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Comparison of Hybrid Grass Carp and Grass Carp

Janine L. Callahan
University of Central Florida

COMPARISON OF HYBRID GRASS CARP
AND GRASS CARP

BY

JANINE LUCILLE CALLAHAN
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ABSTRACT

The hybrid grass carp, a cross between the male bighead carp (Hypopthalmichthys nobilis) and the female grass carp (Ctenopharyngodon idella), was first produced in the United States in 1979 for biocontrol purposes. Unlike the fish produced in 1979 and 1980, the hybrid grass carp spawned in 1981 were assumed to be uniform, triploid, and to have growth and feeding rates comparable to those of grass carp. A comparison study to determine differences in the morphology of the 1979, 1980, and 1981 hybrid grass carp revealed that the hybrid grass carp spawned in 1981 have a longer relative gut length, fewer deformities of the gill rakers, and fewer diploid fish than the previous spawns. In feeding trials, the growth rate of the 1981 hybrid grass carp (2.5 to 3.9 g $fish^{-1}$ day^{-1}) were similar to that of the 1979 and 1980 fish (2.8 and 3.9 g $fish^{-1}$ day^{-1}, respectively). In field tests, their mortality rate ranged from 20.8 to 97.4% and was similar to that of other hybrid grass carp. Due to the increased gut length, low feeding rate, and high mortality, the 1981 hybrid grass carp were unable to control (eliminate) the growth of aquatic vegetation in field trials in Blue Lake and in a detention pond which had been treated with herbicide prior to stocking. The 1981 hybrid grass carp has proven to be less effective than previous hybrid grass carp spawns as a biocontrol agent.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Hydrilla (*Hydrilla verticillata*), an exotic submersed aquatic plant introduced into Florida from Africa in 1960, remains the most serious weed problem in the state in spite of significant attempts to control it (Miley et al., 1979). Once it has been introduced into an aquatic environment, its rapid vegetative growth (usually by fragmentation) enables the plant to quickly spread throughout a body of water. Because hydrilla is able to utilize light more effectively than native submersed aquatic plants and its ability to produce a surface mat restricts light required by other plants, hydrilla usually dominates the submersed aquatic plant community. According to Haller (1979), hydrilla occupied only 10 ha in the Crystal River and the Miami River in 1960 but by 1967 had become established in approximately 2,000 ha of Florida waters. Ten years later, in 1977, the plant occupied about 25% (250,000 ha) of Florida's 1.01 million ha of freshwater and was present in virtually every major watershed in the state.

Since its introduction into Florida, millions of dollars have been spent by federal and state agencies and waterfront property owners to fund projects to control the spread of hydrilla. Haller (1976) and Marx (1980) reported that $6 to $8 million was spent annually in Florida for chemical treatments designed to provide temporary control of hydrilla infestation.

An organism that has proven to be an effective biocontrol agent for hydrilla is the grass carp (Ctenopharyngodon idella Val.), a fish native to the large rivers in eastern China and Russia that empty into the Pacific Ocean (Cross, 1969). The range of these fish in Asia (from 50° N south to 23° N) is equivalent to that area of North America from Winnipeg, Canada south to Tampico, Mexico (Stanley, 1976). Suitable environmental conditions are found in this area which could enable these hardy fish to adapt to the new environments. In fact, the grass carp has been introduced throughout the world and has become naturalized in Japan, Taiwan, the Philippines, and Mexico (Sutton and Vandiver, 1976). The grass carp was introduced into the United States in 1963 with a shipment of fingerlings brought from Malaysia to the Fish Farming Experimental Station in Stuttgart, Arkansas by the United States Bureau of Sport Fisheries and Wildlife (Stevenson, 1965; Fedorenko and Fraser, 1978). In 1970, the Arkansas Game and Fish Commission began stocking that state with grass carp, and since 1972, the fish has been supplied commercially throughout the United States by fish farmers in Arkansas (Anonymous, 1976a; Lynch, 1979).

The grass carp was first used in Florida in 1969 for early research investigations sponsored by the United States Army Corps of Engineers and conducted by the United States Department of Agriculture and the University of Florida (Miley et al., 1979). Results of these early studies indicated that the grass carp preferred the exotic hydrilla over many of the native plants. In 1970, the Florida

Department of Natural Resources brought the fish into the state for use as a biocontrol agent for hydrilla (Sutton, 1977; Miley et al., 1979).

The grass carp has demonstrated its capability to consume and control (eliminate) a wide variety of aquatic plants under experimental conditions in lakes and ponds. The grass carp has been reported to consume mainly submersed aquatic plants, preferring those plants with soft stems and leaves that fit lengthwise into its mouth (Anonymous, 1976b); however, the fish will consume almost any type of vegetation when their preferred food is not available (Sutton, 1977). Results of various studies (Avault, 1965; Cross, 1969; Sneed, 1971; Opuszynski, 1972; etc.) vary as to exactly what the grass carp 'prefer' to eat, but it is generally agreed that grass carp can consume large amounts of Hydrilla as well as various species of *Ceratophyllum*, *Chara*, *Elodea*, *Myriophyllum*, *Potamogeton*, and *Eleocharis*. Small fish (about 1.2 kg) may consume their body weight of plant material daily and larger fish (2.5 kg in size) are reported to eat almost 2 kg of vegetation per day (Osborne and Sassic, 1981). Adult grass carp begin feeding more frequently on submersed plants when water temperatures reach 12 C (Van Zon et al., 1976) and consume up to 120% of their body weight per day at temperatures between 22-33 C (Fischer, 1968; Opuszynski, 1972).

Successful control (elimination) of hydrilla in Florida by the grass carp has been reported by Miley et al. (1979) in Lake Holden (Orange County), Clear Lake (Pasco County), and Lake Bell (Pasco County); by Osborne (1982a) in Lake Orienta, Clear Lake, Little Lake Fairview, Lake Killarney, and Little Lake Barton (Orange County); by

Beach et al. (1976) in Broward Pond (Broward County); and by Shireman and Maceina (1981) in Lake Baldwin (Orange County). Grass carp have controlled Potamogeton illinoensis and Myriophyllum spicatum in Deer Point Reservoir in Bay County, Florida (Kobylinski et al., 1980). Control of or a reduction in Ceratophyllum, Chara, Potamogeton, Elodea, and Najas has been achieved by grass carp in Arkansas (Bailey and Boyd, 1972), Iowa (Mitzner, 1978), Indiana (Lembi et al., 1978), and Georgia (Shelton et al., 1981).

The ability of the grass carp to consume large quantities of aquatic macrophytes is the main reason for the restriction on its use and introduction in most states. The possibility exists that the grass carp may escape into rivers and streams, reproduce and overpopulate, and reduce the vegetation to the point where native fish, invertebrates, and waterfowl are adversely affected. This controversy persists even though environmental conditions would probably inhibit a successful spawn and overpopulation by this species (Stanley et al., 1978). Although grass carp have been reported to stain the water with fecal material (Avault et al., 1968) and occasionally feed upon macro-invertebrates (Edwards, 1973), there have been no reports of widespread environmental damage resulting from the presence of grass carp throughout the United States. Introduced and escaped grass carp are reported to have found their way into at least 40 states (Pflieger, 1978) and 75% of the fresh waters in the United States (Burkhalter, 1975). Since 1976, this fish has become widely distributed from Louisiana to South Dakota via the Mississippi River and Missouri

River systems (Greenfield, 1973; Pflieger, 1978). Grass carp are reported to have escaped from Deer Point Reservoir in northwest Florida and were found in large numbers in a bay leading to the Gulf of Mexico (Guillory and Gasaway, 1978; Hardin, 1981).

At present, only Arkansas, Mississippi, Alabama, and Kansas allow unrestricted use of the grass carp (Lynch, 1979), although a few states still grant permits for its use by private individuals or in research studies (Osborne, 1982b). Osborne (1982a) noted that prior to 1980, Florida allowed grass carp to be stocked under Florida Rule 16C-21 in freshwater impoundments in Florida that were less than or equal to 10 ha in size. Presently, under Florida Rule 39-8, the grass carp is only permitted for research purposes in waters that were stocked with grass carp prior to 1980.

The hybrid grass carp, resulting from a cross between the female grass carp and the male bighead carp (Hypophthalmichthys nobilis Rich., formerly Aristichthys nobilis), was produced in this country partially in response to the need for a safe, effective biocontrol agent. While the original intent of the European and Russian scientists who developed this cross was to satisfy European culinary demands for a fish with better food quality characteristics (Cassani, 1981), American researchers anticipated that the hybrid grass carp would have a penchant for aquatic macrophytes similar to its maternal parent but would not be capable of reproducing.

According to Sutton et al. (1981), a cross between the grass carp and the bighead carp may result in the production of three types of organisms: (1) a diploid hybrid grass carp, which receives 24 chromosomes from the female grass carp and 24 chromosomes from the male bighead carp and is distinguished by the presence of a ventral keel and a reduced growth rate; (2) a gynogenetic female grass carp, which results from the development of an unfertilized ovum and is fully capable of reproduction; and (3) a triploid hybrid grass carp, which receives 48 chromosomes from the female grass carp and 24 chromosomes from the male bighead carp and is supposedly sterile and unable to reproduce. It is this triploid hybrid grass carp that was heralded as the fish to replace the grass carp as a biological control agent for aquatic weeds. Based upon the 1974 techniques of three Hungarian scientists, the hybrid grass carp was first produced in the United States in 1979 at the J. M. Malone & Son Enterprises fish hatchery in Lonoke, Arkansas (Lynch, 1979).

Sutton et al. (1981) reported that the grass carp and the hybrid grass carp produced in 1979 by the Malone fish hatchery had similar pharyngeal teeth structure, head size, position of the eyes, and a terminal mouth. They found 'intermediate' characteristics which included the number and size of the scales, mouth and caudal fin size, gill raker length, and position of dorsal fin insertion. Other investigators reported that the pharyngeal teeth of the hybrid grass carp appeared to be similar, if not identical, to those of the grass carp and assumed that the hybrid grass carp would have similar capabilities for consuming coarse vegetation (Buck, 1979).

Marian and Krasznai (1978) compared the diploid chromosome number of the F_1 hybrid grass carp (2n=72) with the diploid chromosome number of the parent grass carp and bighead carp (2n=48) and found the hybrid grass carp to be triploid. Based on the frequencies of metacentric and submetacentric chromosomes, Beck et al. (1980) verified with karyological analysis that hybrid grass carp spawned in 1979 at the Malone fish hatchery were triploid (containing 72 chromosomes). They reported that these hybrid grass carp probably received 48 chromosomes from the maternal parent and 24 chromosomes from the paternal parent and were probably sterile. Results of these and other morphological and karyological studies led many investigators to conclude that the hybrid grass carp produced at the Malone fish hatchery was a sterile weed-eating fish.

Results of several growth and feeding studies did not seem to substantiate these beliefs. Sutton (1981) reported that with the exception of large fish (over 1,000 g), hybrid grass carp in general showed an inability to curtail an excessive growth of aquatic weeds in experimental pools. Given a choice of ten plant species during one of his trials, the hybrid grass carp only fed upon Chara and Najas; in another trial in which six plant species were offered to the hybrid grass carp, Hydrilla, Vallisneria, and Potamogeton were eaten in small amounts only. Cassani (1981) reported that hybrid grass carp fingerlings from the 1979 spawn favored Ceratophyllum demersum, Chara spp, and Najas guadalupensis in a feeding trial in which nine plants were used. Feeding damage to Hydrilla verticillata was minor, while only slight damage occurred to Myriophyllum pinnatum; Egeria densa and Potamogeton illinoensis were undamaged. In pool studies, Hestand and Chapman (1980) determined that small hybrid grass carp (averaging 17.8 cm) fed mainly on Chara and Najas, while larger hybrids (27.9 cm and up) seemed to prefer Hydrilla and Chara. Sutton (1980) found that in outdoor pools, hybrid grass carp consumed only small amounts of Hydrilla verticillata and Myriophyllum spicatum. Osborne (1982b) reported that hybrid grass carp ranging from 18-26 g stocked at the rate of 183 fish ha^{-1} in 1979 did not control (eliminate) or reduce Hydrilla verticillata in Blue Lake or Ceratophyllum demersum in Crealde Lake after one year. Osborne (1982a) reported that the hybrid grass carp were unable to control vegetation in eight central Florida study lakes during 1980-1982. However, Hestand and Chapman (1980) reported that the hybrid grass carp appeared to be stabilizing or reducing the density of Hydrilla in Palm Lake (Seminole County) and Lake Diane (Pasco County) during 1979-1981. In their study, an increase in the distance from the lake surface to the top of the hydrilla was noted for several months during the study, which the authors implied "could" be the result of hybrid grass carp feeding.

While the study conducted by Hestand and Chapman (1980) produced results indicating that the hybrid grass carp had the potential to limit hydrilla growth, the high degree of variability in the feeding habits and growth rates led other investigators (Sutton, 1981; Cassani et al., 1982; Osborne, 1982b; etc.) to conclude that overall the 1979 and 1980 hybrid grass carp had feeding and growth rates much lower than those of the grass carp.

In February, 1982, J. M. Malone & Son Enterprises revealed that developmental problems plagued the hybrid grass carp spawned in 1979 and 1980 (Malone, 1982). Because fish from these spawns had been used in every study involving hybrid grass carp conducted in the United States up to that time, the effect of these production problems on the results of these studies was a major concern. These problems included numerous morphological deformities, low feeding rates, low survivability, and the production of thousands of diploid fish along with the triploids. In 1981, personnel at the Malone fish hatchery concentrated on producing triploid hybrid grass carp in commercial quantities and correcting deformities and variances to provide a more standardized, functional triploid hybrid grass carp (Malone, 1982). Production of the 1981 hybrid grass carp supposedly resulted in a "new and different fish" that, according to Malone, had growth rates and feeding rates comparable to those of the grass carp (Malone, 1982).

A two-part study was devised to characterize the major similarities and differences between hybrid grass carp spawned in 1979, 1980, and 1981 and grass carp spawned in 1980 and 1981. The first part of this study involved a morphometric comparison of these hybrid grass carp and grass carp. The second part of the study was composed of a series of feeding trials in experimental ponds, a small lake, and a detention pond. The growth rates, feeding rates, and food conversion efficiencies of the 1981 hybrid grass carp used in these trials were then compared to those of the grass carp and previous spawns of hybrid grass carp. Results of the morphometric comparison and feeding trials were used to evaluate the potential of the 1981 hybrid grass carp as a biological weed control agent.

METHODS AND MATERIALS

The hybrid grass carp and grass carp used in this study were produced at the J. M. Malone & Son Enterprises fish hatchery in Lonoke, Arkansas. The 1979 hybrid grass carp were selected from those fish remaining after a 20-month feeding study in Blue Lake. The 1980 hybrid grass carp were from a group of fish that had been previously used in growth and feeding studies in the University of Central Florida experimental ponds. Hybrid grass carp from the 1981 spawn were donated by the Orange County Pollution Control Department, the Florida Game and Fresh Water Fish Commission, and the Lee County Hyacinth Control District. The 1980 and 1981 grass carp had been previously obtained from the Malone hatchery for feeding studies.

Morphometric Comparison

A total of 73 fish were selected for the morphometric comparison; this included 14 hybrid grass carp from the 1979 spawn, 25 hybrid grass carp from the 1980 spawn, 15 hybrid grass carp from the 1981 spawn, eight grass carp from the 1980 spawn, and 11 grass carp from the 1981 spawn. Because a large number of morphological features were to be recorded for each fish, the amount of time required to examine each fish precluded that they be examined at the time of collection. Consequently, the fish were identified with a tag, individually wrapped in wet toweling and aluminum foil to minimize dessication, and frozen at 0 C. The fish were removed from the freezer and allowed to thaw at 4 C for 36 hours prior to being measured.

Body lengths and widths were measured to the nearest 0.1 cm, in accordance with the methods outlined by Berry and Low (1970), Needham and Needham (1978), and Eddy and Underhill (1978). Snout length, orbit length, mouth width and height, upper jaw length, and fin lengths and widths were measured with calipers to the nearest 0.1 cm. Scale and fin ray counts were made in accordance with Needham and Needham (1978). Both paired fins were measured, but all other external measurements were made on the left side of each fish (Table 1).

After removing the left side of the body, the digestive tract of a fish was detached by cutting through the gut immediately behind the pharynx and above the rectum (Hickling, 1966). The gut length was determined to the nearest 0.1 cm by lightly stretching it along the length of a fish board (Berry and Low, 1970).

The right and left branchial arches were removed as a group and rinsed in 1% bleach to remove excess blood and mucus (Berry and Low, 1970). The five individual gill arches in each group were carefully separated to prevent damage to or loss of the gill rakers. Measurements of lengths (to the nearest 0.01 cm) and numbers of rakers and filaments on the gill arches were obtained using a Wild Heerbrugg binocular dissecting microscope (Table 1).

The two pharyngeal arches (fifth gill arches) were boiled in water for five minutes to aid in the removal of muscle tissue. They were soaked overnight in 90% acetone and then placed in hydrogen

Table 1. Morphological measurements recorded for 1979, 1980, and 1981 hybrid grass carp and 1980 and 1981 grass carp. Numbers in parentheses indicate number of measurements of that feature per fish.

- Weight
- Total length
- Standard length
- Scales above lateral line
- Scales below lateral line
- Scales in lateral line
- Caudal length
- Caudal peduncle length
- Caudal peduncle height
- Trunk length
- Head length
- Head width
- Snout length
- Upper jaw length
- Orbit length
- Mouth width (extended)
- Mouth height (extended)
- Body depth
- Body width
- Dorsal fin length
- Dorsal fin width at base
- Dorsal fin - no. rays
- Pectoral fin length (2)
- Pectoral fin width at base (2)
- Pectoral fin - no. rays (2)
- Pelvic fin length (2)
- Pelvic fin width at base (2)
- Pelvic fin - no. rays (2)
- Anal fin length
- Anal fin width at base
- Anal fin - no. rays
- Caudal fin length
- Caudal fin width
- Caudal fin - no. rays
- Esophagus - internal diameter
- Intestinal swelling - internal diameter
- Intestine - internal diameter
- Rectum - internal diameter
- Gut length
- Filament length - first four gill arches (8)
- Gill raker length - minimum and maximum - five gill arches (20)
- No. gill rakers per arch - distal and medial surface (20)
- Gill arch length - both arches (10)
- Gill arch breadth - both arches (10)
- Pharyngeal teeth - no. per arch (2)
- Pharyngeal teeth - no. rows per arch (2)
- Pharyngeal teeth height - minimum and maximum - both rows (8)
- Pharyngeal teeth width at base - minimum and maximum - both rows (8)
- Erythrocyte nucleus volume (mean)
- Erythrocyte cell volume (mean)

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peroxide for one hour (Berry and Low, 1970). After drying, the arches were measured using a binocular dissecting microscope to determine the size (to the nearest 0.01 cm) and number of teeth per arch (Table 1).

The erythrocyte smears made at the time of collection were examined on a Zeiss phase contrast microscope at 1,000 X using an ocular micrometer. Blood was collected from all fish except the 1981 grass carp. Relative measurements were obtained of the long and short axes of ten randomly selected erythrocytes and their nuclei. These measurements were used to calculate the relative mean erythrocyte nucleus and cell volumes using the following formula:

$$V = 4/3 \cdot pi \cdot L \cdot (W/2)^2,$$

where V is the volume of the nucleus or cell, L is the length of the long axis, and W is the length of the short axis (Vollenweider, 1969; Sutton, 1983). The values for the relative erythrocyte nucleus volume were used to evaluate the diploid or triploid genotype of each fish; the triploid condition was assumed if the relative mean erythrocyte nucleus volume was 50% greater than the relative mean erythrocyte nucleus volume of the grass carp.

A total of 135 measurements were collected for each of the 73 hybrid grass carp and grass carp (Table 1). This large number of measurements was reduced by combining or eliminating values from the data set. For example, measurements of the numbers and lengths of gill rakers on both sides of the ten gill arches were combined and expressed as a mean for each fish. The width of the extended caudal fin was eliminated because several of the fish had damaged caudal fins and accurate measurements could not be made. By combination and elimination the number of variables for each fish was reduced to 47.

To make initial comparisons between fish of varying sizes and ages from the different year classes, some of the parameters had to be standardized by one of two methods. The external measurements and gut dimensions of each fish, such as the caudal length, gut length, and head length, were divided by the standard length to obtain ratios. The gill arch measurements of the individual fish were divided by its mean pharyngeal arch length to produce relative values. The mean pharyngeal arch length was then divided by the standard length to yield a ratio. Counts of morphological structures, such as numbers of scales, fin rays, and gill rakers, did not need to be standardized.

Statistical comparisons of the four fish groups were made using this reduced, standardized data (Table 2). For the statistical comparisons, the 1980 and 1981 grass carp were combined into one group; this group was compared to the 1979, 1980, and 1981 hybrid grass carp using a principal components analysis (Morrison, 1976; SAS, 1982) and an analysis of variance (Haber and Runyon, 1971; Steel and Torrie, 1980). If a morphological feature was shown to be statistically different among the groups, the Student's t-test (Haber and Runyon, 1971) was used to determine between which two groups the characteristic was significantly different. Significance in all tests was expressed at the 0.01 level.

Table 2. Data set with 47 reduced, standardized values that were used in computer analyses to determine statistical differences among the four groups of fish (1979, 1980, and 1981 hybrid grass carp and 1980-81 grass carp). Note that SL = Standard Length and PAL = Pharyngeal Arch Length.

Weight	Pelvic fin length ($\bar{x}$)/SL
Total length/SL	Pelvic fin width ($\bar{x}$)/SL
Standard length	Pelvic fin - no. rays ($\bar{x}$)
Scales above lateral line	Anal fin length/SL
Scales below lateral line	Anal fin width/SL
Scales in lateral line	Anal fin - no. rays
Caudal length/SL	Caudal fin length/SL
Caudal peduncle length/SL	Caudal fin - no. rays
Caudal peduncle height/SL	Gut length/SL
Trunk length/SL	Filament length ($\bar{x}$)/PAL
Head length/SL	Gill raker length ($\bar{x}$)/PAL
Head width/SL	No. gill rakers ($\bar{x}$) per gill arch
Snout length/SL	No. gill rakers ($\bar{x}$) per pharyngeal arch
Upper jaw length/SL	Gill arch length ($\bar{x}$)/PAL
Orbit length/SL	Gill arch breadth ($\bar{x}$)/PAL
Mouth width/SL	Pharyngeal arch length ($\bar{x}$)/SL
Mouth height/SL	Pharyngeal arch breadth ($\bar{x}$)/PAL
Body depth/SL	Pharyngeal teeth - no. per arch ($\bar{x}$)
Body width/SL	Pharyngeal teeth height ($\bar{x}$)
Dorsal fin length/SL	Pharyngeal teeth width ($\bar{x}$)
Dorsal fin width/SL	Erythrocyte nucleus volume ($\bar{x}$)
Dorsal fin - no. rays	Erythrocyte cell volume ($\bar{x}$)
Pectoral fin length ($\bar{x}$)/SL	
Pectoral fin width ($\bar{x}$)/SL	
Pectoral fin - no. rays ($\bar{x}$)	

Blue Lake

Blue Lake, a 0.45 ha lake located in Seminole County, Florida, was used to evaluate the ability of the 1981 hybrid grass carp to limit the regrowth of hydrilla in the absence of predation. Three hundred pounds of the herbicide Aquathol K was applied in June, 1981 to reduce biomass; the hybrid grass carp were stocked six months later at a density of 1,666.7 fish ha^{-1}. These fish had a mean standard length of 14.4 cm and a mean weight of 76.7 g. Erythrocyte samples were taken from the gill area of the fish with a syringe and used to determine triploidy.

Vegetation biomass sampling was begun in October, 1981 using the Osborne submersed aquatic plant sampler (APHA et al., 1981) and was conducted at bimonthly intervals through October, 1982. Fifteen sampling stations were randomly selected using a grid map (Figure 1). The vegetation samples were washed, spun in a garment washer at 540 rpm for four minutes to remove excess water, and weighed to the nearest 0.001 kg on a Sartorius Model 1264 MP balance. The fresh weight biomass (kg m^{-2}-FW and mt-FW $lake^{-1}$) and the percent frequency of occurrence of vegetation (the number of samples with vegetation/the number of stations sampled · 100) were determined.

The hybrid grass carp were removed from the lake on October 9, 1982 with a 0.1 ppm concentration of 5% emulsified rotenone; to confirm that all of the hybrid grass carp had been removed, rotenone at a concentration of 5 ppm was added to the lake. After reviving the fish in aerated water containing potassium permangenate (0.1 ppm), the

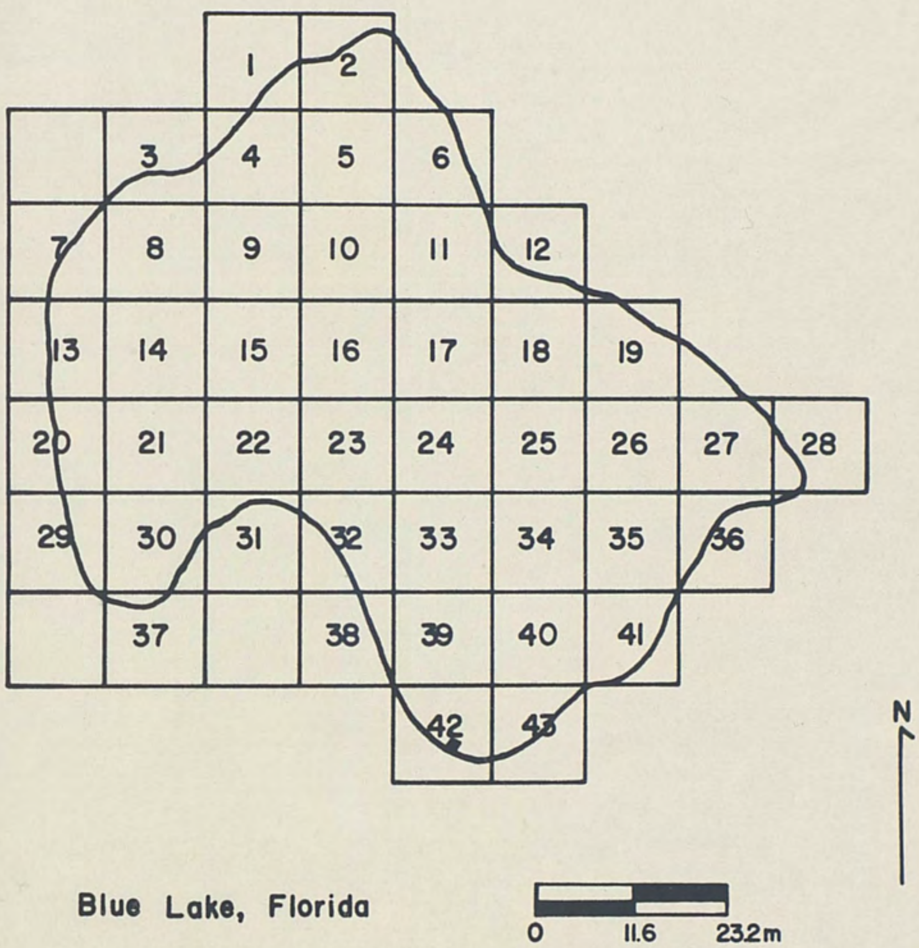

Figure 1. Sampling grid map of Blue Lake, Seminole County, Florida.

hybrid grass carp were stocked in a 0.13 ha experimental pond on the campus of the University of Central Florida.

Detention Pond

A 0.42 ha stormwater detention pond, located in southwest Orange County, Florida, was used to determine the ability of the 1981 hybrid grass carp to prevent the regrowth of hydrilla following its eradication with the herbicide Hydout (56 kg/ha) in August, 1981. To evaluate the effects of predation on the hybrid grass carp, the wild fish population was not removed from the pond. Metal fish barriers were constructed to prevent the escape of the fish. The Osborne submersed aquatic plant sampler (APHA et al., 1981) was used to obtain the biomass of the aquatic vegetation at the time of stocking and at bimonthly intervals throughout the study. Vegetation was collected from 15 sampling stations that had been randomly selected from a grid map (Figure 2).

The hybrid grass carp were stocked on April 29, 1982 at a rate of 833.3 fish ha^{-1}; the mean standard length was 21.1 cm and the mean weight was 199.4 g. The fish were removed with 0.1 ppm rotenone on March 8, 1983 and their survival and growth rates were determined.

Experimental Ponds

The experimental ponds used to conduct controlled feeding experiments are located on the campus of the University of Central Florida in Orlando, Florida. The 0.13 ha ponds were drained to remove fish and

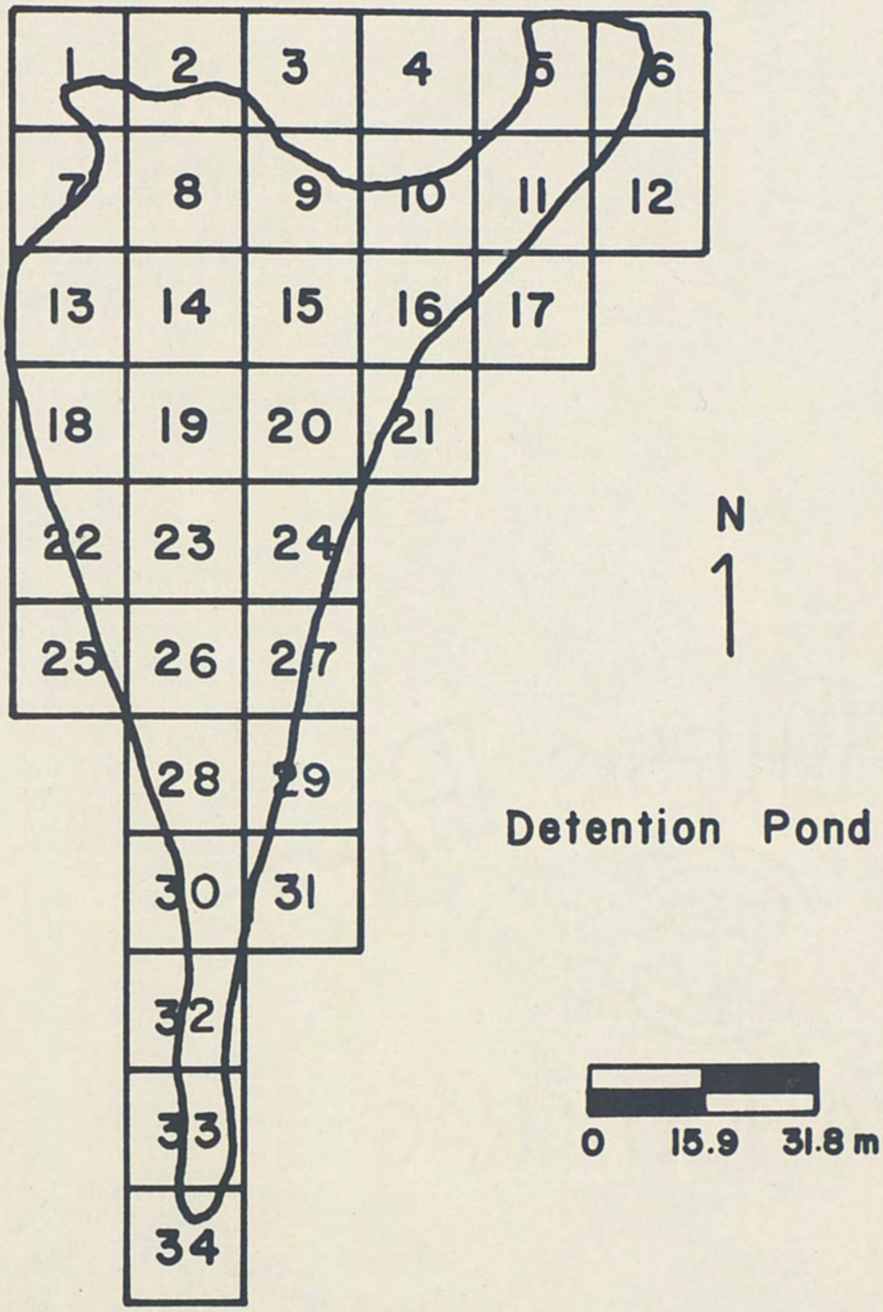

Figure 2. Sampling grid map of detention pond, Orange County, Florida.

vegetation prior to each experiment; water for refilling the ponds was provided from a deep water well. Square exclosures made of 2.5 cm mesh wire, which enclosed an area of 13.4 m^2, were placed midway in the ponds and used to monitor vegetation growth in the absence of fish. Electric livestock fence was placed along the perimeter of the ponds to eliminate predation by wading birds.

Three feeding experiments using the 1981 hybrid grass carp were conducted in the ponds between April, 1982 and February, 1983. On May 15, 1982, Egeria densa Planchon was collected from the Wekiva River, washed, spun to remove excess water, weighed to the nearest 0.01 kg, and broadcast into one experimental pond. In the first trial, the pond received 25 kg of Egeria while 5 kg of vegetation was placed into the exclosure. Sixteen of the 1981 hybrid grass carp, with a mean standard length of 21.3 cm and a mean weight of 201.9 g, were placed in the pond on May 15 at a density of 123.1 fish ha^{-1}. At the end of 14 days, the pond was drained and all fish and vegetation removed. The fish were counted and weighed; the E. densa was washed, spun to remove excess water, and weighed.

Nine of the fish that were used in the first feeding trial were stocked into the experimental pond (69.2 fish ha^{-1}) to begin the second feeding trial. The mean standard length of these hybrid grass carp was 22.5 cm and the mean weight was 247.4 g. Egeria densa that had been collected from the Wekiva River was weighed into the pond; in this trial, 12.5 kg of E. densa was placed in the pond and 2.5 kg was added to the exclosure. This trial was terminated on June 20, 1982 due to a filamentous algae bloom that covered much of the *Egeria*.

To remove the algae and *Egeria* prior to conducting the third trial, the pond was stocked with 50 grass carp (approximately 2 kg each). The vegetation was eliminated within three weeks and the grass carp were removed on July 24, 1982. In the final feeding trial, 20 hybrid grass carp from the 1981 spawn were used. The fish, with a mean standard length of 21.8 cm and a mean weight of 211.8 g, were added to the pond at a stocking rate of 153.8 fish ha^{-1}, along with 30.0 kg of *Egeria densa* (25.0 kg in the pond and 5.0 kg in the exclosure). A dense filamentous algae bloom interrupted this trial after 10 days; the pond was drained at the end of 28 days and the fish were recovered, weighed, and measured.

The 1981 hybrid grass carp that had been removed from Blue Lake on October 9, 1982 were stocked at the rate of 2,253.8 fish ha^{-1} into one of the 0.13 ha experimental ponds at the University of Central Florida. These fish were fed commercial catfish food to supplement the filamentous algae and *E. densa* already present in the pond. The pond did not contain any other species of fish and was electrically wired with cattle fencing to eliminate predation by wading birds. At the end of 115 days (February 1, 1983) the pond was drained and the fish were collected, counted, and weighed.

Two hundred 1981 hybrid grass carp, with a mean standard length of 16.0 cm and a mean weight of 86.3 g, were stocked into an experimental pond at a density of 1,538.5 fish ha^{-1} on April 14, 1982.

Egeria densa, Hydrilla verticillata, Potamogeton illinoensis, Hydrocotyl umbellata, and Panicum repens were present in ample quantity; Lemna minor was added on three separate occasions between April, 1982 and February, 1983. No other species of fish was present and the pond was protected against predation by birds with electric cattle fencing. On February 26, 1983, after 318 days, the pond was drained and the fish were collected, counted, and weighed.

RESULTS AND DISCUSSION

A computer program, based on the General Linear Models Procedure (PROC GLM) with the Multivariate Analysis of Variance option (MANOVA), was used to examine the relationships between the four groups of fish using the principal components analysis (Morrison, 1976; SAS, 1982). Four test statistics (Hotelling-Lawley Trace, Pillai's Trace, Wilk's Criterion, and Roy's Maximum Root Criterion) were used to calculate the F approximations, associated degrees of freedom, and the probability levels to determine significant differences at the 0.01 level (SAS, 1982). Results of the principal components analysis confirmed that there was a significant difference between the grass carp group and all groups of hybrid grass carp, as well as a statistical difference between the 1979 and 1981 hybrid grass carp and the 1980 and 1981 hybrid grass carp. Tests were not conducted between the 1979 and 1980 hybrids to determine significant differences.

The analysis of variance (ANOVA) was used to determine specific morphological features that were statistically different among the groups of fish (Haber and Runyon, 1971; Steel and Torrie, 1980). The analysis of variance detected a significant difference at the 0.01 level among the four groups of fish in 23 of the 47 characteristics examined (Table 3). Results of the Student's *t*-test, shown in Table 4, depict between which of the groups a statistical difference occurred.

Table 3. Results of analysis of variance (ANOVA) used to determine which variables were significantly different among the four groups of fish. N=72, df=(3,68), and P=26.277 for all but RBCCV and RBCNV, where N=62, df=(3,54), and P=26.297 at 0.01 level. Refer to Appendix 1 for explanation of abbreviated variable names.

Variable Name	Sum X_{tot}	Sum X^2_{tot}	F
TL	89.950	112.414	41.50*
SALL	638.000	5,852.000	174.34*
SBLL	418.000	2,462.000	29.97*
SILL	3,445.000	166,593.000	60.53*
CL	22.801	7.303	28.11*
CPL	14.137	2.790	27.01*
CPH	9.134	1.161	15.00
TRL	34.264	16.384	45.20*
HL	18.374	4.722	45.37*
HW	11.772	1.929	7.50
SNL	4.664	0.306	23.30
UPL	5.312	0.396	23.30
OL	2.998	0.128	70.07*
MW	5.456	0.414	-34.00
MH	6.781	0.650	20.00
BD	18.580	4.810	17.00
BW	11.223	1.759	17.00
DFH	15.039	3.185	53.50*
DFW	7.740	0.840	7.00
DFR	573.000	4,587.000	1.13
PCFL	17.082	4.123	96.50*
PCFW	4.087	0.238	3.00
PCFR	1,236.500	21,250.750	3.56
PLFL	12.749	2.285	77.00*
PLFW	3.137	0.137	-20.40
PLFR	636.000	5,625.500	11.72
AFH	11.116	1.733	43.00*
AFW	7.771	0.845	43.30*
AFR	685.000	6,561.000	13.80
CFL	18.661	4.880	50.00*
CFR	1,361.000	25,733.000	2.19
GL	191.814	526.907	26.60*
ALGA	9.963	1.390	33.00*
ABGA	15.081	3.344	26.60*
PTNR	314.000	1.386.000	1.04
PTHT	333.000	1,557.000	0.71
PTWD	98.000	150.000	23.78

Table 3--Continued.

Variable Name	Sum X_{tot}	Sum X^2_{tot}	F
PNRAVG	112.000	192.000	29.30*
BNRAVG	2,272.000	77,072.000	151.80*
FLAVG	22.048	6.841	19.60
RLAVG	3.402	0.166	3.00
ALAVG	89.128	1b1.926	33.80*
ABAVG	3.006	0.136	20.00
RBCCV	491.022	4,139.478	54.13*
RBCNV	51.834	48.211	30.74*

*Indicates significant difference.

Table 4. Results of Student's t-test to determine between which two groups a variable was significantly different. P=2.655, df=68 for all but RBCCV and RBCNV, where P=2.673 and df=54 at 0.01 level. Refer to Appendix 1 for explanation of abbreviated variable names.

Variable Name	79HC:81HC[a]	80HC:81HC	79HC:GC[b]	80HC:GC	81HC:GC
TL	4.947*	4.546*	5.159*	7.090*	10.518*
SALL	1.546	0.069	18.638*	19.696*	17.357*
SBLL	1.809	0.624	6.871*	6.405*	5.082*
SILL	3.059*	2.875*	11.095*	12.148*	8.058*
CL	0.000	1.123	4.116*	3.558*	4.196*
CPL	2.960*	0.000	8.418*	6.145*	5.435*
TRL	0.404	2.449	9.962*	8.409*	9.726*
HL	0.951	3.464*	7.937*	6.632*	9.103*
OL	10.213*	8.713*	2.662*	6.137*	13.569*
DFH	2.664*	4.762*	8.730*	8.233*	11.732*
PCFL	4.757*	1.515	15.080*	13.264*	10.316*
PLFL	0.269	2.142	12.347*	11.643*	12.301*
AFH	1.345	3.979*	8.699*	7.439*	10.298*
AFW	2.456	1.677	10.759*	7.676*	8.356*
GL	4.729*	4.023*	5.338*	4.719*	0.415
ALGA	3.498*	1.837	1.684	4.205*	5.435*
ABGA	1.798	4.009*	4.950*	7.779*	3.135*
PNRAVG	1.080	1.853	7.786*	9.634*	6.789*
BNRAVG	0.871	0.012	15.616*	19.059*	16.846*
ALAVG	3.691*	1.737	9.233*	8.039*	5.488*
CFL	1.713	3.897*	8.135*	7.776*	10.518*
RBCCV	1.890	2.401	2.917*	3.102*	4.987*
RBCNV	0.596	2.050	2.691*	1.652	3.377*

[a]HC=hybrid grass carp
[b]GC=grass carp
*Indicates significant difference

Weights of the fish were not included in the final statistical analyses because the amounts and types of food and the frequency of feeding varied greatly among the groups. However, as shown in Table 5, a large difference was seen between the weight of the grass carp and the weights of the hybrid grass carp. The mean weight of the grass carp was much greater than any of the mean weights of the hybrid grass carp, even though the mean total length of the 1979 hybrid grass carp was not significantly different from that of the grass carp (Table 5). The mean head length, trunk length, and caudal length illustrate the vast differences in size between the 1980-81 grass carp group and the 1979, 1980, and 1981 hybrid grass carp.

The standard length ratios given in Table 6 show the relationship of the three body regions in the grass carp and the hybrid grass carp. The relative mean head lengths, trunk lengths, and caudal lengths of the three year classes of hybrid grass carp were quite similar, but were found to be statistically different from those of the grass carp. Compared to the results obtained in this study, slightly different values for the head length/standard length ratio were reported by Sutton et al. (1981) and Kilambi and Zdinak (1981), but in each study the relative values were found to be greater for the hybrid grass carp than the grass carp. In general, the grass carp had smaller head length/standard length and caudal length/standard length ratios, while hybrid grass carp were found to have a smaller trunk length/standard length ratio (Table 6, Figure 3).

Table 5. Mean length and weight measurements obtained in morphometric comparison.

	1980-81 Grass Carp	1979 Hybrid Grass Carp	1980 Hybrid Grass Carp	1981 Hybrid Grass Carp
Mean head length (cm)	7.78	8.79	7.05	4.87
Range (cm)	6.6-9.2	7.0-9.9	5.8-8.4	3.5-5.9
Mean trunk length (cm)	17.90	14.88	12.96	8.14
Range (cm)	14.6-21.4	10.6-18.2	10.1-16.1	5.8-10.5
Mean caudal length (cm)	9.88	10.94	8.82	5.92
Range (cm)	8.5-11.6	7.9-13.2	6.8-11.3	4.4-9.0
Mean total length (cm)	41.87	41.11	34.44	22.81
Range (cm)	35.3-49.8	31.2-49.2	27.5-40.9	17.2-28.9
Mean weight (g)	837.82	713.06	441.72	127.15
Range (g)	477.8-1,272.1	274.1-1,117.2	214.7-743.5	52.3-227.1

Table 6. Mean length/standard length ratios of external measurements obtained in morphometric study.

	1980-81 Grass Carp	1979 Hybrid Grass Carp	1980 Hybrid Grass Carp	1981 Hybrid Grass Carp
Head length/standard length	0.23	0.27	0.26	0.27
SE	0.0022	0.0037	0.0022	0.0040
Trunk length/standard length	0.52	0.45	0.47	0.45
SE	0.0029	0.0033	0.0051	0.0030
Caudal length/standard length	0.29	0.33	0.32	0.33
SE	0.0027	0.0030	0.0029	0.0151
Caudal peduncle length/ standard length	0.18	0.21	0.20	0.20
SE	0.0021	0.0017	0.0026	0.0020
Orbit length/standard length	0.04	0.04	0.04	0.05
SE	0.0007	0.0010	0.0008	0.0022

Figure 3. 1981 hybrid grass carp (top) and 1981 grass carp (bottom).


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A significant difference was determined for the relative mean orbit length and caudal peduncle length between the four groups of fish (Table 6). Although Kilambi and Zdinak (1981) found the orbit length to be significantly greater in grass carp when compared to similar sized hybrid grass carp, in this study no significant difference was found between the orbit length/standard length ratio of the 1979 hybrid grass carp and the grass carp. A significant difference was not found for the relative mean caudal peduncle length between the 1980 and 1981 hybrid grass carp, but the caudal peduncle length/standard length ratio for the three year classes of hybrid grass carp were statistically different from that of the grass carp.

The dorsal, pectoral, pelvic, anal, and caudal fin length to standard length ratios were significantly larger for the hybrid grass carp compared to grass carp (Table 7). The anal fin width/standard length ratio was found to be statistically larger for hybrid grass carp. The pectoral fin length/standard length ratio was found to be significantly longer in hybrid grass carp than grass carp by Kilambi and Zdinak (1981). Sutton et al. (1981) found that the caudal fin was an intermediate feature between the parental grass carp and bighead carp. The larger fin sizes of the hybrid grass carp were quite obvious when compared to those of the grass carp (Figure 3). Although the fin length ratios were quite similar among the year classes of hybrid grass carp, the dorsal, anal, and caudal fin length/standard length ratios were found to be significantly different between the 1980 and 1981 hybrid grass carp. This may be due to the high incidence of diploidy

Table 7. Fin length/standard length ratios determined in morphometric comparison.

	1980-81 Grass Carp	1979 Hybrid Grass Carp	1980 Hybrid Grass Carp	1981 Hybrid Grass Carp
Dorsal fin length/SL[a]	0.18	0.22	0.21	0.23
SE	0.0016	0.0031	0.0035	0.0031
Pectoral fin length/SL	0.19	0.27	0.25	0.24
SE	0.0026	0.0060	0.0025	0.0021
Pelvic fin length/SL	0.15	0.19	0.18	0.19
SE	0.0017	0.0026	0.0022	0.0017
Anal fin length/SL	0.13	0.16	0.16	0.17
SE	0.0015	0.0021	0.0015	0.0014
Anal fin width/SL	0.10	0.12	0.11	0.11
SE	0.0012	0.0016	0.0018	0.0013
Caudal fin length/SL	0.23	0.27	0.26	0.28
SE	0.0036	0.0039	0.0032	0.0030

[a]SL=Standard Length

which occurred in the 1980 spawn (Malone, 1982; Magee and Phillip, 1982).

Differences in the size and number of scales in the lateral lines of the hybrid grass carp and grass carp were readily apparent (Table 8). The three year classes of hybrid grass carp had a significantly greater number of scales above, below, and in the lateral line than the grass carp. The number of scales in the lateral line of the hybrid grass carp ranged from 45-63, with 9-11 scales above the lateral line and 5-7 below the lateral line. The number of grass carp scales in the lateral line ranged from 37-44, with 6-7 scales above the lateral line and 5 scales below the lateral line. The scales in the hybrid grass carp were generally smaller and more numerous than those of the grass carp (Figure 4), but were more variable between individuals.

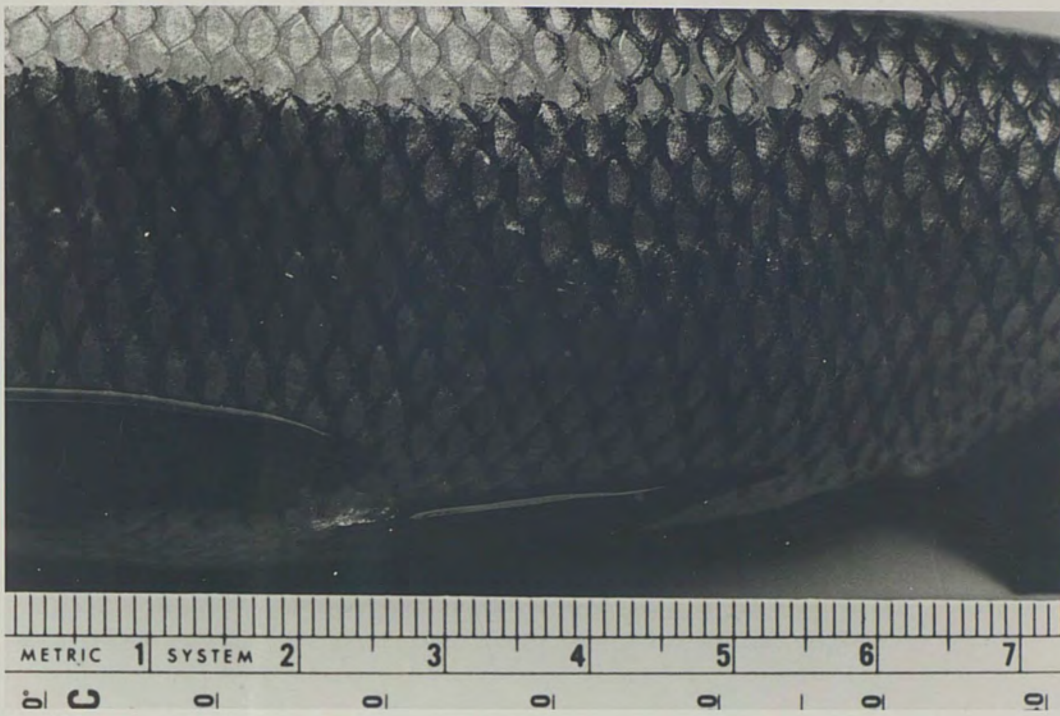
The relative gut length of the 1981 hybrid grass carp more closely resembled that of the grass carp than those of the 1979 and 1980 hybrid grass carp (Table 9). Michewicz et al. (1972) and Anonymous (1976b) reported that the gut length of the grass carp ranged from two to three times the standard length; the same relationship was found for the fish examined in this study. The grass carp and 1981 hybrid grass carp had significantly longer relative gut lengths than the 1979 and 1980 hybrid grass carp. The relative gut length was a major difference found between the 1979 and 1980 year classes and the 1981 year class of hybrid grass carp.

The 1981 hybrid grass carp had a reduced number of gill raker deformities when compared to the 1979 and 1980 hybrid grass carp. The

Table 8. Numbers of scales in lateral line region recorded during morphometric comparison.

	1980-81 Grass Carp	1979 Hybrid Grass Carp	1980 Hybrid Grass Carp	1981 Hybrid Grass Carp
Mean number of scales above lateral line	6.16	10.00	9.68	9.67
Range	6-7	9-11	9-11	9-11
s^2	0.1392	0.2857	0.3776	0.4889
SE	0.0879	0.1429	0.1229	0.1805
Mean number of scales below lateral line	5.00	6.29	6.04	5.93
Range	5	5-7	5-7	5-7
s^2	0.0000	0.3469	0.4384	0.1956
SE	0.0000	0.1574	0.1324	0.1142
Mean number of scales in lateral line	40.74	51.29	50.76	48.27
Range	37-44	48-53	45-63	45-52
s^2	3.3952	2.0612	13.3024	3.7956
SE	0.4343	0.3837	0.7295	0.5030

Figure 4. Scales in trunk region of a 1981 hybrid grass carp (top) and a 1981 grass carp (bottom).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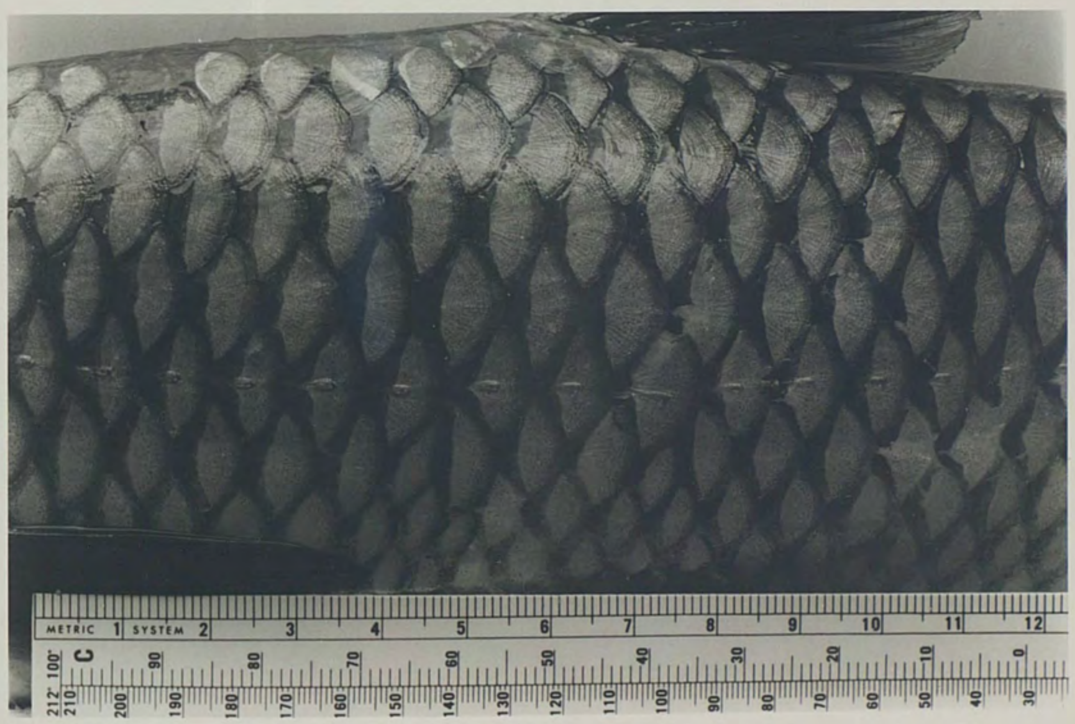
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Table 9. Gut length measurements obtained during morphometric comparison.

	1980-81 Grass Carp	1979 Hybrid Grass Carp	1980 Hybrid Grass Carp	1981 Hybrid Grass Carp
Mean gut length (cm)	103.4	75.1	67.5	51.5
Range (cm)	80.9 - 129.8	44.8 - 90.2	52.5 - 101.1	34.7 - 60.0
Gut length/standard length ($\bar{x}$)	3.01	2.29	2.46	2.95
s^2	0.0742	0.0517	0.0928	0.3446
SE	0.0642	0.0607	0.0609	0.1516

gill rakers in the 1980 hybrid grass carp were frequently deformed (probably caused by the high incidence of diploidy in that spawn), while fewer deformities were found in the rakers of the 1979 hybrids; the gill rakers of the 1981 hybrids were usually well formed. Berry and Low (1970) reported that gill rakers in the bighead carp are long and closely arranged, as opposed to those of grass carp, which have very small and sparsely arranged gill rakers. The gill rakers of hybrid grass carp appear to be intermediate, differing from both parental forms in length and number. In general, the gill rakers on the five branchial arches were found to be longer and more numerous for all year classes of hybrid grass carp when compared to those of the grass carp (Table 10). The gill filament/pharyngeal arch length ratio for the hybrid grass carp were smaller than for grass carp. The relative branchial arch length of the 1981 hybrid grass carp was slightly less than for the other year classes, but was significantly greater than that for the grass carp (Table 10). The grass carp were found to only have a slightly larger relative mean branchial arch breadth when compared to the hybrid grass carp. In general, the branchial arches in the grass carp tended to be short and broad, while those of the hybrid grass carp were thin and long. The teeth on the pharyngeal arches of the grass carp were generally larger than those of the hybrid grass carp (Table 11, Figure 5) and were always in two rows, with teeth formulas of either 2,4-5,2 or 2,5-4,2. Hybrid grass carp have either one or two rows of teeth on each arch (more often only one row) and nine teeth formulas. The number of teeth on the pharyngeal arches of the grass carp was generally greater than those of the

Table 10. Gill arch measurements calculated for hybrid grass carp and grass carp examined in morphometric study.

	1980–81 Grass Carp	1979 Hybrid Grass Carp	1980 Hybrid Grass Carp	1981 Hybrid Grass Carp
Raker length/PAL[a]	0.04	0.05	0.05	0.05
SE	0.0020	0.0015	0.0013	0.0017
Filament length/PAL	0.34	0.30	0.28	0.31
SE	0.0056	0.0076	0.0055	0.0061
Branchial arch breadth/PAL	0.06	0.04	0.04	0.04
SE	0.0024	0.0017	0.0018	0.0013
Branchial arch length/PAL	1.05	1.37	1.30	1.24
SE	0.0086	0.0262	0.0252	0.0201
Mean number of rakers per branchial arch	19.60	37.90	39.18	38.80
Mean number of rakers per pharyngeal arch	12.50	28.29	28.48	29.50

[a]PAL=Pharyngeal Arch Length

Table 11. Pharyngeal teeth and arch measurements for hybrid grass carp and grass carp obtained during morphometric comparison.

	1980-81 Grass Carp	1979 Hybrid Grass Carp	1980 Hybrid Grass Carp	1981 Hybrid Grass Carp
Height of larger teeth/PAL[a]	0.26	0.18	0.19	0.19
Height of smaller teeth/PAL	0.12	0.07	0.08	0.08
Width of larger teeth/PAL	0.09	0.06	0.06	0.07
Width of smaller teeth/PAL	0.04	0.02	0.03	0.04
Pharyngeal arch length/SL[b]	0.13	0.13	0.14	0.15
Pharyngeal arch breadth/PAL	0.26	0.20	0.17	0.22

[a]PAL=Pharyngeal Arch Length

[b]SL=Standard Length

Figure 5. Teeth on pharyngeal gill arches of 1980 hybrid grass carp (top) and 1981 grass carp (bottom).


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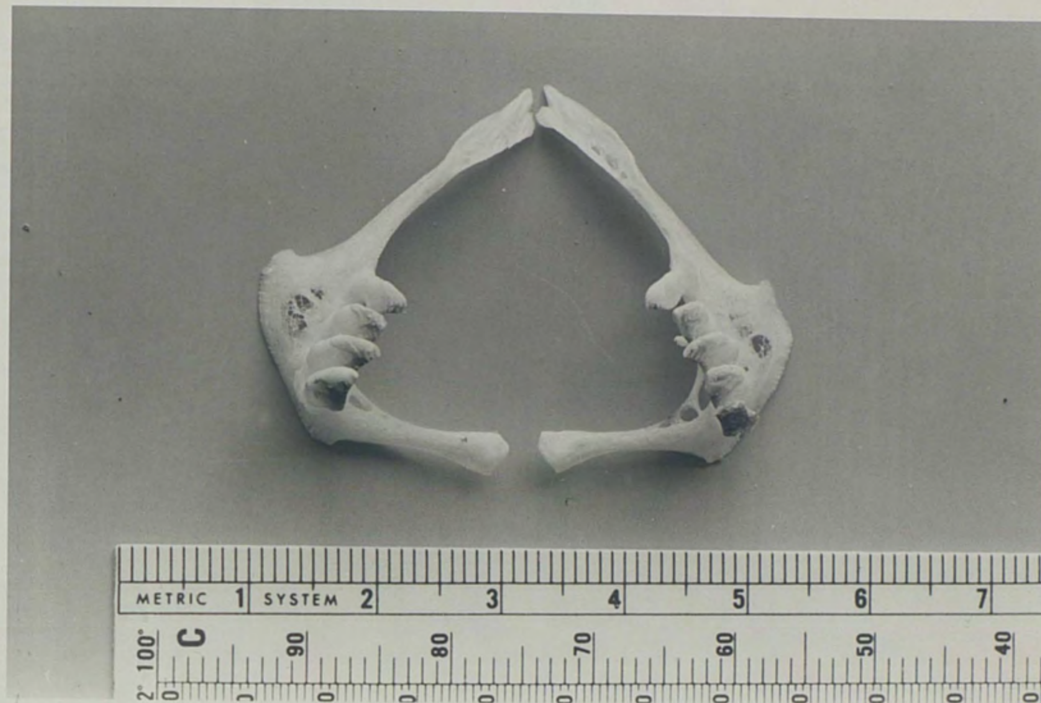
hybrid grass carp. The pharyngeal arches of the grass carp were generally longer and broader (Figure 6), although no significant difference was found between the relative pharyngeal arch lengths of the grass carp and the 1979 hybrid grass carp (Table 11).

The relative mean erythrocyte volumes were significantly larger in all year classes of hybrid grass carp compared to that of the grass carp (Table 12), while the relative mean erythrocyte nucleus volumes of the 1979 and 1981 hybrid grass carp were significantly larger than that of the grass carp. The lack of a significant difference for the relative mean erythrocyte nucleus volume between the 1980 hybrid grass carp and the grass carp probably reflects the high number of diploid fish in this year class, as indicated by Magee and Philipp (1982). The triploid condition was assumed if the mean nucleus volume was 50% greater than that for the grass carp; this condition was found in the 1981 hybrid grass carp. These fish had a relative mean erythrocyte nucleus volume that was 52.2% larger than that of the grass carp, indicating that most if not all of these fish were triploid. The relative mean erythrocyte nucleus volume of the 1979 and 1980 hybrid grass carp were only 37.3 and 11.9% greater than that of the grass carp group. These low values are probably due to a greater number of diploid hybrid grass carp produced with the triploid fish in those spawns or to a mixture of diploid and triploid cells in the individual fish (the "mosaic" condition). When the actual sizes of the nuclei of the hybrid grass carp and grass carp were compared, it was found that 100% of the 1979, 46% of the 1980, and 93% of the 1981 hybrid grass carp had

Figure 6. Pharyngeal arches of 1980 hybrid grass carp (top) and 1981 grass carp (bottom).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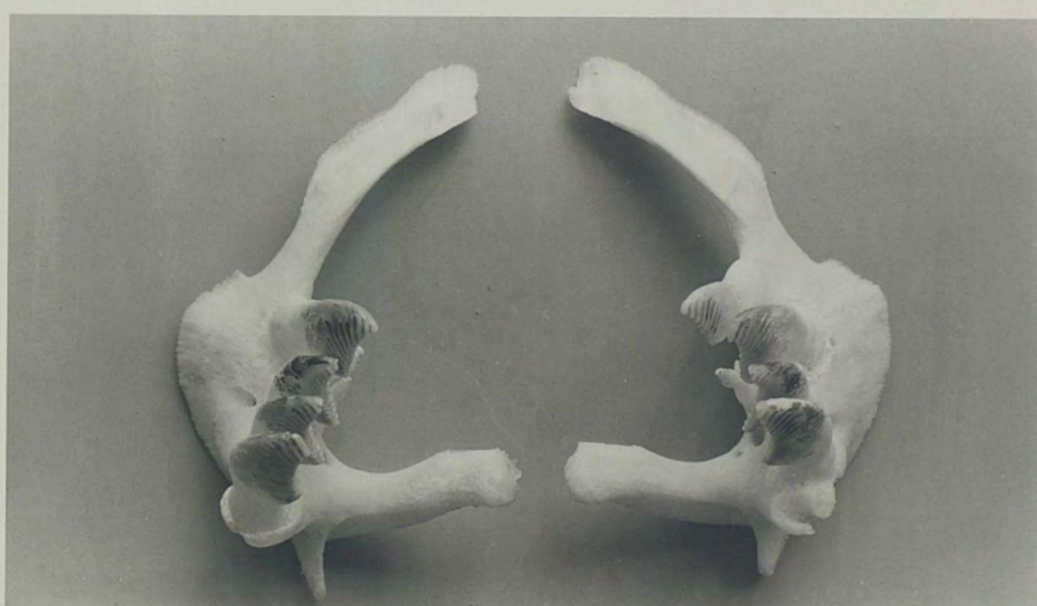
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Table 12. Erythrocyte and erythrocyte nucleus volume measurements for hybrid grass carp and grass carp.

	1980 Grass Carp	1979 Hybrid Grass Carp	1980 Hybrid Grass Carp	1981 Hybrid Grass Carp
Mean relative cell volume[a]	4.4	8.0	7.6	10.1
Mean relative nucleus volume[a]	0.67	0.92	0.75	1.02
Mean amount that hybrid grass carp ENV[b] is larger than grass carp ENV (%)	---	37.3	11.9	52.2
% hybrid grass carp with larger ENV than mean grass carp ENV	---	100.0	46.2	93.3

[a] In units of ocular micrometer, observed at 1,000 X
[b] ENV=Erythrocyte Nucleus Volume

larger nucleus volumes than the mean grass carp value (Table 12). This difference in size would seem to indicate that these hybrids were triploid. Although the comparison of the relative sizes of erythrocyte nucleus volumes (rather than the number of hybrids with larger nuclei) is more accurate, it should be noted that Magee and Philipp (1982) found an average of 44% of their 1980 hybrid grass carp were triploid, while 100% of their 1979 and 1981 fish were triploid. Based on electrophoresis and histochemical procedures, they determined that the bighead carp and grass carp more closely resembled subspecies or sibling species rather than distinct genera. They concluded that there may be some question as to the sterility of the hybrid grass carp due to the presence of diploid fish in the 1980 hybrid grass carp spawn.

The larger relative gut length, fewer deformities of the gill rakers, and a reduction in the number of diploid fish in the 1981 hybrid grass carp spawn were the major morphological differences between the year classes of hybrid grass carp. There was little or no change in the growth rate or mortality rate of the 1981 hybrid grass carp from those of the 1979 and 1980 fish; there was, however, a major difference in the feeding rate and food conversion efficiency of the 1981 hybrid grass carp and those of the 1979 and 1980 hybrids.

The amount of *Egeria densa* consumed fish^{-1} day^{-1} by the 1981 hybrid grass carp in the 14-day trial was only 30.0 g, which is equivalent to approximately 15% of their body weight per day; this was less than one-half that consumed by the 1980 hybrid grass carp and about one-tenth that consumed each day by the 1979 hybrids (Tables 13 and

Table 13. Results of feeding and vegetation control trials using 1981 hybrid grass carp in experimental ponds.

	Trial 1	Trial 2	Trial 3
Initial mean weight (g)	201.9	247.4	211.8
Initial mean standard length (cm)	21.3	22.5	21.8
Final mean weight (g)	253.7	299.7	321.5
Final mean standard length (cm)	22.7	24.8	24.5
Mean weight gain $fish^{-1}$ day^{-1} (g)	3.7	2.5	3.9
Mean length gain $fish^{-1}$ day^{-1} (cm)	0.10	0.11	0.10
Egeria consumed $fish^{-1}$ day^{-1} (g)	30.0	---	---
Efficiency (%)	14.8	---	---
Initial stocking rate (fish ha^{-1})	123.1	69.2	153.8
Initial number of fish	16	9	20
Final number of fish	10	3	20
Mortality (%)	37.5	66.7	0.0
Duration of trial (days)	14	21	28

14). Sutton (1983) reported that small hybrid grass carp (with a mean weight of 550 g) were less effective than large hybrid grass carp (with a mean weight of 2,800 g) at eliminating hydrilla in a closed recirculating system. Results of five trials indicate that the large hybrid grass carp consumed 5.4 g (dry weight) $fish^{-1}$ day^{-1} of plant material while the small hybrids only consumed 0.4 g $fish^{-1}$ day^{-1} of hydrilla. As shown in Table 14, grass carp with initial mean weights of 0.858 kg and 1.270 kg were able to consume significantly larger amounts of Egeria in the experimental pond studies, consuming more than 37 times as much as the 1981 hybrid grass carp.

Grass carp fry have been observed feeding on algae, rotifers, crustaceans, and chironomid larvae (Michewicz et al., 1972; Opuszynski, 1979; Watkins et al., 1981). These fish begin the transition from animal to plant food when they reach approximately 25 mm in length (De Silva and Weerakoon, 1981). Between 30-49 mm in length, they usually make the transition complete (Michewicz et al., 1972; Opuszynski, 1979), although Watkins et al. (1981) found grass carp 50-100 mm in length which consumed invertebrates. Small hybrid grass carp have also been observed feeding on animal material; Cassani (1981) reported that hybrid grass carp fingerlings held in a 143 l aquarium readily consumed leeches (Hirudinea) and mosquito larvae (*Culex quinquefasciatus* Say). Kilambi and Zdinak (1982) found hybrid grass carp which preferred zooplankton over *Chara*. Freshwater shrimp (*Palemonetes paludosus*) and mosquitofish (*Gambusia affinis*) were removed from the digestive tracts of four hybrid grass carp from the

Table 14. Weight gain, consumption rate and feeding efficiency of 1979 and 1980 hybrid grass carp and 1981 grass carp in experimental ponds.[a]

	1979 Hybrid Grass Carp	1980 Hybrid Grass Carp	1981 Grass Carp	1981 Grass Carp
Initial mean weight (g)	754.0	178.0	858.0	1,270.0
Final mean weight (g)	812.0	316.0	1,270.0	1,600.0
Mean weight gain $fish^{-1}$ day^{-1} (g)	2.8	3.9	20.7	29.7
Egeria consumed $fish^{-1}$ day^{-1} (g)	278.0	76.1	983.3	1,117.9
Efficiency (%)	1.0	5.2	2.1	2.4
Duration of trial (days)	20	35	14	14

[a]From Osborne, 1982b.

0.13 ha experimental ponds in this study. These hybrid grass carp were from the 1979, 1980, and 1981 spawns and ranged in size from 17.8 to 43.7 cm in length and 119.8 to 1,383.6 g in weight; the largest fish had both freshwater shrimp and mosquitofish in its intestine. None of the grass carp examined in this study had animal material in its gut. Animal material may possibly be a more important factor in the diet of large hybrid grass carp than it is in large grass carp.

According to Osborne (1982b), grass carp stocked in hydrilla-infested lakes generally grow at a rate of 15 g $fish^{-1}$ day^{-1}. In Blue Lake, where there was an abundance of vegetation, the 1981 hybrid grass carp had a mean weight gain of only 1.3 g $fish^{-1}$ day^{-1}, only slightly better than that of the 1979 hybrids during a previous study in Blue Lake (Table 15). When stocked into this lake, the mean weight of the 1981 hybrid grass carp was almost three times that of the initial mean weight of the 1979 hybrids, but the 1981 fish only reached a mean weight of 504.8 g while the 1979 hybrids reached a mean weight of 580.3 g. The hybrid grass carp stocked in the detention pond decreased in size during the 313 day study (Table 15), in spite of the presence of vegetation in the pond throughout the study, although this is probably a reflection of the high mortality rate that occurred in this situation. The 1981 hybrid grass carp used in the field trials in the experimental ponds had growth rates that were up to 75 times less than those of the grass carp (Tables 14 and 15). The 1981 hybrid grass carp used in the 115 day and the 318 day trials had extremely low growth rates; these fish increased only 0.4 and 0.8 g $fish^{-1}$ day^{-1},

Table 15. Results of field trials in experimental ponds, Blue Lake, and detention pond using 1981 hybrid grass carp.

	Experimental Pond		Blue Lake		Detention Pond
	Trial 1	Trial 2	1979[a]	1981	
Initial mean weight (g)	504.8	86.3	25.8	76.7	199.4
Final mean weight (g)	546.7	333.1	580.3	504.8	198.5
Initial mean standard length (cm)	26.7	16.0	---	14.4	21.1
Final mean standard length (cm)	28.4	23.9	---	26.7	19.6
Mean weight gain $fish^{-1}$ day^{-1} (g)	0.4	0.8	0.9	1.3	-0.003
Mean length gain $fish^{-1}$ day^{-1} (cm)	0.01	0.02	---	0.04	-0.005
Initial stocking rate (fish ha^{-1})	2,253.8	1,538.5	166.7	1,666.7	833.3
Initial number of fish	293	200	75	750	350
Final number of fish	232	71	57	308	9
Mortality (%)	20.8	64.5	24.0	58.9	97.4
Duration of trial (days)	115	318	598	330	313

[a]From Osborne, 1982b.

respectively, even though numerous types of food were offered. Sutton (1981) reported that hybrid grass carp with a mean initial weight of 58 g increased 0.6 g $fish^{-1}$ day^{-1} when fed duckweed and trout chow, while fish with a mean initial weight of 80 g gained 2.8 g $fish^{-1}$ day^{-1}. The amount gained by the smaller fish is comparable to that obtained in this study by the 1981 hybrid grass carp supplied with a variety of food (Table 15), while the 2.8 g $fish^{-1}$ day^{-1} increase of the larger fish is similar to that obtained in the feeding trials in this study (Table 13). The 1981 hybrid grass carp in these feeding trials had a growth rate only slightly greater than that of the 1979 hybrids and almost identical to that of the 1980 hybrids, the spawn that contained fish characterized by a low rate of growth (Tables 13 and 14). Growth of the 1981 hybrid grass carp in the short-term trials was six to eight times less than that of the 1981 grass carp used in previous experimental pond studies, which reached gains of 29.7 g $fish^{-1}$ day^{-1}. The 1981 hybrid grass carp had a mean increase in weight ranging from 2.5 g to 3.9 g $fish^{-1}$ day^{-1}, while their standard length increased only about 0.10 cm $fish^{-1}$ day^{-1}. These data indicate that after an initial period of low growth, the growth rate of the 1981 hybrid grass carp decreased significantly throughout the duration of the trial.

Even though there was a limited amount of predation in Blue Lake (water snakes and wading birds), the 1981 hybrid grass carp still incurred a high rate of mortality. The mortality rate of the 1981 hybrids was 58.9%, more than twice as high as the 24.0% of the 1979 hybrid grass carp that had been stocked previously in this lake (Table 15). The majority of these deaths was probably due to the hybridization process, since there was no competition or predation pressure from other fish species and vegetation was plentiful in the lake.

A spillway built from the detention pond to the adjacent lake in October, 1982 may have allowed the escape of the 1981 hybrid grass carp when the water level in the pond was periodically reduced for flood control purposes. Only nine of the 350 fish stocked into the pond were recovered (Table 15); the final mean weight and standard length measurements were actually less than the original mean weight and length. Although there was no way to distinguish those fish that escaped into the lake from those fish eliminated by predation and hybridization problems, five of six marked 1981 hybrid grass carp that had been placed in the pond on February 1, 1983 were recovered 35 days later on March 8, 1983. This indicates that fish stocked into the pond tended to remain in the pond and did not move to the lake during the drawdowns. It is assumed that a majority of the fish stocked into the pond for the study were eliminated by predation and hybridization problems. Sutton (1983) reported a mortality rate of 100% for the 12 hybrid grass carp that were stocked in a 0.23 ha canal for weed control on February 3, 1982. He also reported that of the 1,200 hybrid grass carp (with a mean length of 20 cm) put into four research ponds on July 20 and 21, 1981, only 79 were recovered on April 16, 1982; these fish had been fed catfish chow and alfalfa pellets in addition to the vegetation in the ponds, but the mortality rate was

93.4%. These results coincide with the loss of 97.4% of the 1981 hybrid grass carp stocked in the detention pond.

The mortality rate for the 1981 hybrid grass carp in the field trials and feeding and growth trials in the experimental pond studies ranged from 0.0–66.7% (Tables 13 and 15), in spite of the absence of other fish species in the ponds and the protection against predation provided by the electric fencing. The stocking density of these fish did not appear to be correlated with the high mortality rates; at densities of 69.2 and 1,538.5 fish ha^{-1}, the mortality rates were 66.7% and 64.5%, while fish stocked at 153.8 and 2,253.8 ha^{-1} had mortality rates of 0.0% and 20.8%. It is assumed that the high rates of mortality in these 0.13 ha ponds, in the presence of adequate food (both plant and animal) and in the absence of competition and predation, were the result of the hybridization process.

The stocking rate of the 1981 hybrid grass carp at 1,666.7 fish ha^{-1} was not adequate to control (eliminate) the hydrilla in Blue Lake during this trial. Sutton (1983) reported that, with stocking densities of 2,000 fish ha^{-1}, the standing crop usually was not eliminated or even decreased, but in some cases actually increased in the presence of these fish. Osborne (1982b) reported that leaf feeding by the 1979 hybrid grass carp in Blue Lake from November, 1979 to June, 1981 apparently stimulated heavy growth of *Hydrilla verticillata*, resulting in high biomass and a lack of control or a reduction in the amount of vegetation, even during the winter months. With a monthly mean hydrilla biomass greater than 3.0 kg m^{-2}, vegetation remained at a high level throughout that study. The monthly biomass in Blue Lake in 1981-1982 also averaged about 3.0 kg m^{-2}. The hydrilla biomass in Blue Lake at the time of stocking in November, 1981 was approaching its lowest level of the study (Table 16). After the introduction of the 1981 hybrid grass carp, the biomass of the hydrilla steadily increased until it finally reached a maximum of 3.997 kg m^{-2}-FW (almost 18.0 mt-FW in the lake) in August, 1982. The percent frequency of occurrence of hydrilla in Blue Lake was at 100% for all sampling months (October, 1981 - August, 1982). Chara spp and Najas guadalupensis were found in Blue Lake during 1979-1980, but these species gradually declined in biomass in 1981-1982 until they were no longer found by the end of the study; probably the heavy growth of hydrilla in the lake caused the elimination of these species.

Construction of a shopping mall across the road from the south end of the detention pond caused large amounts of silt to be introduced into this body of water. Turbidity in the pond greatly increased and vegetation became covered with silt; from October, 1982 until the conclusion of the trial, no more vegetation was collected with the Osborne submersed aquatic plant sampler (Table 17). Until the influx of silt into the pond, the Eleocharis baldwinii and Hydrilla verticillata had increased in percent frequency of occurrence from 60-93% and 0-60%, respectively; after the influx, the frequency of occurrence dropped to 0% for both species. Hydrilla was found in the shallows at the southern end of the pond in depths of 0.6-0.9 m from October, 1982 until March, 1983. These plants were accessible to the hybrid grass

Table 16. Percent frequency of occurrence (% freq) and monthly and annual mean biomass (kg m^{-2}-FW and mt-FW) of submersed vegetation in Blue Lake, Florida from October, 1981 - September, 1982.

	Hydrilla verticillata		
	% freq	kg m^{-2}-FW	mt-FW
October	100.0	2.081	9.4
November			
December	100.0	1.880	8.5
January			
February	100.0	2.643	11.9
March			
April	100.0	3.199	14.4
May			
June	100.0	3.522	15.9
July			
August	100.0	3.997	17.9
September			
ANNUAL	100.0	2.887	13.0

Table 17. Percent frequency of occurrence (% freq) and monthly and mean biomass (kg m^{-2}-FW and mt-FW) of submersed vegetation in the detention pond located in Orange County, Florida from May, 1982 - March, 1983.

	Hydrilla verticillata			*Eleocharis baldwinii*		
	% freq	kg m^{-2}-FW	mt-FW	% freq	kg m^{-2}-FW	mt-FW
May	0	0.000	0.00	60	0.412	1.7
June	53	0.002	0.01	73	0.458	1.9
July						
August	60	0.003	0.01	93	0.346	1.8
September						
October	0	0.000	0.00	0	0.000	0.0
November						
December	0	0.000	0.00	0	0.000	0.0
January						
February	0	0.000	0.00	0	0.000	0.0
MEAN	18.8	0.001	0.003	37.7	0.203	0.9

carp in the pond, although not to the plant sampler, but the fish were unable to eliminate even this little amount of vegetation.

The 1981 hybrid grass carp in the experimental pond trials that lasted 115 days and 318 days were unable to control (eliminate) the vegetation in those 0.13 ha ponds. Although no measurements were recorded for the growth of vegetation in these two ponds, observations made during the studies confirmed that vegetation increased and spread throughout the ponds with little indication of feeding by the hybrid grass carp. The lack of control of the vegetation in the experimental ponds was probably due to the greater efficiency for converting vegetation into fish flesh. In the one feeding trial not hindered by the development of an algal bloom, the 1981 hybrid grass carp exhibited an efficiency of 14.8%, which was six to seven times larger than that of the 1981 grass carp and up to 15 times more efficient than the 1979 and 1980 hybrid grass carp rates (Tables 13 and 14). This ability to make more efficient use of the food consumed by the 1981 hybrid grass carp is probably a major factor in its low feeding and growth rates.

Although the statistical analyses showed the 1981 hybrid grass carp to be significantly different from the 1979 and 1980 hybrids, the few morphological and karyological characteristics found to be different do not seem to support the contention that the 1981 fish is a "new and different" hybrid grass carp. The high mortality rate, low growth and feeding rates, and greater food conversion efficiency appear to have made the 1981 hybrid grass carp less effective than the 1979 and 1980 hybrids as biocontrol agents; this effectively limits their usefulness against submersed aquatic vegetation, especially hydrilla.

SUMMARY

1. Statistical analyses showed that the 1981 hybrid grass carp differed significantly from both the 1979 and 1980 hybrids; all three year classes of hybrid grass carp differed statistically from the grass carp.
2. Results of the morphological and karyological comparisons between the 1979, 1980, and 1981 hybrid grass carp showed that the 1981 fish had a longer relative gut length, fewer deformities of the gill rakers, and a reduction in the number of diploids in the spawn.
3. The increased relative gut length in the 1981 hybrid grass carp appears to be related to an increased food conversion efficiency compared to that of the 1979 and 1980 hybrids. The increased efficiency is probably related to or the cause of the lowered feeding rate found in the 1981 hybrid grass carp.
4. In feeding and field trials the growth and mortality rates of the 1981 hybrid grass carp were similar to those of the 1979 and 1980 fish; the growth rate was low and the mortality rate was high.
5. Several hybrid grass carp from all three year classes were found with small fish or invertebrates in their intestines; none of the grass carp examined contained any recognizable animal material.
6. The 1981 hybrid grass carp was unable to control (eliminate) aquatic vegetation in the experimental ponds, a small lake, and a detention pond under a wide range of conditions. These varied

conditions included waters with and without prior herbicide treatment, with and without predators, trials of long and short duration, high and low stocking rates of the hybrid grass carp, and various sizes and ages of fish.

7. The high mortality rate, low feeding and growth rates, and high food conversion efficiency effectively limit the usefulness of the 1981 hybrid grass carp as a biocontrol agent.

8. The proven ineffectiveness of the hybrid grass carp at weed control will hopefully cause a return to the use of grass carp as biocontrol agents for aquatic plants. Although it may have the potential, I do not believe and have found no reports that grass carp have "ruined" rivers and streams in the United States to the extent predicted in the early 1970's. Strictly controlled studies in a river system may now be in order to determine precisely the effect of grass carp in those waters.

9. The production of a sterilized grass carp may also result in an effective biocontrol agent to replace the hybrid grass carp, but further testing needs to be conducted to determine the feasibility of production and use of this organism.

Appendix

Description of Contents:

Abbreviated variable names used to illustrate results of analysis of variance and Student's *t*-test analysis between the four groups of hybrid grass carp and grass carp.

Table 18.

Variable	Abbreviation
Weight	WT
Total length/SL[a]	TL
Standard length	SL
Scales above lateral line	SALL
Scales below lateral line	SBLL
Scales in lateral line	SILL
Caudal length/SL	CL
Caudal peduncle length/SL	CPL
Caudal peduncle height/SL	CPH
Trunk length/SL	TRL
Head length/SL	HL
Head width/SL	HW
Snout length/SL	SNL
Upper jaw length/SL	UPL
Orbit length/SL	OL
Mouth width/SL	MW
Mouth height/SL	MH
Body depth/SL	BD
Body width/SL	BW
Dorsal fin length/SL	DFH
Dorsal fin width/SL	DFW
Dorsal fin - no. rays	DFR
Pectoral fin length ($\bar{x}$)/SL	PCFL
Pectoral fin width ($\bar{x}$)/SL	PCFW
Pectoral fin - no. rays ($\bar{x}$)	PCFR
Pelvic fin length ($\bar{x}$)/SL	PLFL
Pelvic fin width ($\bar{x}$)/SL	PLFW
Pelvic fin - no. rays ($\bar{x}$)	PLFR
Anal fin length/SL	AFH
Anal fin width/SL	AFW
Anal fin - no. rays	AFR
Caudal fin length/SL	CFL
Caudal fin - no. rays	CFR
Gut length/SL	GL
Gill raker length ($\bar{x}$)/PAL[b]	RLAVG
Filament length ($\bar{x}$)/PAL	FLAVG
No. gill rakers ($\bar{x}$) per branchial arch	BNRAVG
No. gill rakers ($\bar{x}$) per pharyngeal arch	PNRAVG
Gill arch length ($\bar{x}$)/PAL	ALAVG
Gill arch breadth ($\bar{x}$)/PAL	ABAVG
Pharyngeal arch length ($\bar{x}$)/SL	ALGA
Pharyngeal arch breadth ($\bar{x}$)/PAL	ABGA

Table 18--Continued.

Variable	Abbreviation
Pharyngeal teeth - no. per arch ($\bar{x}$)	PTNR
Pharyngeal teeth height ($\bar{x}$)	PTHT
Pharyngeal teeth width ($\bar{x}$)	PTWD
Erythrocyte nucleus volume ($\bar{x}$)	RBCNV
Erythrocyte volume ($\bar{x}$)	RBCCV

[a]SL=Standard Length
[b]PAL=Pharyngeal Arch Length

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