Triploid Grass Carp: Status and Management Implications

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ABSTRACT

Triploid grass carp are becoming readily available from commercial producers, who are capable of examining the fish individually to ensure that only triploids are shipped. Moreover, carefully controlled programs in Florida and other states have demonstrated that permitting procedures that require 100% triploids can work. Federal involvement in certification has lessened the load at the state level. Research has indicated that triploids feed and behave similarly to their diploid parents, which makes them effective biological plant control agents. Theory and the available empirical evidence suggest that triploid grass carp will be functionally sterile and will not establish self-sustaining populations. Judicious use of the triploid grass carp is advised because of their propensity to migrate and their predicted longevity (10–15 years). Their greatest benefit will be in reducing costs and reducing the quantities of potentially hazardous herbicides introduced into waters where plants historically have been eliminated using chemicals.

Worldwide distribution of grass carp (*Ctenopharyngodon idella*) has come about because of their ability to control aquatic vegetation (Shireman and Smith 1983) and their importance as a food fish (Bardach et al. 1972). Importation of grass carp into the United States was first suggested by Swingle (1957), and broodstock were first introduced into Arkansas and Alabama in 1963 (Guillory and Gasaway 1978). However, there remains a great deal of concern over inadvertent naturalization of grass carp in the United States. In this article, we will review the status of triploid grass carp for biological weed control and outline the implications that are posed to the fisheries manager.

Diploids

Stocked into suitable situations and at appropriate rates, grass carp provide effective control of aquatic macrophytes such as *Hydrilla verticillata*, *Potamogeton illinoensis*, *Elodea* spp., *Ceratophyllum demersum*, and *Najas quadalupensis* (Leslie et al. 1986; Shireman and Smith 1983; Von Zon 1979), that may last 10 years or more. Suitable situations for grass carp introduction are those in which the grass carp are of a sufficient size to avoid predation, have a preferred plant available for food, and have suitable water quality and temperatures. Appropriate stocking rates depend on the plant biomass to be consumed, the plant species, the size of the grass carp, relative growing season of the fish and plants, as well as the overall size of the site. Although effective stocking rates vary widely from 5 to 500 fish/ha, rates of 25–60 fish/ha are used generally.

Unfortunately, experience in Florida has indicated that in most moderate sized areas (e.g., < 25 ha) with a monoculture of plants, grass carp represent an "all-or-none" solution. Either stocking densities are high enough to exceed the growth potential of the plants, thereby eliminating them, or stocking densities are too low, and plant growth continues. In larger bodies of water (e.g., < 200 ha), or in flowing water where the fish cannot be contained, grass carp may eliminate plants from certain areas while problematic quantities of plants thrive in other areas. Research in large bodies of water is still needed to evaluate this potential problem and determine appropriate management schemes.

Grass carp are not a panacea for plant control: total elimination of aquatic plants (especially submergent vegetation) can interfere with sport fisheries because of the eradication of habitat for invertebrates and juvenile fish. Therefore, grass carp are best adapted to situations where total elimination of the macrophyte community is desirable. Examples of these are golf course ponds, small urban lakes, irrigation canals, or other areas in which plants are typically eliminated by herbicides. In these environments, grass carp are cost effective and do not pose the health hazards associated with the use of chemicals. Shireman et al. (1985) estimated that the cost of aquatic plant control using grass carp was initially \$158–247/hectare and that benefits extended for more than 7 years. In contrast, mechanical harvesting and herbicide treatment costs about \$1000 and \$418–1339/(ha · yr), respectively. A new herbicide, fluoridane (Sonar) may be more cost effective in some circumstances.

Triploids

Stanley et al. (1978) concluded that diploid grass carp would reproduce in the United States. Recently, fingerlings from natural spawning have been observed in the lower Mississippi River (Conner et al. 1980), as well as several Mexican rivers (Anonymous 1976). Concern in the United States about unwanted introductions is so great that most of the 50 states have some form of restriction on the use of grass carp. Of primary concern is the impact that grass carp populations will have on endemic flora and fauna, especially the ecology of wetlands dependent on aquatic vegetation.

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Uncontrolled populations could lead to elimination of aquatic plants in affected areas which are important to fish nursery areas, as substrate for invertebrates, and as forage for waterfowl.

Initial attempts to prevent unwanted naturalization of pure grass carp were focused on creating all female populations through gynogenesis (Stanley 1976) and, more recently, through hormonal implants followed by mating sex-reversed, XX males to normal females (Boney et al. 1984). Monosex populations are still fertile, however, and juveniles cannot be sexed readily. Accidental introduction or the occasional production of males (Chourrout and Quillet 1982) could lead to reproduction. Surgical gonadectomies have failed owing to the rapid regeneration of gonads (Clippinger and Osborne 1984; Underwood et al. 1986).

In 1978 Marian and Krasznai reported on the intergeneric cross between grass carp (female) and bighead carp (male; *Hypophthalmichthys nobilis*). The F1 hybrids that survived were apparently 100% triploid. A new wave of interest followed, focusing on the triploid hybrid as a biological weed control (Buck 1979). Commercial producers were attracted to the financial possibilities of producing triploid hybrids, since it originally appeared that triploids occurred spontaneously. Malone and Son Enterprises duplicated the intergeneric cross made by Marian and Krasznai (1978) and produced a spawn of hybrids in 1979 that were nearly all triploid. In 1980 they repeated the cross but exercised considerable caution in rearing the yolk-sac fry. After this careful rearing, the hybrid larvae were ponded. Subsequent analysis revealed only about 67% triploidy, which was apparently due to the survival of some of the less vital diploid hybrids.

There are several examples of hybrid crosses that yield subvital diploids but healthy triploids. The grass x bighead carp is one of these; the tiger trout (brown trout *Salmo trutta* female x brook trout *Salvelinus fontinalis* male; Scheerer and Thorgaard 1983) is another. The 100% triploid progeny seen by Marian and Krasznai and the United States grower were the result of larval selection in a harsh environment (i.e., handling and ponding after three days). Most of the subvital diploid hybrids died leaving primarily healthy triploids.

The need to increase the unacceptably low spontaneous production of triploid hybrids led directly to the current technology surrounding the production of pure (unhybridized) grass carp triploids. Induced polyploidy has become increasingly popular with aquaculturists since the first trials in the early 1970s (Purdom 1972). The process, a relatively simple one, involves inhibiting the 2nd maturation division of meiosis in the fertilized egg, thereby causing retention of the extra chromosome set contained in the second polar body of the ovum. Two techniques—thermal and pressure shocks—have been described specifically for grass carp. Cassani and Caton (1985) used cold shocks that resulted in between 50–100% triploidy with survival of eggs generally less than 20%. Thompson et al. (in press) used heat shocks which yielded up to 87% triploids and up to 50% survival. Commercial producers, whose techniques are understandably proprietary, probably use similar methods. More recently, using hydrostatic pressure treatments of 7000 or 8000 psi, Cassani and Caton (1986) consistently produced yields of nearly 100% triploids; about 30% mortality resulted from such treatments.

The major obstacle to using triploid hybrids for weed control was their failure to adequately consume macrophytes. The triploid hybrid consumes about a third as much plant material as diploid grass carp (Wattendorf and Shafland 1983; Beaty et al. 1984). Pure triploid grass carp seem to consume weeds about as efficiently as diploids (Wattendorf and Anderson 1984; Sutton 1985), so growth is nearly equivalent.

Triploid Verification

Morphological and meristic characters of triploids and diploids are very nearly identical. This identity is both an advantage and a constraint. Bonar et al. (1984) examined 34 morphologic and meristic characters of diploid and triploid grass carp. Of these, six (body depth, caudal peduncle width, suborbital width, cheek height, circumferential scale count, and caudal peduncle scale count) were significantly different, but no single measurement was sufficient to separate diploids from triploids with 100% accuracy. The important difference between the two is in chromosome number and hence DNA content. Triploid grass carp have three haploid sets of chromosomes (3N = 72). The presence of these extra chromosomes in the nucleus requires a corresponding increase in nuclear (and cellular) size which provides the key to separating diploids and triploids efficiently.

The use of triploid grass carp as a legitimate alternative to herbicide application will undoubtedly occur only if triploids can be used. Because no known induction technique can produce 100% triploidy consistently, the ability to identify diploids and triploids becomes imperative. Because tremendous numbers of fish will be required, the technology should be rapid and cost effective. However, because there is potential for abuse or human error, or both, in the supply of triploids, producers and regulatory agencies will need access to the technology.

Of the techniques available today, the one most amenable to grass carp is ploidy determination by a Coulter Counter. The technique was developed independently and almost simultaneously, although for different fish species, in Newfoundland, Canada, by T. J. Benfey (1984), in Seattle, Washington, by O. W. Johnson (1984), and in Boca Raton, Florida, by R. J. Wattendorf (1986). A Coulter Counter is an electronic particle size analyzer. The particles, cells or nuclei of fish erythrocytes, are suspended in an electrolyte solution and passed through an appropriately sized orifice located between two electrodes. The change in resistance generated by particles passing through the electrodes is proportional to the volume of the particle. The result is a frequency-volume histogram, indicating the size distribution of the particles. Because cells maintain a constant nuclear: cytoplasm ratio, triploids have larger erythrocytes (and nuclei) than diploids. Benfey et al. (1984) and Johnson et al. (1984) used the cell size differences in diploids and triploids to separate the two. Wattendorf (1986) first lysed erythrocytes and examined just nuclei which, based on measurements, Beck and Biggers (1983) concluded to be better indicators of ploidy than the whole cell. This extra step in sample preparation is rapid and decreases within sample variability. The technique is virtually 100% effective.

Implications of Triploid Grass Carp for Fisheries Managers

With a pure triploid grass carp, fisheries managers seem to have the best of both worlds: non-reproducing biological weed control with considerable potential to eliminate weeds. We assume that predicting stocking rates for effective macrophyte control will be adequately addressed by extension of work already completed on diploids or through new studies with triploids. The major considerations for the use of triploids, then, will be assuring their exclusive use.

There are four general regulation concepts used by state agencies to deal with grass carp, according to a phone survey in January 1987 (Figure 1). First, 12 states allow any and all grass carp to be imported and stocked in their waters, generally without constraint. For the most part, these are Alabama and Arkansas, the initial importers of the fish, and the states that are either contiguous with them or have no aquatic plant problems. Second, 15 states now have specific policies that allow the use of 100% grass carp. This trend started with Florida. Also adopting this strategy were those states that have significant aquatic plant control problems and that originally banned diploid grass carp. Third, four states permit experiments with triploid grass carp, but do not yet allow their widespread use. Finally, 19 states still technically prohibit the importation of grass carp, but several are experimenting (or have experimented) with triploids, to determine if a change in policy is appropriate.

States that allow triploid grass carp require 100% triploidy, and the issues raised by this requirement include certification and enforcement. The decision to accept triploids or not may rest on the likelihood that triploids will reproduce. We will address these areas of certification, enforcement, and reproductive likelihood.

Certification

What quality controls are needed between producers and users of triploid grass carp? In a recent survey by the Chinese Carp Committee (formerly Grass Carp/Hybrid Committee) of the Introduced Fish Section, American Fisheries Society, all producers of triploid grass carp who responded indicated that they were equipped to verify 100% triploids. The method of choice was nuclear volume via Coulter Counter. All shipments should in theory be 100% triploids, since producers are required to examine grass carp individually for ploidy. States allowing 100% triploids frequently require that each shipment originate from an approved grower, and that all shipments be verified via random sampling.

The first state certification process for ensuring the use of 100% triploid populations of grass carp was implemented in Florida during the summer of 1984. Regulations required that any site to be stocked with triploid grass carp be

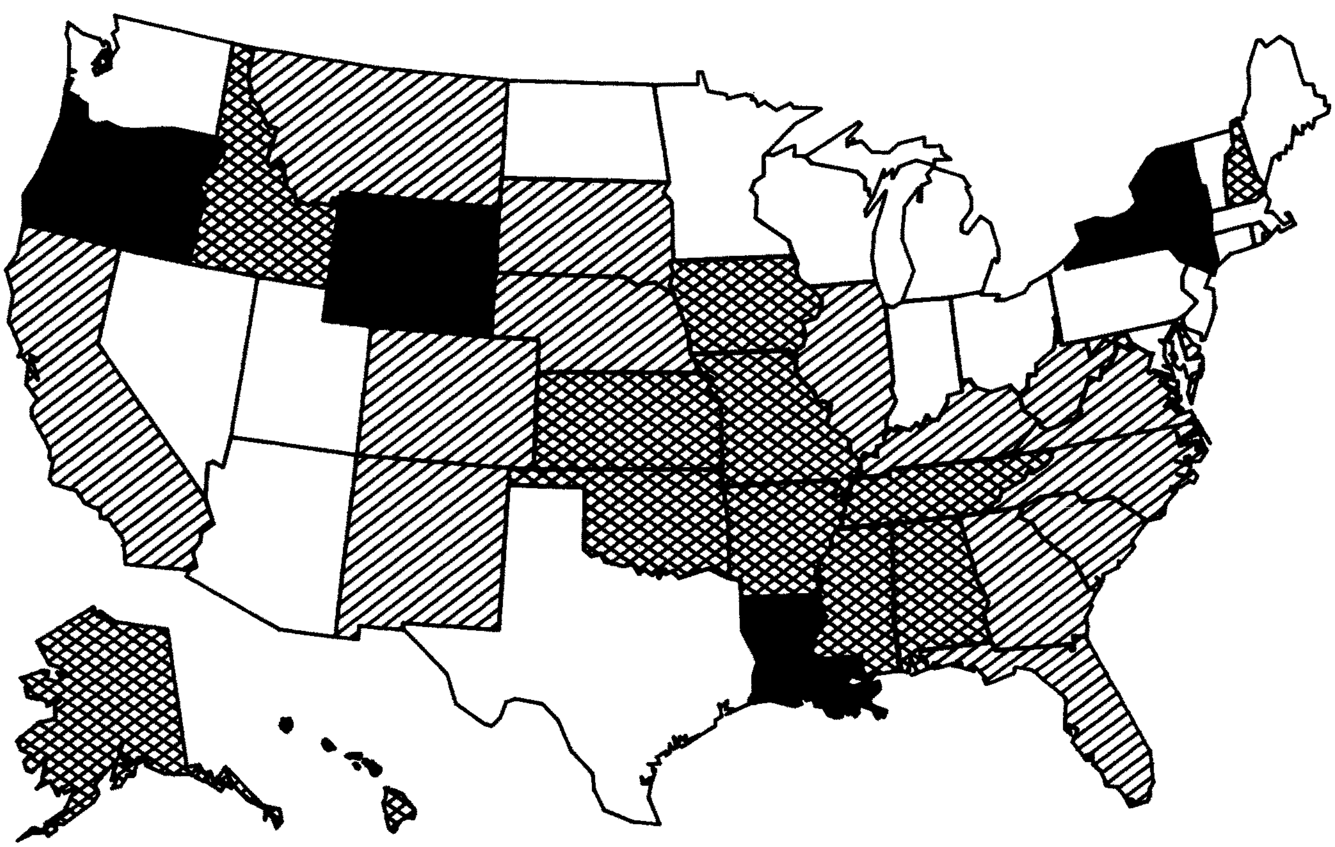

Figure 1. There are four general categories used by the state fisheries agencies to regulate grass carp, based on a January 1987 survey. Grass carp (diploid and triploid) are allowed without substantial constraint—cross-hatch; only 100% triploid grass carp are permitted for management of aquatic vegetation—single-hatch; 100% triploid grass carp are permitted, but only for closely regulated research—black; grass carp (diploid and triploid) are prohibited (although some of these states have conducted limited grass carp research)—white.

inspected by a Florida Game and Fresh Water Fish Commission biologist. If appropriate, a permit would be issued specifying a maximum number of fish to be used. The shipper then brought the fish to a state hatchery where 120 randomly selected fish were examined with the Coulter Counter system. Only 100% triploid shipments were accepted. Subsequently, the U.S. Fish and Wildlife Service began to inspect shipments that originated near the Stuttgart Laboratory in Arkansas for ploidy and parasites as the shipper loaded them. Consequently, state inspection in Florida is now generally used as a quality control assurance. Unannounced spot-checks are conducted occasionally, when the fish enter the state, to ensure the shipment has not been adulterated.

An area where a national effort might be warranted is to certify the growers themselves and to expand their program to check grass carp shipments in other states besides Arkansas. Approval would be contingent upon the ability of growers to produce triploids and distinguish them from diploids.

Enforcement

Once a state allows triploids, the presence of grass carp in impoundments will no longer be the sole criterion for determining illegality. Diploids look like triploids, hence illegally stocked diploid grass carp will become more difficult to detect and will require capture and ploidy determination. Therefore, states will need the capability, either directly or through a consulting arrangement, to monitor shipments and to follow up stocking programs occasionally by checking previously stocked ponds. We believe that enforcement efforts might be reduced greatly if only triploids were allowed in every state. That is, other than the broodstock kept at spawning facilities, all grass carp in the United States would be triploid.

Reproductive Likelihood

From the standpoint of gonadogenesis, triploid fish are not sterile, that is, males and females can be readily distinguished. Females produce rudimentary gonads; however, egg production is almost non-existent (see Thorgaard 1983 for review). Triploid grass carp exhibit the same quality (Doroshov 1986). On the other hand, triploid males often produce substantial testes, and some species actually produce spermatozoa (Lincoln 1981).

Early results indicated that triploid male grass carp only produced spermatids. However, in 1986, researchers at the Imperial Valley Irrigation District showed that triploid males could be induced to spermiate (R. Stocker and N. Hagstrom, personal communication). Furthermore, sperm obtained from artificially stimulated triploids was capable of fertilizing eggs from a normal diploid female. The vast majority of embryos that developed beyond gastrulation were malformed; many more were unable to hatch properly; and only a few larvae out of about 250,000 eggs survived more than a week. These larvae died within a month.

Decreased survival of embryos derived from backcrossing triploid x diploid grass carp may be the result of aneuploid sperm. Allen et al. (1986) have examined the milk produced from spermiating triploid male grass carp cytologically. From data on DNA content in cells found in milt and from cell morphology they concluded that the extra chromosome set segregates at random, and that this random segregation is close to that predicted theoretically. The mean DNA content of triploid sperm is 1.5N (aneuploid); only 60 cells in every billion produced by random segregation in a triploid will be a true euploid (i.e., haploid or diploid) gamete. The spermatocrit (cells/volume of milt) is also 50 times less in triploids than in diploids.

Given the extremely small likelihood that triploid gametes will unite, produce viable offspring, and do so with a fecundity that will lead to sufficiently large populations to reproduce, triploids appear to be functionally sterile. Logically, if only males produce gametes, then by necessity triploid males will have to mate with diploid, female grass carp. Under these circumstances, we predict that triploids could reproduce in extremely limited numbers, only if there is a mature population of diploids and ideal spawning habitat present. In fact if triploid grass carp do exhibit spawning behavior, and triploid males can spermiate naturally and inseminate normal eggs, they may have the potential to reduce diploid populations. The overwhelming majority (0.99999988) of gametes produced by a triploid will be aneuploid. Union of aneuploid and normal gametes produced inviable offspring in plaice *Pleuronectes platessa* (Lincoln 1981), rainbow trout *Salmo gairdneri* (Lincoln and Scott 1984), and apparently grass carp (N. Hagstrom, personal communication). This type of population control has been used widely with insects (Smith and von Borstel 1972).

Will even an infinitesimally small chance of reproduction be too great a risk to take? Few of our alternatives today are risk free. The common sense approach would dictate that a situation-by-situation approach is warranted. It seems to us that the use of deadly herbicides, such as Acrolein, to kill aquatic plants has a greater potential for environmental calamity. The technology is available today to monitor, control, and possibly reverse a decision to use triploid grass carp.

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