

**Morphological comparisons of diploid and triploid hybrid grass carp, *Ctenopharyngodon idella* ♀ × *Hypophthalmichthys nobilis* ♂**

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Morphological and karyological comparisons of 25–28 hybrid grass carp from each of 3 year-classes (1979–1981) indicated that diploid fish resulted during 1979 (82.1%) and 1980 (76.0%) the others being triploid. All of the fish tested from the 1981 year-class were triploid. Most triploid fish differed from diploid fish by having a faster growth rate, fewer scales in the lateral line and transverse series below the lateral line, a relatively longer gut, and fewer deformities.

## I. INTRODUCTION

The use of exotic herbivorous fish as biological control agents for aquatic weeds has not gained widespread acceptance in the U.S.A. Much of the controversy centres on the possibility of reproduction, especially for the grass carp, *Ctenopharyngodon idella*, which is banned from 35 states (Sutton, 1977). In 1979, a commercial fish farmer, J. M. Malone and Son, Inc., began commercial production of a hybrid grass carp which was theoretically sterile and was considered a potential replacement for the grass carp. The hybrid is the result of a cross of female grass carp ( $2n=48$ ) and male bighead carp, *Hypophthalmichthys nobilis* ( $2n=48$ ). Beck *et al.* (1980) demonstrated that the hybrid was triploid ( $2n=72$ ) based on a sample of 25 fish produced during 1979 from the Malone hatchery. Beck *et al.* (1980) concluded that triploidization was most likely caused by retention of a polar body and not by dispermy due to the hybrid having two maternal sets and one paternal set of chromosomes. The triploid hybrid is considered sterile due to the production of aneuploid gametes theoretically disallowing normal development. Magee & Philipp (1982) assessed 100 fish from each year-class with electrophoretic techniques and found the 1979 and 1981 year-classes to be 100% triploid. However, only 32% to 60% of fish from the 1980 year-class were triploid, the others were diploid.

Hybrid grass carp purchased from the 1979 and 1980 year classes of Malone and Son were mostly slow growing (80–90%), many of which were deformed. Chromosomal analysis of six slower growing fish indicated that they were diploid ( $2n=48$ ).

Kilambi & Zdinak (1981) compared grass carp with hybrid grass carp based on 12 morphometric measurements, but no discussion of the hybrid ploidy was made. Ten similar sized grass carp, bighead carp and hybrid grass carp were compared on a morphological basis by Sutton *et al.* (1981) although the ploidy of these

fishes was not determined. European researchers have also reported conflicting results concerning growth rate and morphological traits for hybrids from the grass carp  $\times$  bighead cross (Aliev, 1967; Andriyasheva, 1968; Marian & Krasznai, 1978). Our field observations of hybrid grass carp feeding behaviour indicate a high degree of inconsistency, possibly a result of genetic differences.

The purpose of this study is to determine morphological differences that could be used to differentiate diploids from triploids thereby enabling easier screening of inferior or potentially fertile fish.

## II. MATERIALS AND METHODS

Morphological measurements were made on 28, 25 and 25 fish from each of the 1979, 1980 and 1981 year classes respectively. Measurements of total length (T.L.), standard length (S.L.), head width, head length, height, snout length, interorbital width, lateral line scales, scales in the transverse series above and below the lateral line, gut length, fin ray counts, gill rakers and pharyngeal teeth counts were made as described by Hubbs & Lagler (1949).

Several thousand fish from each year class were kept in separate ponds and fed duckweed and an artificial catfish feed. All fish used were between 1 and 2 years old and were selected at random from a seine holding 100–200 fish at a time.

For determining chromosome numbers, individual fish were given intraperitoneal injections of a 0.1% solution of colchicine at a dosage of 0.01 ml g<sup>-1</sup> body weight. Gill epithelial and splenic tissues were removed 3–6 h after injection, and mascerated in a hypotonic solution (1.0% sodium citrate) and centrifuged at 750 g. This hypotonic treatment was repeated with total exposure not exceeding 45 min. After the final centrifugation in the hypotonic solution, cellular pellets were fixed by adding 0.5 ml of 3:1 methanol-acetic acid, and refrigerated for 30 min, followed by two additional fixations for 15 min each. Final cellular suspensions were dropped onto cold slides, air-dried and stained in 10% Giemsa.

A modification of this technique was used for 19 of the 1981 fish. This involved an intraperitoneal injection of 0.05% colchicine at a dosage of 0.01 ml g<sup>-1</sup> body weight. Gill epithelial and kidney tissues, rather than spleen, were removed 17–18 h after injection, followed by the same procedure described above after tissue removal.

Chromosomes were counted from photographic negatives and prints to establish modes for each specimen. Karyotypes were prepared from photographic enlargements of the best metaphase cells, i.e. those displaying the least chromosomal contraction, and no overlap.

Centromeric position, determined by short arm–long arm ratios, was the basis for the classification of chromosomes. Terminology for the centromeric positions followed the criteria established by Levan *et al.* (1964). The grouped metacentric and submetacentric chromosomes of the final photokaryotypes were placed in metacentric, submetacentric and acrocentric groups, in order of decreasing length.

Where appropriate, mean values were compared for significance ( $P < 0.05$ ) with Duncan's Multiple Range Test preceded by a one-way analysis of variance (ANOVA). Means for gill raker and scale counts were normalized by converting to the square root prior to the above analysis.

## III. RESULTS AND DISCUSSION

Of the 78 fish tested for ploidy, 36 had a modal chromosome number of 72 (triploid) and 42 had a modal chromosome number of 48 (diploid). Non-modal variations were generally less than 48 or 72, most likely a result of cell rupture and consequent chromosome loss during slide preparation. All of the fish tested from the 1981 year-class were triploid, while five (17.9%) of the 1979 year-class and six (24.0%) of the 1980 year-class were triploid (Table I). The triploid karyotype for three fish consisted of 39 metacentric and 33 submetacentric chromosomes (Fig. 1). The diploid karyotypes from three individuals contained

TABLE I. Morphological measurements for hybrid grass carp produced during 1979 and 1980 ( $\pm$ s.d.). Values within parentheses are ranges.

Character	1979		1980		1981	
	Triploid	Diploid	Triploid	Diploid	Triploid	Diploid
<i>n</i>	5	23	6	19	25	
$\bar{x}$ T.L. (cm)	39.9 $\pm$ 6.7 (32.8 - 47.5)	29.7 $\pm$ 5.9 (21.0 - 44.5)	30.6 $\pm$ 4.9 (22.5 - 36.5)	24.8 $\pm$ 7.9 (14.4 - 38.9)	27.1 $\pm$ 3.83 (21.3 - 36.2)	
$\bar{x}$ S.L. (cm)	31.7 $\pm$ 5.8 (25.4 - 38.1)	23.9 $\pm$ 5.0 (16.4 - 36.6)	24.5 $\pm$ 5.0 (16.6 - 27.6)	19.2 $\pm$ 6.9 (11.0 - 32.6)	22.1 $\pm$ 3.2 (17.0 - 29.6)	
$\bar{x}$ Weight (g)	690.6 $\pm$ 329.7 (321 - 1025)	301.3 $\pm$ 231.4 (91 - 950)	386.0 $\pm$ 185.3 (138 - 682)	243.5 $\pm$ 236.2 (37 - 765)	206.8 $\pm$ 99.1 (102 - 508)	
$\bar{x}$ Head width*	16.5 $\pm$ 0.6a (15.7 - 17.3)	16.9 $\pm$ 0.9a (14.7 - 18.9)	18.2 $\pm$ 1.2b (16.6 - 19.9)	18.2 $\pm$ 1.6b (16.2 - 21.9)	17.1 $\pm$ 0.68a (16.0 - 18.8)	
$\bar{x}$ Head length*	26.6 $\pm$ 1.6a (25.4 - 29.3)	27.1 $\pm$ 1.3a (23.7 - 29.1)	27.1 $\pm$ 3.4a (24.2 - 32.5)	30.0 $\pm$ 2.8b (26.1 - 36.0)	27.9 $\pm$ 0.85a (26.4 - 29.3)	
$\bar{x}$ Height*	22.4 $\pm$ 1.2ab (20.9 - 24.3)	22.3 $\pm$ 1.0a (21.0 - 24.1)	23.5 $\pm$ 1.7b (22.0 - 27.0)	25.2 $\pm$ 1.5c (22.5 - 27.7)	23.7 $\pm$ 1.1bd (21.3 - 26.4)	
$\bar{x}$ Snout length*	7.6 - 0.5a (6.9 - 8.2)	7.6 $\pm$ 0.6a (6.5 - 8.8)	7.3 $\pm$ 1.6a (5.4 - 9.4)	8.0 $\pm$ 0.7a (6.7 - 9.8)	7.4 $\pm$ 0.6a (6.0 - 8.5)	
$\bar{x}$ Interorbital width*	11.8 $\pm$ 0.5a (11.3 - 12.7)	12.0 $\pm$ 0.6ab (10.9 - 14.2)	12.9 $\pm$ 1.4bc (11.1 - 14.6)	14.2 $\pm$ 1.6d (11.7 - 17.7)	13.4 $\pm$ 0.61cd (12.1 - 14.7)	
$\bar{x}$ Scales in lateral line	49.0 $\pm$ 1.9a (47 - 51)	49.4 $\pm$ 1.9ab (46 - 53)	50.8 $\pm$ 5.1ab (46 - 59)	51.5 $\pm$ 3.0b (47 - 56)	44.7 $\pm$ 1.67c (42 - 50)	
$\bar{x}$ Scales in transverse series above lateral line	10.8 $\pm$ 0.4a (10 - 11)	10.9 $\pm$ 0.6a (8 - 12)	10.7 $\pm$ 0.8ab (10 - 12)	11.2 $\pm$ 0.9a (10 - 13)	10.1 $\pm$ 0.3b (10 - 11)	
$\bar{x}$ Scales in transverse series below lateral line	8.0 $\pm$ 0.7ab (7 - 9)	7.3 $\pm$ 0.8b (6 - 8)	8.2 $\pm$ 1.6a (7 - 11)	8.1 $\pm$ 0.8ab (7 - 10)	6.0 $\pm$ 0.2c (6 - 7)	
$\bar{x}$ Gut length*	2.4 $\pm$ 0.2a (2.0 - 2.5)	2.1 $\pm$ 0.2ab (1.6 - 2.6)	2.0 $\pm$ 0.5b (1.6 - 3.1)	1.5 $\pm$ 0.3c (0.9 - 2.1)	2.0 $\pm$ 0.1b (1.8 - 2.5)	
Keel	3 slight 1 none 1?	2 distinct 10 slight 6 none 5?	2 distinct 4 none	13 distinct 4 slight 2 none	11 distinct 13 slight 1 none	

\*Expressed as a percentage of s.l.

Means followed by the same letter are not significantly different at the ( $P < 0.05$ ) level.

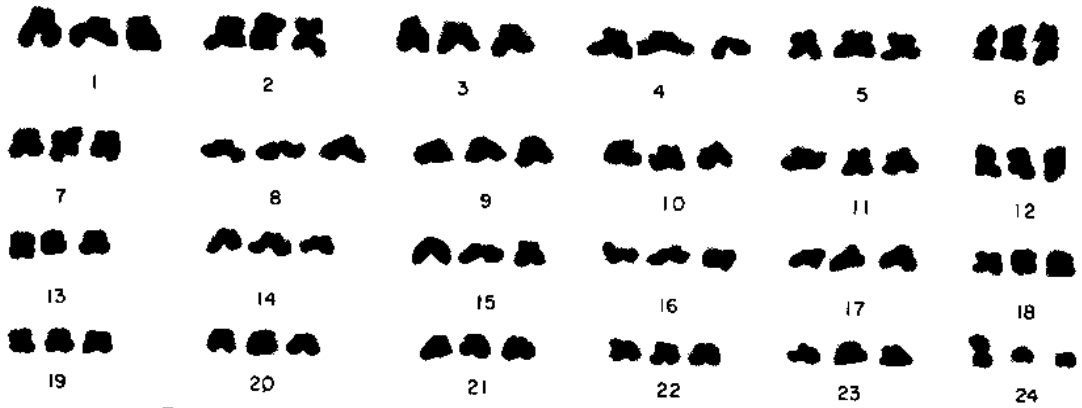


FIG. 1. Triploid karyotype of *C. idella* ♀ × *H. nobilis* ♂ hybrid. Scale = 5 µm.

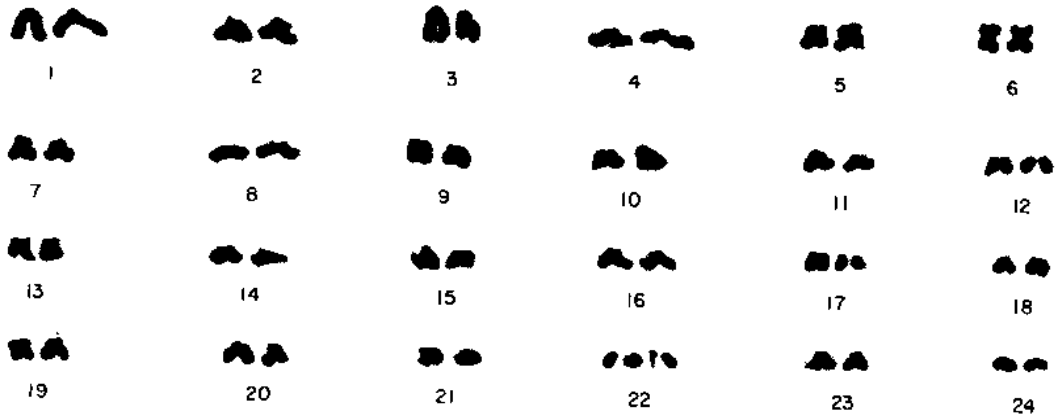


FIG. 2. Diploid karyotype of *C. idella* ♀ × *H. nobilis* ♂ hybrid. Scale = 5 µm.

15 pairs of metacentric, and nine pairs of submetacentric chromosomes (Fig. 2). Modal chromosome numbers did not vary between tissue types, indicating that mosaicism is not likely a common outcome from this cross.

Reducing the colchicine concentration from 0.1% to 0.05% and increasing the exposure resulted in substantially more chromosome sets per preparation, especially from kidney tissue.

Results of the morphological comparisons are listed in Tables I-IV. No morphological traits were found that could be used consistently to differentiate triploid from diploid fish (Table I). This is especially evident in the 1979 year-class where ploidy had no significant effect on measurements of the standardized traits (Table I). Significant differences were found between year-classes for fish with the same ploidy number, further complicating the comparisons. The 1981 year-class which was the most uniform with respect to ploidy (100% of our sample) showed significant differences from the other year-classes regardless of ploidy in the number of scales in the transverse series below the lateral line and the lateral line scale count.

Differences in gill raker numbers were found to be more consistent but again there were no significant differences between diploid and triploid fish from the

TABLE II. Modal fin ray numbers and ranges for hybrid grass carp

	No.	Dorsal	Pectoral	Pelvic	Anal	Caudal
1979		1*/7†	1/19	1/7	1/9	1/17/1
Triploid	5	—	(1/18,19)	(1/7-8)	—	(1/17,18/1)
1979		1/7	1/18	1/8	1/9	1/17/1
Diploid	23	(1/7-8)	(1/18-20)	(1/7-8)	(1/8-10)	(1/16,17/1)
1980		1/7	1/18	1/8	1/9-10‡	1/17/1
Triploid	6	—	(1/18-20)	(1/7-8)	(0-1/9,10)	—
1980		1/7	1/18	1/7-8‡	1/9	1/17/1
Diploid	19	—	(1/18-20)	(0-1/7-8)	(0-1/9-11)	(1/16,17/1)
1981		1-7	1/18	1/8	1/9	1/17/1
Triploid	25	(1/6-7)	(1/16,18,19)	(1/7-8)	(1/8,9)	(1/16-18/1)

\*Unbranched primary rays.

†Branched primary rays.

‡Equal numbers of both combinations.

TABLE III. Arrangement of pharyngeal teeth in hybrid grass carp produced during 1979, 1980 and 1981

1979	No.	1979	No.	1980	No.	1980	No.	1981	No.
Triploid		Diploid		Triploid		Diploid		Triploid	
0.4-4.0	1	0.4-5.1	1	1.4-5.0	1	0.4-5.0	6	0.4-4.0	8
1.4-5.0	1	0.4-4.0	5	0.4-4.0	2	0.3-4.0	1	0.4-5.0	4
1.4-5.1	2	0.4-5.0	1	0.4-5.0	2	0.4-4.0	5	0.5-4.0	8
0.5-4.0	1	1.4-4.0	3	1.5-4.1	1	1.4-4.1	1	0.5-4.1	1
		1.4-4.1	1			0.5-4.0	4	1.4-4.0	2
		1.4-5.0	1			0.5-4.1	1	1.5-4.1	2
		1.4-5.1	2			1.5-4.0	1		
		0.5-4.0	4						
		0.5-4.1	2						
		1.5-4.0	1						
		1.5-4.1	2						

1979 year-class (Table IV). Significant differences did occur for gill raker numbers at all branchial arches for the 1980 year-class compared to the 1979 and 1981 year-classes (Table IV).

Sutton *et al.* (1981) concluded that diploid hybrid grass carp should be distinguishable from triploid fish by the presence of a keel and a reduced growth rate based on the findings of Andriyasheva (1969); Berry & Low (1970); Verigin *et al.* (1973) and Marian & Krasznai (1978). However, many of our triploid fish had distinct keels while some diploids had no keels, making this character an unreliable indicator of ploidy.

Despite inconsistencies in certain morphological traits, several trends could be noted. With the exception of fin rays which did not show consistent differences between year-classes or ploidy (Table II), the 1980 year-class, especially the

TABLE IV. Mean ( $\pm$ S.D.) gill rakers per gill arch

No.	Branchial arch							
	I		II		III		IV	
	Inner	Outer	Inner	Outer	Inner	Outer	Inner	Outer
1979 Triploid	41.0 $\pm$ 3.4 <sup>a</sup>	31.4 $\pm$ 1.1 <sup>a</sup>	41.2 $\pm$ 5.3 <sup>a</sup>	43.2 $\pm$ 2.9 <sup>a</sup>	39.6 $\pm$ 2.7 <sup>a</sup>	43.2 $\pm$ 2.7 <sup>a</sup>	30.0 $\pm$ 3.3 <sup>a</sup>	39.8 $\pm$ 2.8 <sup>a</sup>
1979 Diploid	41.3 $\pm$ 3.0 <sup>a</sup>	30.7 $\pm$ 3.5 <sup>a</sup>	42.2 $\pm$ 2.9 <sup>a</sup>	41.1 $\pm$ 2.4 <sup>a</sup>	38.2 $\pm$ 3.9 <sup>a</sup>	41.0 $\pm$ 2.9 <sup>a</sup>	30.5 $\pm$ 1.6 <sup>a</sup>	39.1 $\pm$ 2.2 <sup>a</sup>
1980 Triploid	51.8 $\pm$ 15.3 <sup>b</sup>	38.3 $\pm$ 10.5 <sup>b</sup>	51.3 $\pm$ 15.3 <sup>b</sup>	51.7 $\pm$ 15.3 <sup>b</sup>	49.8 $\pm$ 17.4 <sup>b</sup>	52.2 $\pm$ 19.4 <sup>b</sup>	38.3 $\pm$ 13.0 <sup>b</sup>	48.8 $\pm$ 16.4 <sup>b</sup>
1980 Diploid	55.7 $\pm$ 9.5 <sup>b</sup>	42.4 $\pm$ 8.2 <sup>b</sup>	58.8 $\pm$ 10.7 <sup>c</sup>	56.6 $\pm$ 9.8 <sup>b</sup>	54.7 $\pm$ 10.2 <sup>b</sup>	59.3 $\pm$ 10.2 <sup>c</sup>	46.6 $\pm$ 9.4 <sup>c</sup>	54.8 $\pm$ 8.7 <sup>c</sup>
1981 Triploid	41.1 $\pm$ 1.1 <sup>a</sup>	29.3 $\pm$ 6.3 <sup>a</sup>	41.5 $\pm$ 1.6 <sup>a</sup>	41.3 $\pm$ 1.2 <sup>a</sup>	38.2 $\pm$ 1.9 <sup>a</sup>	41.3 $\pm$ 1.6 <sup>a</sup>	31.8 $\pm$ 1.8 <sup>a</sup>	38.7 $\pm$ 2.3 <sup>a</sup>

Means in each column followed by the same letter are not significantly different at the ( $P < 0.05$ ) level.

diploid fish, were closer morphologically to the bighead parent compared to the 1979 and 1981 year-classes. This is evidenced by a longer head, smaller and more numerous scales in the lateral line and in the transverse series above and below the lateral line and more gill rakers per gill arch. The bighead differs from the grass carp in these features (Berry & Low, 1970), the reason(s) is unknown to us, however, the breeder stated that no shocking of the eggs occurred to induce a change in ploidy during 1980 (Malone pers. comm.). Also, the 1980 year-class had more deformities and to a greater degree especially of the gill rakers, a possible cause for poor feeding on aquatic plants and slow growth.

Another trend is uniform ploidy and greater consistency in most morphological traits of the 1981 year-class. Eggs were shocked to induce triploidy in this year-class (Malone pers. comm.).

The number and arrangement of pharyngeal teeth varied considerably, although those with four teeth on one inner side, and five on the other inner side was the most common combination regardless of ploidy or year-class. The most common formulae reported by Marian & Krasznai (1978) were 1·4-4·1 and 1·5-3·1.

Intrayear-class comparisons of weight and length indicate that triploid fish are faster growing although there were exceptions (Table I). A few of the diploid fish were larger than most triploid fish. Conversely, some triploid fish were shorter than the average for the year-class in question. The trend toward an increase in size for triploids is also evident in means for relative gut length; in general, triploids had longer guts. Herbivorous fish with shorter gut lengths would be expected to have a larger percentage of undigested plant material in their faeces (Michewicz *et al.*, 1972). Since diploid fish had shorter gut lengths overall than triploid fish, it is possible that diploids would have to exert more energy consuming and digesting food than would triploid fish, resulting in their relatively slower rate of growth. Kilambi & Zdinak (1981) found the digestive tract length of the hybrid grass carp did not differ significantly from grass carp, although comparisons to our data would be difficult because ploidy was not determined and their figures were based on T.L. not S.L. as ours were.

Various types of deformities were common among the fish from the 1979 and 1980 year-classes. Deformities of the gill rakers (clubbed, branched and fused), especially those on the first branchial arch, were the most common deformity type (Table V). Those fish in the 1981 year-class were considerably more consistent regarding normal development of the gill rakers.

Parental contributions to the hybrid genome are difficult to determine by comparing karyotypes due to similarities in chromosome morphology between grass carp and bighead carp (Marian-Krasznai & Krasznai, 1978). Different applications of colchicine involving various dosages and exposure times can affect chromosome morphology which is reflected in the literature by differing results (Marian-Krasznai & Krasznai, 1978; Beck *et al.*, 1980). However, our karyotypes for the triploid hybrid shows the same chromosome morphology as reported by Beck *et al.* (1980) (39 metacentric and 33 submetacentric). Triploid hybrids are expected to be sterile if aneuploid gametes are produced resulting in abnormal development.

Biochemical analyses of triploid hybrid carp resulted in a maternal : paternal allele ratio of 2 : 1 (Magee & Philipp, 1982). This confirms the hypothesis of Beck

TABLE V. Number and percentage (in parentheses) of fish having various types of deformities according to ploidy and year-class

Deformity location	1979 Triploid	1979 Diploid	1980 Triploid	1980 Diploid	1981 Triploid
Gill rakers	4 (80)	16 (70)	6 (100)	19 (100)	3 (12)
Head (shape of opercle and mouth)		1 (4)	4 (67)	8 (42)	
Eye				1 (5)	3 (12)
Lateral line	1 (20)	1 (4)		1 (5)	5 (20)
Fins			1 (17)		
Spine				2 (11)	

*et al.* (1980) in that the female parent (grass carp) contributes a diploid complement, most likely through polar body retention. However, three of six 1980 triploids studied by us may have been an exception to this trend. All three fish had morphological characters indicating a greater paternal likeness with relatively more gill rakers (up to 85 per side) and 55–59 scales in the lateral line. These fish were possibly the result of dispermic fertilization. Excessive chromosome contraction made it impossible to substantiate this with regard to chromosome morphology. Another possibility is that these fish developed with paternal traits despite having predominantly maternal inheritance, as reported by Makeeva (1972) for hybrid embryos of a bighead  $\times$  carp, *Cyprinus carpio* L., cross.

Magee & Philipp (1982) and Beck *et al.* (1980) both report that 100% of the 1979 fish tested were triploid. We found that a substantial number (82.1%) of our sample of fish from the 1979 year-class were diploid and probably represents a more accurate account of most of the fish sold from this year-class for aquatic weed control. None of the 1979 fish tested by Beck *et al.* (1980) or Magee & Philipp (1982) were from random collections of fish produced by Malone and Son Inc.

Of greater interest with regard to sterility are diploid hybrid grass carp. The number of metacentric and submetacentric chromosomes in our karyotype for a diploid hybrid is the same as Beck *et al.* (1980) reported for grass carp. Sutton *et al.* (1981) reports that gynogenesis is a possible outcome from this cross. The morphological characteristics of diploid fish in question are intermediate between both parent species, ruling out gynogenesis. The most probable outcome is both parents contributing a haploid complement resulting in a fish that could theoretically produce viable gametes. Also, diploids have been reported from grass carp  $\times$  silver carp, *Hypophthalmichthys molitrix*, hybrids (Mantelman, 1973). Magee & Philipp (1982) did not discuss the parental allele dosage for their diploid hybrid (grass carp  $\times$  bighead carp) carp.

In summary, hybrid grass carp did not show consistent differences in various morphological traits between diploid and triploid fish. The most consistent trend differentiating the morphology of diploid and triploid fish was that triploid fish grew faster, had fewer scales in the lateral line and the transverse series below the lateral line and overall fewer deformities. As a result of these data, morphological traits are poor determinants for an absolute method of screening diploid from



triploid fish. Screening methods such as flow cytometry or measurements of the erythrocyte nucleus will be more accurate methods for ploidy determinations but considerably more time-consuming (Allen & Stanley, 1983; Beck & Biggers, 1983). Assuming the information given here is representative of each year-class, then the 1979 and 1980 year-classes were predominantly diploid with predominance of triploid for the 1981 year-class. We feel that genetic inconsistencies have led to conflicting observations of feeding behaviour of fish from year-classes 1979–1981. Until production techniques (e.g. egg shocking) can be refined so that a uniform product results, or accurate screening measures are incorporated, the efficacy and practicality of hybrid grass carp as a biological control agent for aquatic weeds will remain uncertain.

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