1872-73. P. IXXVI.

__________. 1876. Report of the Commissioner. U.S. Commission of Fish and Fisheries for 1873-74. P. XXXIIV.

__________. 1879a. Report of the Commissioner. U.S. Fish Commission Report for 1877. P. 43.

__________. 1879b. Report of the U.S. Fish Commission. Annual Report of the Smithsonian Institute. P. 73.

__________. 1880. Report of the Commissioner. U.S. Commission of Fish and Fisheries for 1878. P. XLI.

__________. 1882. Report of the U.S. Fish Commission. Annual Report of the Smithsonian Inst. 1882.

__________. 1883. Report of the U.S. Fish Commission. Annual Report of the Smithsonian Inst. 1883, P. 83.

Balon, E.K. 1974. Domestication of the carp Cyprinus carpio. Roy. Ont. Mus. Life Sci. Misc. Pub. ISBN: 0-8854-147-3. 37 p.

Battle, H.I. 1940. The embryology and larval development of the goldfish Carassius auratus (L) from Lake Erie. Ohio J. Sci. 49(2): 82-91.

Baylock, B.C. 1971. A laboratory technique for spawning carp. Prog. Fish Culturist. 33(1):48-50.

Berg, L.S. 1964. Freshwater fishes of the USSR and adjacent countries. Israel program for Scientific translations, Jerusalem. 377-405.

Berry, P.Y. and M.P. Low. 1970. Comparative studies on some aspects of the morphology and histology of Ctenopharyngodon idellus, Aristichthys nobilis and their hybrid. Copeia 1970 (4):708-726.

Buss, K. and K. Corl. 1966. The viability of trout germ cells immersed in water. The Progressive Fish Culturist. 28(3):152-153.

Childers, W.F. 1967. Hybridization of four species of sunfishes (Centrarchidae). Illinois Natural History Survey Bulletin, Vol. 29, Art. 3, 158-214.

Cole, L.J. 1905. The German carp in the United States. Rept. U.S. Bur. Fish. 1904. pp. 525-641.

DeKay, J.E. 1842. Zoology of New York or New York Fauna, Part I. Zoology (4) 415 pp.

Eastman, J.T. and J.C. Underhill. 1973. Intra-specific variation in the pharyngeal tooth formulae of some cyprinid fishes. Copeia 1973 (1):45-53.

Fedotova, L.A. 1971. The carp Cyprinus carpio of Bukhtarma Reservoir. Journal of Ichthyology. 11(3):363-370.

Finsch, O. 1882. Bulletin of the U.S. Fish Commission for 1881. pp. 220-225.

Fribourgh, J.H. 1966. An application of a differential staining method to low-temperature studies on goldfish spermatozoa. Prog. Fish Culturist 28(4):227-231.

__________. 1970. Ultra structure of the goldfish, Carassius auratus (Cyprinidae), spermatozoan. Copeia. 1970 (2):274-279.

Goodman, M.M. 1967. The identification of hybrid plants in segregating populations. Evolution 21:334-340.

Greenfield, D.W., F. Abdel-Hameed, G. Deckert, and R. Flinn. 1973. Hybridization between Phoxinus erythrogaster and Notropis cornutus (Pisces: Cyprinidae). Copeia 1973 (1):54-60.

Greenfield, W.F. 1972. Introgressive hybridization between Gila orcutti and Hesperoleucus symmetricus in the Cuyama River Basin, California. I. meristics morphometrics and breeding. Copeia 1972 (4):84-859.

Gupta, C. and C.H. Meske. 1976. Abnormalities of the gonads of carp. J. Fish Biol. (9):75-77.

Henry, J. 1877. Report of the Secretary. Annual Rept. of the Smithsonian Inst., 1882. p. 48.

Hessel, R. 1878. The carp and its culture in rivers and lakes, and its introduction into America. Rept. U.S. Fish Commission, 1875-1876. p. 865-900.

Hocutt, C.H. and P.H. Hambrick. 1973. Hybridization between the darters Percina crassa roanoka and Percina oxyrhyncha (Percidae, Etheostomatini), with comments on the distribution of Percina crassa in New River. The American Midland Naturalist. 90(2): 397-405.

Hubbs, C.L. 1955. Natural hybridization in fishes. Syst. Zool. 4: 1-21.

Hubbs, C.L. and K. Kuronuma. 1942. Analysis of hybridization in nature between two species of Japanese flounders. Papers Michigan Acad. Sci. Arts, Letters 27:267-306.

_________. 1957. Duration of sperm viability as a factor in frequency of fish hybridization. Texas Journal of Science. 9(4):472-474.

Hubbs, Clark. 1961. Differences between the gamete compatibility of Etheostoma lepidum and sperm and eggs of related percid fishes from allopatric and sympatric populations. Texas Journal of Science. 13(4):427-431.

Ivanov, S.N. 1971. An analysis of the fecundity and intermittent spawning of the Lake Balkhash wild carp Cyprinus carpio (L.). Journal of Ichthyology. 11(5):666-673.

Keenleyside, M.H., R.K. Misra, and D. Bateson. 1973. Extended analysis of hybridization in sunfishes (Centrarchidae) using an adjusted hybrid index method. J. Fish. Res. Bd. Can. 30:1901-1904.

Littlejohn, J.J. 1969. The systemic significance of isolating mechanisms. 459-489. In: Systematic Biology. Nat. Acad. Sci. Publ., 1962.

Makeyeva, A.P. and V.V. Vergin. 1974. Hybridization of the pond carp Cyprinus carpio with the grass carp Ctenopharyngodon idella. Journal of Ichthyology. 14(2):254-260.

Makino, S. 1939. The chromosomes of the carp, Cyprinus carpio, including those of some related species of cyprinidae for comparison. Cytologia 9:430-440.

Makino, S., Ojima, and Y. Matsui. 1958. Cytogenetical and cytochemical studies in carp-funa hybrids. The Nucleus 1(2):153-162.

Mayr, E. 1963. Population, Species, and Evolution. Harvard University Press, Cambridge, Mass. 453 pp.

McAllister, D.E. 1972. Morphological and myogen comparison of Johnny and tesselated darters and their hybrids, Genus Etheostoma, near Ottawa, Canada. J. Fish. Res. Bd. Can. 29(8):1173-1180.

McCrimmon, H.R. 1968. Carp in Canada. Fisheries Research Board of Canada, Bulletin 165, 93 p.

McDonald, M. 1887. Bulletin U.S. Fish Commission 1886. pp. 385-394.

Nelson, J.S. 1966. Hybridization between two cyprinid fishes, Hybopsis plumbea and Rhinichthys cataractae in Alberta. Canadian Journal of Zoology. 44:963-967.

Nikolskii, G.V. 1961. Special Ichthyology. Israel Program for Scientific Translations, Jerusalem. 288-294.

Ohno, S. 1967. Diploid-tetraploid relationship among old world members of the fish family Cyprinidae. Chromosoma 23:1-9.

Ojima, Y. 1966. Cytogenetic studies in lower vertebrates. Proceedings of the Japan Academy. 42(1):62-66.

________. 1973. Recent studies of hybrids in fresh water teleosts. Proceedings of the ICLA Asian Pacific Meeting of Laboratory Animals. September 20-25, 1971, Tokyo and Inuyama.

Ojima, Y., M. Hayashi, and K. Ohno. 1975. Triploidy appeared in the back-cross offspring from funa-carp crossing. Proc. Japan Academy. 51(8):702-706.

Orlov, Y.A. 1967. Fundamentals of Paleontology Vol. XI Agnatha, Pisces English Translation, Israel Program for Scientific Translations, Jerusalem, 1967. p. 641-642.

Poppe, R.A. 1880. Report of the U.S. Commissioner of Fish and Fisheries for 1878. pp. 661-666.

Romer, A.S. 1966. Vertebrate paleontology. The University of Chicago Press, Chicago. p. 356.

Shikhshabekov, M.M. 1969. Some data on the spawning ecology of the roach Rutilus rutilus caspicus (Jak), and bream Abramis brama (L.) and the carp Cyprinus carpio (L.) in the Arakum Lakes of Dagestan. Journal of Ichthyology. 9(5):771-779.

Shikhshabekov, J.J. 1972. The annual cycle of the gonads in wild carp Cyprinus carpio (L.) from the Terek Delta. Journal of Ichthyology. 12(5):855-860.

Smiley, C.W. 1883. Bulletin of the U.S. Fish Commission for 1883, pp. 305-332.

________. 1884a. Bulletin of the U.S. Fish Commission for 1884, p. 307.

________. 1884b. Report of the U.S. Commissioner of Fish and Fisheries for 1882, pp. 943-1008.

________. 1886. Report of the U.S. Commissioner of Fish and Fisheries for 1884, pp. 657-890.

Smith, G.R. 1973. Analysis of several hybrid cyprinid fishes from Western North America. Copeia 1973 (3):195-410.

Smith, H.M. 1924. Goldfish and their cultivation in America. National Geographic Mag. Vol. XLVI, p. 375-400.

Stacey, N.E. and N.R. Liley. 1974. Regulation of spawning behavior in the female goldfish. Nature 247:71-72.

Swee, H.B. and H.R. McCrimmon. 1966. Reproductive biology of the carp, Cyprinus carpio L. in Lake St. Lawrence, Ontario. Trans. Amer. Fish. Soc. 95(4):172-380.

Trautman, M.B. 1957. The Fishes of Ohio. Ohio State University Press, Columbus, Ohio, 683 pp.

Whiteside, B.G. and F.J. Richan. 1969. Repressive factors controlling reproduction in goldfish. Prog. Fish Culturist. 31(3):165.

Woodruff, D.S. 1973. Natural Hybridization and hybrid zones. Syst. Zool. 22(3):213-218.