

# **A Risk Analysis Pertaining to the Use of Triploid Grass Carp for the Biological Control of Aquatic Plants**

## **Steering Committee**

John Cassani  
Resource Manager  
Lee County Hyacinth Control District  
P.O. Box 60005  
Fort Myers, Florida 33906  
Telephone: 239-694-2174  
[cassani@lchcd.org](mailto:cassani@lchcd.org)

Scott Hardin  
Invasive Species Coordinator  
Florida Fish and Wildlife Conservation  
Commission  
South Meridian Street  
Tallahassee, Florida 32399-1600  
Telephone 850-488-4066  
[scott.hardin@myfwc.com](mailto:scott.hardin@myfwc.com)

Vince Mudrak  
Center Director  
U.S. Fish and Wildlife Service  
Warm Springs Fish Technology Center  
5308 Spring Street  
Warm Springs, Georgia 31830  
Telephone: 706-655-3382 ext 223  
[Vincent\\_Mudrak@fws.gov](mailto:Vincent_Mudrak@fws.gov)

Paul Zajicek (Project Manager)  
Biological Administrator  
Florida Department of Agriculture  
and Consumer Services  
1203 Governor's Square Boulevard,  
Fifth Floor  
Tallahassee, Florida 32309  
Telephone 850-488-4033  
[zajicep@doacs.state.fl.us](mailto:zajicep@doacs.state.fl.us)

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## Introduction

At least three biotypes of hydrilla (*Hydrilla verticillata*) in 20 water bodies throughout Central Florida ranging in size from 120 to 8,500 ha (e.g., Kissimmee Chain of Lakes, Lake Istokpoga, Polk County lakes, and other water bodies) have developed a resistance to fluridone (Michel et al 2004; Hoyer et al 2005). Lakes Tohopekaliga, Cypress, Hatchineha, and Istokpoga have demonstrated the capacity to support hydrilla with as high as 60 to 90 percent surface acreage coverage. Each of these lakes is dominated by fluridone resistant hydrilla (Hoyer et al 2005). These lakes and other fluridone resistant hydrilla impacted water bodies are “open” in the sense that means to prevent escape of stocked triploid grass carp may be impossible (i.e., stocked fish will leave the target water body).

In response to spreading fluridone resistance, a hydrilla management workshop was organized by Florida LAKEWATCH<sup>1</sup> to summarize management, control and research options and recommendations (Hoyer et al 2005). The use of triploid grass carp as a control tool was a persistent workshop theme. Research and experience provided by the participants revealed several management challenges:

- When stocked in sufficient densities, at a large enough size to avoid predation (approximately >30 cm total length), grass carp are very efficient at controlling all submersed aquatic vegetation.
- When grass carp are stocked into a lake at densities high enough to control all submersed aquatic vegetation they are difficult (time, money and labor) to remove from a system.
- Less clear was the potential for grass carp to be used after herbicides have decreased aquatic plant biomass to selectively control hydrilla re-growth and maintain desirable levels of native aquatic vegetation.
- Some research suggests that stocking low levels of grass carp can achieve control of problem plant species while maintaining moderate levels of native aquatic vegetation in ponds and small impoundments.
- Other research reported an apparent stocking level break point suggesting an elimination of all submersed vegetation or very little to no control (i.e., an “all or none” result).
- Stocking rates to achieve a certain level of submersed vegetation control are difficult to maintain in individual lakes because of varying grass carp mortality rates, fish mobility, and changing limnological and climatological conditions.
- A goal for effective aquatic plant management is the ability to manage plant density and composition. A critical aspect to the use of triploid grass carp to achieve this goal is to cost effectively remove grass carp when aquatic vegetation conditions approach desired equilibrium levels.

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<sup>1</sup> Florida LAKEWATCH (<http://lakewatch.ifas.ufl.edu/>) is a volunteer citizen lake monitoring program that facilitates "hands-on" citizen participation in the management of Florida lakes through monthly monitoring activities. Coordinated through the University of Florida's Institute of Food and Agricultural Sciences/Department of Fisheries and Aquatic Sciences, the program has been in existence since 1986.

A qualified grass carp recommendation from the workshop report stated:

“...if there was some cost-effective and selective method of removing grass carp from a lake system before complete eradication of submersed aquatic vegetation was accomplished, then triploid grass carp would be an excellent method of hydrilla control for large and small lakes. Therefore, we recommend making funds available for more research on new techniques for removing grass carp from lakes. Research on this and other methods may be expensive but a successful method would pay great dividends to aquatic plant management in Florida lakes. (page 13)”

Much of the triploid grass carp and large lake stocking information cited in by the workshop was derived from grass carp and aquatic vegetation management publications and a report entitled, *Proceedings of the Grass Carp Symposium* (USACOE 1994). As noted in the introduction of the Proceedings:

“As with technology, it is necessary to assess the status of not only the state of the art, but the general view of its applications in the real-world environments. It is intended that this workshop serve at least a portion of that assessment. It is anticipated that another will serve the same need again in the future. (page 1)”

To address this complex issue, a research proposal was submitted to the Florida Department of Environmental Protection, Bureau of Invasive Plant Management, with the objectives of implementing a proven risk analysis methodology to re-evaluate the risks of introducing triploid grass carp into State of Florida freshwater resources, determining the risk management steps required to mitigate risk, and developing a risk management operational plan.

### **Methodology**

The *Generic Nonindigenous Aquatic Organisms Risk Analysis Review Process: For estimating risk associated with the introduction of nonindigenous aquatic organisms and how to manage for that risk* (hereafter referred to as “Generic Analysis”) (ANSTF 1996) was utilized as a framework to achieve the project objectives. The Generic Analysis consists of a three steps. The first step is to complete a risk assessment that consists of: 1) identification of interested parties and their input to serve as an expert panel; 2) collect and summarize biological and ecological information concerning the triploid grass carp and its use to manage aquatic plants; 3) qualitative risk assessment using high, medium and low rankings to judge the probability and consequences of establishment along with the assignment of uncertainty ratings to the risk rankings; and 4) recommendations. The second step is risk management that has an objective of determining appropriate policies, operational measures, and adaptive management components built upon the foundation of published information, expert and stakeholder input, and risk prioritization created by the risk assessment. The third and iterative step is to develop a realistic risk management operational plan that includes a monitoring system to revise and update mitigation over time.

Three hydrilla management scenarios were identified to be separately considered by the expert panel using the Generic Analysis methodology:

- 1) Stocking open, freshwater systems with triploid grass carp.
  - a) Implementing stocking rates to eliminate hydrilla rapidly followed by efforts to reduce and manage triploid grass carp populations to achieve maintenance control.
  - b) Implementing stocking rates at low levels to achieve hydrilla management objectives over the long term and the methodologies to assess and adjust stocking to meet aquatic plant management objectives.
- 2) Herbicide application to achieve hydrilla management objectives followed by stocking low levels of triploid grass carp to maintain hydrilla management objectives.
- 3) Integrated hydrilla management: triploid grass carp, chemical, water level, mechanical, and biological.

The services of aquatic plant, grass carp, angler, waterfowl, freshwater ecology and freshwater fishery experts were recruited from state or federal agencies; national or state universities; and triploid grass carp producers to participate in two intensive workshops held on April 2, 2007 at the UF-Tropical Aquaculture Laboratory and June 29, 2007 at the UF-Department of Fisheries and Aquatic Sciences (see Appendix A for the list of invited stakeholders).

The initial expert workshop consisted of a: 1) review and commentary on the draft literature review distributed to the participants prior to the workshop and prepared by a Ms. Tina Weier, Other Personal Services-Biological Scientist, engaged by the Division of Aquaculture to assemble and summarize pertinent triploid grass carp management practices, biology, ecological effects, and management practices and 2) Generic Analysis presentation and discussion to familiarize the expert panel with the risk analysis methodology.

During the second workshop the draft literature review was presented by Ms. Weier for a second review and comment and the participants completed the risk analysis. The literature review provides a succinct summary of the triploid grass carp origin, use, management, and effectiveness that provides background and support to the deliberations of the expert panel.

## **Grass Carp Literature Review**

### **History / Creation of Triploid Grass Carp**

Grass carp (*Ctenopharyngodon idella*) have been distributed worldwide to over twenty countries spanning five continents since 1961 for the biological control of aquatic vegetation (van Zon 1977; Opuszynski and Shireman 1995). Native to large East Asian rivers with Pacific drainage between latitudes 23 - 50 degrees north and longitudes 100 –

140 degrees east (Fischer and Lyakhovich 1973), grass carp provide not only a source of control for nuisance vegetation but also valuable fish flesh consumed as an important form of protein in many cultures, especially in the tropical regions. Importation of grass carp into the United States was first suggested by Swingle (1957) and implemented in 1963 by importation of broodstock to Arkansas and Alabama (Guillory and Gasaway 1978; Mitchell and Kelly 2006). However, despite the presence of grass carp in 40 states by 1976, a great deal of reservation remains about the possibility and implications of inadvertent naturalization of grass carp in the U.S. waterways.

Initial attempts to prevent unwanted naturalization of grass carp focused on producing all female populations through gynogenesis (Stanley 1976) and hormonal implants followed by mating XX males to normal females (Boney 1984). However monosex populations are still fertile and the process of sexing juveniles is tedious and time consuming. Clippinger and Osborne (1984) attempted surgical gonadectomies but failed due to the rapid regeneration of gonads in grass carp. Studies then focused on creating triploid hybrids through grass carp x bighead carp (*Hypophthalmichthys nobilis*) intergeneric crossing (Marian and Krasznai 1978; Buck 1979) but the low spontaneous production of triploid hybrids and the failure of the cross to survive led directly to the current focus on the production of pure, unhybridized triploid grass carp (Allen and Wattendorf 1987).

Induced polyploidy for the purpose of sterilization has become increasingly popular with aquaculturists since the first trials of the technology in the 1970's (Purdom 1972). The process involves inhibiting the second 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 (Allen and Wattendorf 1987) by temperature and pressure shock to the eggs (Cassani and Caton 1985a; Cassani and Caton 1986a). This causes the triploid condition in grass carp giving them a chromosome number of 72 (3N) instead of the natural 48 (2N) and makes them functionally sterile. The presence of these extra chromosomes in the nuclei requires a corresponding increase in nuclear size which allows for the efficient separation of diploids and triploids (Allen and Wattendorf 1987).

The coulter counter, a particle size analyzer, works by passing particles suspended in an electrolyte solution through an orifice between two electrodes. The change in resistance generated by particles passing through the electrodes is proportional to the volume of the particles. 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 nuclei than diploids and can therefore easily be detected (Allen and Wattendorf 1987). Wattendorf (1986) first lysed erythrocytes and examined just the nuclei which Beck and Biggers (1983) determined to be the best indicator of ploidy, being nearly 100 percent effective. An analysis of U.S. Fish and Wildlife Service inspections over the last four years of 121,080 fish yielded 11 diploid grass carp, a 0.0000908 incidence rate of diploids occurring in a population of blood tested triploid grass carp (Mike Freeze, KeoFish Farm, personal communication). Unfortunately, sophisticated and costly methods are needed (i.e., coulter counter or flow cytometry) for identifying triploid grass carp. Bonar et al. (1984) examined 34 morphological and meristic characters and found only 6

of them to be significantly different between diploids and triploids, with no single trait being sufficiently different to separate the two with 100 percent accuracy.

### **Reproduction**

In order to reproduce grass carp begin their ascension of rivers to spawn as water flows increase during spring and temperatures rise from 18 to 23 degrees Celsius (Aliev 1976; Shireman and Smith 1983). They reportedly require a flow rate of 0.23 – 1.6 m/s and a river length of 50 – 180 km because grass carp eggs are only semi-buoyant and must remain in suspension 24 – 36 hours until hatched (Aliev 1976; Stanley et al. 1978; Leslie et al. 1982). Nursery habitats, such as inundated vegetation, must also be available and persist for 2 months or more until fish are of sufficient size to survive in main stem habitats (Vladimirov 1975; Nezdolty and Mitrofanov 1975).

A large portion of the controversy related to grass carp is due to their potential for natural reproduction and subsequent colonization in undesirable locations (Sutton 1977). Stanley et al. (1978) predicted substantial reproduction would occur in United States rivers despite grass carp's stringent spawning requirements, and since 1990 evidence of natural reproduction in grass carp has been documented in the Illinois and Mississippi rivers (Raibley et al. 1995), Trinity river (Elder and Murphy 1997) and in Red and Washita rivers (Hargrave and Gido 2004).

Triploid grass carp have been proposed as a sterile substitute to diploid grass carp for the management of nuisance aquatic vegetation, but the working definitions of sterility for aquaculture and management purposes are very different and must be considered. In aquaculture, reduced development of gonads may suffice as sterility. However, in management the definition of sterility must include an estimate of reproductive likelihood (Allen et al. 1986).

Doroshov (1986) found that female triploid grass carp produce only rudimentary gonads and egg production is non-existent. On the other hand, triploid males produce substantial testes, can be induced to spermiate and will attempt to (Allen and Wattendorf 1987). Allen et al. (1986) determined that for every  $10^9$  cells undergoing meiotic reduction in triploid males approximately 60 euploid sperm could be formed. Therefore the reproductive potential of a single male grass carp is approximately  $4 \times 10^{-11}$  for every meiotic reduction of hexaploid spermatogonia, meaning one F1 adult from a triploid X diploid backcross will result from every  $.25 \times 10^{11}$  of these reductions (Allen et al. 1986), and a mature population of diploid females must be present due to the complete infertility of female triploids

In summary, it has been predicted that triploids could reproduce in extremely limited numbers if a mature diploid population and ideal spawning habitat were present. However, if triploid males do exhibit spawning behavior, can spermiate naturally and successfully inseminate normal eggs they have potential to reduce diploid populations due to the majority (.99999988) of gametes produced being aneuploid and therefore forming unviable offspring (Allen et al. 1986).

## **Movement**

The movement patterns of triploid grass carp have many possible implications in the quality of vegetation control they can provide and are strongly influenced by many factors. In their native habitat juvenile grass carp undergo a period of relative inactivity and growth in the lower Amur River until sexual maturity is reached (anywhere from 1 to 9 years of age depending on water temperature and food conditions) at which point they begin a slow northward migration until they arrive at the spawning grounds in the upper river (Gorbach and Krykhtin 1988). It was suggested that because of this natural tendency grass carp would not migrate downstream of their release point, but several studies have demonstrated that this is not the case (Bain et al. 1990; Chilton and Poarch 1997; Kirk et al. 2001). However, Bain et al. tracked the movements of both immature and mature grass carp and found that while the mature grass carp exhibited no directional preference, the juveniles had a significant directional trend to the northeast. Bain et al. also observed that the juvenile grass carp had a relatively small home range of 0.10 square kilometers and stayed in vegetated areas dominated by hydrilla. On the other hand, several studies have observed that mature grass carp move extensively (range .7 – 71.1 km) and as much as 179.3 m/h (Bain et al. 1990; Cassani and Maloney 1991; Chilton and Poarch 1997; Maceina et al. 1999; Kirk et al. 2001), are not seasonally affected, lack directional tendency and due to the high level of dispersion will occasionally move beyond preferred plant communities (Bain et al. 1990; Cassani and Maloney 1991; Clapp et al. 1993; Chilton and Poarch 1997;). Clapp et al. also found that where plants are present in abundance grass carp can be expected to feed in areas from 250 to 750 hectares in size, however, if areas of abundant plants are small or widely dispersed the fish can be expected to move longer distances and have multiple home ranges varying in size up to 4,954 hectares.

Some other movement characteristics of grass carp include an intense period of heightened activity for 1-12 weeks after release when relatively high levels of dispersion occur at all ages (Bain et al. 1990; Cassani and Maloney 1991; Chilton and Poarch 1997). Also, when Cassani and Maloney observed grass carp movements in morphologically diverse reservoirs with surface areas of 45.3 and 20.2 hectares and maximum depths of 1.8 and 2.9 meters respectively, they found that grass carp avoided shallow, narrow areas of the reservoirs which may explain the findings of Clapp et al. that fish did not move through waterways connecting Lake Harris to other lakes in the chain. Martyn et al. (1986) and Hockin et al. (1989) studied grass carp schooling behavior and observed the potential for creating density gradients with high concentrations of fish in some areas and low concentrations of fish in other areas. Grass carp have also been noted to “escape” from systems which are associated with flowing water (Ellis 1974; Nixon and Miller 1978)

These movement patterns suggest that grass carp are affected by many factors including life-history, social behavior, climate, time from release, plant coverage and the physical characteristics of the water body. With so many factors to consider, it is clear that every release must be individually evaluated on a case-by-case basis based on the conditions of release and the desired aquatic plant management goals.

### **Salinity Tolerance**

Due to their ability to consume large volumes of aquatic vegetation (Bennett 1974; Guillory and Gasaway 1978), inhabit large rivers, migrate long distances and tolerate a wide range of environmental conditions triploid grass carp have great potential for being a nuisance in open aquatic systems (Bain 1996). Of special concern is their ability to tolerate brackish water. The coastal marshes of the states bordering the Gulf of Mexico provide breeding grounds for marine and freshwater fish and invertebrates, resting areas for migratory birds and important feeding grounds for many commercially important species (Maceina and Shireman 1979).

Pavlov and Nelovkin (1963) and Kilambi (1980) documented the migration of grass carp from the Volga to the Ural River through the brackish Caspian Sea suggesting relatively high tolerance levels for salinity. In Texas smooth cordgrass has disappeared in all but fenced in enclosures in parts of Galveston Bay – an area downstream of a documented population of diploid grass carp (Leslie et al. 1996). Webb et al. (1994) is investigating the possible links between grass carp populations and the estuarine habitat loss in Galveston Bay.

Maceina and Shireman (1979) found decreasing survival, weight, muscle tissue water content, feeding rate and growth in fingerlings exposed to salinities up to 15.7 ppt – however they predicted that grass carp could inhabit and feed efficiently in brackish water-bodies up to a salinity of 9 ppt. Cross (1969) observed maximum survival times ranging from 24 days in 10.5 ppt salinity (30% seawater) to 5 hours in 17.5 ppt salinity (50% seawater) in 2+ year old grass carp. Maceina et al. (1980) suggests that signs of physiological stress may be begin between 3.1 – 6.6 ppt. It is unknown whether or not grass carp have physiological mechanisms to adjust their osmoregulation on exposure to subtoxic salinities; however, Kilambi and Zdinak (1980) found that the 24 hour LC-50 of grass carp held in 3-7 ppt salinity for 15 days to be 14.0 – 14.2 ppt whereas the 24 hour LC-50 of grass carp acclimated at 9 ppt for 15 days was 16.0 ppt. Von Zon (1977) observed that salinities of just .9 – 3.6 ppt decreased daily food intake of grass carp by 33 percent. Maceina and Shireman (1979) found that at 9 ppt salinity daily consumption of duckweed decreased 79 percent at 9 ppt and there was no consumption at 12 ppt. In contrast to that, Kilambi (1980) reported that grass carp in 3-9 ppt salinity consumed 1.5 times more vegetation than fish in freshwater but that growth rates were on third of that of fresh water fish.

### **Mortality**

Because grass carp mortality can greatly influence the degree of aquatic plant control achieved, knowledge of longevity, and mortality rates are essential to determining the proper stocking density necessary to achieve the desired management goals. Many factors affect the mortality of grass carp - most notably stocking size, water temperature at stocking, hauling distance and density (Carmichael et al. 1984). Clapp et al. (1994) observed hauling mortality rates of 6 percent to 27 percent in winter and 15 percent to 50 percent in spring for triploid grass carp, which are similar to the results obtained by Kirk (1992) and in the range reported for diploid grass carp (Colle et al. 1978; Hill 1986; Shireman et al. 1986). Clapp et al. (1994) also found that in the winter survival of larger

fish (mean TL=371mm) was more than four times better than that of smaller fish (mean TL = 298mm) from the same stocking center, and that hauling time did not significantly affect mortality during the winter, but did during the spring.

Another major factor affecting the mortality of grass carp is predation. Gasaway (1977) and Hatton (1977) both found that largemouth bass (*Micropterus salmoides*) displayed selectivity for stocked grass carp over other typically available forage when stocked at vulnerable sizes. By looking at the size of cleithrum bones and total length in largemouth bass, Shireman et al. (1977) determined the maximum size grass carp a bass could possibly consume is approximately 450mm in length. If stocked at this size grass carp could theoretically completely avoid largemouth bass predation; however, to rear grass carp to this size is prohibitively expensive. Shireman et al. (1980) found that if smaller grass carp were stocked at temperatures warmer than 20° C growth rates are significantly increased and therefore potential predation is minimized.

Kirk and Socha (2003) studied the longevity and persistence of triploid grass carp in the Santee Cooper Reservoir in South Carolina and found that over a period from 1989 to 2002 the oldest of 477 fish collected were age 11 and total annual mortality rates of 22-39 percent suggest a population persistence (period where 10 percent of a cohort would still survive) of 5 to 9 years.

### **Parasites and Disease**

Shireman and Smith (1983) listed a large number of diseases and parasites which afflict grass carp including over forty species of protozoa, twenty species of trematodes, five species of cestodes, nematodes, crustaceans, fungi, bacteria and viruses. Most of the parasites are reported from pond-raised grass carp where high densities and enriched waters are common and a majority of the afflictions which affect grass carp have no impact other than increased mortality in grass carp (Shireman and Smith 1983). However, the grass carp is an intermediate host for *Posthodiplostomum cuticola* which begins development in mollusks and ends in piscivorous birds (Musselius and Stelkov 1968). *Opisthorchis sinensis*, the Chinese liver fluke, encysts in grass carp and ends its life cycle in mammals, including humans (Faust and Khaw 1927). *Bothriocphalus acheilognathi*, a cestode for which copepods are the intermediate host, inhabits the gut of grass carp of all ages and is the major parasitic introduction to have serious effects on other fish (Ivasik et al. 1969).

Parasites and disease represent a real concern in grass carp, both for the management implications of potentially increased mortality and for their potential environmental impact. Introductions of grass carp have spread parasites to new countries and continents. (Musselius and Strelkov 1968; Ivasick et al. 1969) suggested maintenance of disease-free facilities is necessary. Sinderman (1986) provided practical methods for maintaining disease-free hatcheries and reducing the likelihood of introducing diseases and parasites. Inspection and treatment of fish in shipment are also potential mitigation measures (Bain 1996). However, since open systems are connected to a wide range of habitats and sources of diseases and parasites they should have limited vulnerability to new diseases and parasites (Bain 1996).

## Feeding

When considering grass carp for weed control it is important to remember that when an aquatic plant is consumed a niche becomes available and what, if any, species will fill that niche will be determined by grazing pressure and its duration (Pipalova 2006). In this manner the potential effects of grass carp may be either positive or negative. Plant species spread not eaten by grass carp (Fowler and Robson 1978; Madsen and Beck 1997; Li 1998), or regrowth of a preferred species (Fowler and Robson 1978; Leslie et al. 1994; Cassani et al. 1995) can occur following grass carp introduction. On the other hand, grass carp feeding, if selective for the palatable native plants, might facilitate the spread of less palatable alien species (Leslie et al. 1993; Catarino et al. 1997).

Grass carp have been shown to have an order of preference for certain aquatic plants (Fischer 1968; Cross 1969; Michewicz et al. 1972; Edwards 1974; Fowler and Robson 1978; Leslie et al. 1987; Pine and Anderson 1991) which varies with their age (Osborne and Sassic 1981; Pine et al. 1990) and climate (Leslie et al. 1993). Several studies have shown that feeding selectivity of grass carp decreases with their increasing age and size (Opuzynski 1972; Catarino et al. 1997). It has been suggested that since young grass carp have weak and small pharyngeal teeth their preferred foods are soft, immature plants (Fischer 1968; Opuszynski 1972; van Zon 1977).

Pine and Anderson (1991) found that the most preferred plant species for consumption by adult grass carp were American pondweed (*Potamogeton nodosus*), dioecious hydrilla, and elodea (*Elodea nuttallii*). The fish tend to avoid poisonous plants (Murphy et al. 2002), plants with a strong taste and/or with rough, tough or large floating leaves (Von Zon 1977). Von Zon (1977) also found that while such plants are not preferred, older fish will consume these plants if none of their preferred species are available. In Deer Point Lake Florida grass carp were stocked to control mixed stands of Eurasian water-milfoil (*Myriophyllum spicatum*) and Illinois pondweed (*Potamogeton illinoensis*) and after three years the pondweed was eliminated but the Eurasian water-milfoil had increased in abundance above prestocking levels (Van Dyke et al. 1984). In contrast to Deer Point Lake, selective browsing in lakes Clear, Lucerne and Sybelia led to achieving desired management goals because the target plants were more palatable than the native plants and control was successfully achieved (Leslie et al. 1996). In Lake Conway Florida repeated grass carp stockings to control an incipient hydrilla population were successful over a 15 year period while native plants, Illinois pondweed, nitella (*Nitella megacarpa*) and tapegrass (*Vallisneria americana*), were minimally impacted. Limnological and biological factors unique to Lake Conway were probably critical to this successful outcome (Leslie et al. 1994). Grass carp plant consumption rates are highest at temperatures ranging from 18.3 to 29.4°C (Kilambi and Robison 1979).

As grass carp increase in body weight their rate of growth decreases and so does their requirement for food due to changing from a diet for growth to a maintenance diet (Osborne and Sassic 1981). Sutton (1974) observed that small grass carp of approximately 100g were much more efficient at converting hydrilla to fish flesh than were larger fish of approximately 1kg. Furthermore, Osborne and Sassic (1981) found

that at approximate weights of 14kg or more grass carp become ineffective grazers and discontinue causing a significant reduction in plant biomass.

It has also been observed that differences in water chemistry affect plant palatability to grass carp. In New Zealand studies grass carp of similar age and size (3 kg) repeatedly rejected Brazilian elodea (*Egeria densa*) from western springs but would eat plants of the same species collected from the Waikato River (Chapman and Coffey 1971). Several studies have been conducted in which mixed results of plant preference were observed where the same species is reported to be both preferred and non-preferred including Brazilian elodea, Eurasian water-milfoil and coontail (*Ceratophyllum demersum*) (Wiley et al. 1986; Bowers et al. 1987; Leslie et al. 1987; Bonar et al. 1990; Pine and Anderson 1991). Bonar et al. (1990) found significant differences in grass carp consumption for the same plant species collected from different sites – consumption was positively correlated to the concentrations of calcium and lignin and negatively correlated to iron and cellulose. Another finding of the Bonar et al. (1990) study was that water chemistry may affect the chemical and nutritive composition of resident macrophytes.

A further factor affecting the eating behavior of grass carp is the application of herbicides. Tooby et al. (1980) found that while the application of herbicides (diquat and fluridone) at the recommended levels for plant control were not lethal to fish, grass carp stopped feeding after exposure to the chemicals. Fluridone inhibits the synthesis of carotenoid pigments which protect chlorophyll from photodegradation. Such interference with photosynthesis affects the plants carbohydrate production and may lower the food quality of the plants (McCowen et al. 1979). Supporting this information, Kracko and Nobel (1993) observed that plants treated with fluridone displayed 20 percent lower levels of nonstructural carbohydrates than those in untreated plants and that there was significantly less feeding on hydrilla which was exposed to the treatment. However, field studies which combined use of fluridone and low-density stocking of grass carp for integrated control have been successful (Leslie et al. 1996; Cassani 1996).

The effects of grass carp on aquatic plant diversity are dependant on many factors including the density and size of fish stocked and presence or absence of preferred versus non-preferred plant species. However, the use of grass carp to convert hydrilla and other unwanted plant species into fish flesh is possible if correct planning and management decisions are carried out.

### **Effects on Waterfowl Habitat**

Although food habits studies have been conducted for wintering waterfowl in the southeastern states there has been little emphasis placed on the interrelations of wintering waterfowl species to other parts of the biotic community (Gasaway et al. 1977). Arner et al. (1974) and Chabreck et al. (1975) conducted studies in the southeastern United States and concluded that abundance of aquatic plants and vegetation types affected waterfowl habitat quality. In agreement with this, Hoyer and Canfield (1994) found that removal of aquatic macrophytes may have no effect on annual average bird abundance or total species richness, but that the bird species composition will change. Birds that use aquatic macrophytes will be replaced by species that use open-water habitats. Rybicki and

Landwehr (2007) analyzed long-term submerged aquatic vegetation (native and nonnative) and waterfowl trends in the Potomac estuary and reported a positive response by waterfowl to monoecious biotype hydrilla dominated aquatic vegetation. Duke and Chabreck (1974) found that ducks and coots avoid lakes covered with water hyacinth (*Eichhornia crassipes*). Ryan (1972) surmised that the takeover of Eurasian water-milfoil in Seneca Lake New York influenced weight-loss in diving ducks. However, Florschutz (1972) reported the importance of Eurasian water-milfoil as a waterfowl food plant. Leslie et al. (1993) noted that aquatic plants also provide habitat for the macroinvertebrate communities which are vital foods for waterfowl and they did not recommend grass carp use in areas managed for waterfowl. McKnight and Hepp (1995) discouraged the use of grass carp to control aquatic vegetation in areas dominated by poorly consumed species (i.e., Eurasian watermilfoil) without consideration of the potential effects on more palatable native or nonnative species.

Though mixed results have been observed for the effects of other plants on habitat quality for waterfowl, the results appear clear for hydrilla. Gasaway et al. (1977) observed a decline in numbers of multiple waterfowl species in Lake Wales Florida which was significantly correlated with decreases in hydrilla abundance due to the combined effects of grass carp and water level. Furthermore, Gasaway and Drda (1977) concluded that grass carp stocked at 67 kg/ha degraded waterfowl habitat in four Florida lakes. Johnson and Montalbano (1984) found that in Lake Okeechobee Florida waterfowl did not use the various habitat types proportionately to their abundance but most strongly preferred communities dominated by hydrilla, despite their generally preferred native alternatives being abundant. They also found that hydrilla supported a higher diversity of waterfowl species than all other habitat types.

Although these studies do not show direct relationships between grass carp and degraded waterfowl habitat, several Florida studies have shown that hydrilla is important food for ducks, coots (Montalbano et al. 1979; Hardin et al. 1984), and common moorhens (O'Meara et al. 1982; Mulholland 1983). From these studies it is clear that hydrilla abundance plays a role in habitat quality for waterfowl in Florida and must be considered when management goals are being discussed.

### **Effects on Fish Communities**

Macrophytes provide spawning substrate, food and shelter for fish (van Zon 1977). Removal of aquatic vegetation following grass carp introduction caused complete elimination of perch (*Perca fluviatilis*) and pike (*Esox lucius*) (Stanley et al. 1978) and also a decline in numbers of crucian carp (*Carassius carassius*) and roach (*Rutilus rutilus*) (Vinogradov and Zolotova 1974). Krzywosz et al. (1980) also reported major reduction of phytophilous fish species including rudd (*Scardinius erythrophthalmus*), roach and tench (*Tinca tinca*) due competition with grass carp for the few remaining aquatic macrophytes in the lake.

However some fish species experience increased growth in the presence of grass carp due to increased availability of forage fish (Gasaway 1977; Shireman et al. 1977; Colle and Shireman 1980; Shireman et al. 1985; Shireman and Hoyer 1986; Colle et al. 1989;

Kilgore et al. 1998) and/or due to feeding on fecal pellets (Hickling 1966; Stott et al. 1971; Edwards 1974; Takamura et al. 1993). Bailey (1978), Colle and Shireman (1980), and Shireman (1985) all found that large amounts of vegetation negatively impact coefficients of condition for largemouth bass, bluegills (*Lepomis macrochirus*) and redear sunfish (*Lepomis microlophus*) among all size groups. This probably results from reduced predation success due to increased environmental complexity caused by macrophyte structure (Heck and Thoman 1981; Savino and Stein 1982). Shireman and Maceina (1981) observed that black crappies (*Pomoxis nigromaculatus*) did not reach harvestable size until ages 3 and 4 when hydrilla coverage exceeded 50 percent because of reduced threadfin shad (*Dorosoma petenense*); however, following hydrilla removal the threadfin shad population was re-established and black crappies reached harvestable size by age 2.

Bennett (1954) presented an important point pertaining to the introduction of potential forage fish – predation on natural populations is necessary in maintaining good reproduction and stocks. In his study Bennett demonstrated that changes in predator pressure could cause insufficient culling of the fish population, overpopulation of forage, excessive food competition and stunting. Gasaway (1977) found that introduced grass carp were highly susceptible to predation by largemouth bass and that largemouth bass displayed selectivity for stocked grass carp over other available forage. Shireman et al. (1985) also found that total standing crop of largemouth bass averaged three times higher in years without vegetation than in years with abundant vegetation. They also noted that during years with large amounts of vegetation greater numbers and biomass of small and intermediate sized largemouth bass existed while during macrophyte free years there were greater numbers of harvestable largemouth bass. Shireman et al. (undated) also found that in Orange Lake chain pickerel (*Esox niger*), a direct competitor of largemouth bass, underwent increases in abundance due to the establishment of their preferred habitat (vegetation). Bailey (1978) found that plant removal by grass carp in Arkansas lakes resulted in improvement of largemouth bass coefficients of condition (K), a calculation based on length and weight. Cooper et al. (1963) and Clugston (1964) both observed a strong correlation between coefficients of condition and growth rate - with low coefficients of condition accompanying periods of slow growth in largemouth bass. Colle and Shireman (1980) also noted that once hydrilla attained 40 percent coverage in Lake Baldwin a marked decline in condition factor and growth occurred.

### **Changes in Water Quality**

Most changes in water quality and plankton abundance associated with grass carp appear to be caused not directly by the fish, but due to the removal of aquatic macrophytes (Brooker and Edwards 1975). In studies conducted by Shireman et al. (1983) it was concluded that while changes in the aquatic populations did occur, the method of vegetation control was not the important factor and that the removal of weeds by any means will have similar effects.

Hydrochemistry is strongly affected by a variety of factors including weather conditions, hydrology and morphology of water bodies which makes changes caused by other factors hard to detect. Lembi et al. (1978) reported that as much as 54 percent of the phosphorous and 42 percent of the nitrogen in consumed plants was incorporated into grass carp tissue.

In a four year study of the effects of macrophyte removal by grass carp Canfield et al. (1983) and Maceina et al. (1992) observed initially large increases in concentrations of total alkalinity, calcium, magnesium, potassium, total phosphorous, and chlorophyll a in conjunction with a sharp reduction in secchi disk transparency which coincided with declines in the macrophyte community. However, approximately one year later after complete vegetation control was achieved all chemical changes, with the exception of increased potassium, began declining back to pre-introduction levels and secchi disk transparency increased, a phenomenon also noted by Lembi et al. (1978). This is mostly in agreement with other studies which observed increases in total alkalinity and turbidity, although these studies did not observe substantial changes in calcium, magnesium or phosphorous (Lembi et al. 1978; Small et al. 1985; Leslie 1990; Bonar et al. 2002). Shireman et al. (1985) also observed that these major water quality changes began before macrophyte abundance was reduced below nuisance (10 percent) levels. Leslie et al. (1993) noted that water chemistry effects do not occur to the degree noted following chemical treatment. Aquatic plant removal by grass carp occurs over a longer period of time and half the nutrients in the plant material is assimilated by the carp. The egested bolus sinks quickly and the soluble nutrient fraction is subject to adsorption and assimilation by other biota.

Lembi et al. (1978) suggested that since the potassium levels change directly with amount of vegetation consumed it may be feasible to use potassium levels as indicators of grass carp feeding. Avault et al. (1968) showed that although phosphorous and nitrogen levels were not affected by grass carp introduction potassium increased three-fold over pre-introduction levels with complete elimination of aquatic vegetation. Shireman and Hoyer (1986) suggest that overall lake quality changes will occur in the short term due to aquatic macrophyte removal, especially if macrophyte abundance was high, but will return to their limnological potential based on chemical loading rates, hydrology and sedimentary losses.

An initial decrease in dissolved oxygen levels is related to the disappearance of macrophytes (Michewicz et al. 1972; Lembi et al. 1978; Fowler and Robson 1978; Opuszynski 1997). However, production of oxygen during photosynthesis of subsequent phytoplankton blooms and increased mixing due to lack of vegetation increased its concentration in some studies (Lembi et al. 1978; Leslie et al. 1983; Small et al. 1985; Tomajka 1995).

### **Changes in Phytoplankton**

The effects of grass carp on phytoplankton diversity and abundance vary greatly with the management strategy used. Wetzel (1983) suggests that the inhibition of phytoplankton growth among dense macrophytes is related to competition for light and nutrients. Richard et al. (1984) observed that in four Florida lakes mean phytoplankton abundance increased in all cases with macrophyte reduction, and most sharply with complete elimination. In studies where high densities of grass carp were utilized to quickly eliminate all aquatic vegetation major changes in phytoplankton biomass occurred along with seasonal shifts in the dominating species. Crisman and Kooijman (1981) along with Holdren and Porter (1986) observed shifts from diatoms, cryptophytes and green algae

dominance all year, with the exception of summer-fall, to blue-green cyanophyta becoming dominant throughout all seasons. These changes were also accompanied by a 30 percent reduction in the total number of phytoplankton species encountered in the lake system. Maceina et al (1992) and Kogan (1974) both observed that elimination of aquatic macrophytes in lakes in the southeastern United States caused large increases in blue-green algae.

At low stocking densities however the effects of grass carp on phytoplankton assemblages appears to be minimal. Pipalova (2002) and Bonar et al. (2002) both noted that changes in chlorophyll-a concentrations were not significant in ponds with intermediate control. Cassani et al. (1995) also found that annual mean chlorophyll-a concentrations remained stable in lakes where macrophytes were only suppressed. Buck et al. (1975) suggested that phytoplankton growth in systems with vascular macrophytes present may be limited due to competition for one or more essential nutrients. This is a likely reason that several studies in which intermediate levels of control were achieved observed little change in the phytoplankton communities (Terrell 1975; van Zon et al. 1976; Hestand and Carter 1978; Lembi et al. 1978; Mitzner 1978; Terrell 1982; Leslie et al. 1983; Bonar et al. 2002).

The community structure of the phytoplankton assemblage is often used as an indicator of the trophic state of lakes (Rawson 1956; Frank et al. 1966; Hutchinson 1967; Lund 1969; Hestand and Carter 1978). High species diversity but low overall density accompanied by few seasonal pulses is associated with oligotrophic systems whereas eutrophic systems are characterized by having higher standing crop, lower species diversity and frequent blooms (Richard et al. 1984). Related to this, Lackey and Lackey (1967) observed no cyanophyte blooms in oligotrophic lakes in north central Florida; however, such blooms have been noted in studies of southern eutrophic lakes (Cowell et al. 1975; Conley et al. 1979), suggesting that dominance of blue-green algae may be associated with eutrophic systems.

### **Changes in Zooplankton**

Direct effects of grass carp on zooplankton are minimal since stocked grass carp are of the age that they are primarily herbivorous and no longer consume significant amounts of zooplankton (Greenfield 1971; Kilgen and Smitherman 1971; Opuszynski 1972; Terrell and Terrell 1975; Cassani and Caton 1985). In lakes stocked with herbivorous fish the growth of zooplankton is typically increased through consumption of macrophytes and subsequent increases in rate of nutrient remineralization and plankton biomass (Pipalova 2006). Crisman and Kooijman (1981) observed no major differences in overall abundance of the dominant classes of zooplankton including cladocerans, copepods and rotifers in Lake Conway Florida between the pre and post-stocking periods. While Shireman (1985) also observed a lack of significant changes in community structure and only a slight increase in total biomass of zooplankton in four Florida lakes, he did observe a decline in littoral forms typically associated with macrophytes (crustaceans and arthropods) and an increase in pelagic forms. Several other studies show that zooplankton was unaffected by grass carp introduction (Terrell 1975; Fowler and Robson 1978; Maceina et al. 1992).

### **Changes in Sediment Chemistry**

Studies have shown that approximately 50 percent of the nitrogen and phosphorous consumed by grass carp is incorporated into fish flesh (Lembi et al. 1978). It would therefore be expected that the other 50 percent of these essential nutrients would be released into the water column; however, several studies have shown that this is not the case – that despite the introduction of grass carp and subsequent consumption of available macrophytes little increases were observed in nitrogen and phosphorous levels (Lembi et al. 1978; Small et al. 1985; Richard et al. 1985). Hestand and Carter (1978) and Hodgson and Linda (1984) both found sharp increases in these nutrients following herbicide usage in study ponds. However, in Terrell's (1975) study of water and sediment chemistry following grass carp introduction, sharp increases in iron, magnesium and phosphate-phosphorus concentrations were observed in the sediment of affected lakes. This suggested, along with Hestand and Carter's (1978) data that nutrients released in fecal deposits were precipitated by, or with, organic acids and thus were trapped in the sediment and unavailable to phytoplankton.

### **Changes in Benthic Invertebrates**

Bain (1993) found that removal of aquatic vegetation will cause benthic fauna growth to increase. In agreement with this, many authors (Hickling 1966; Greenfield 1971; Prowse 1971; Stott et al. 1971; Buck et al. 1975; van Zon et al. 1976; Zweerde van der 1982; Kirkagac and Demir 2004) observed significant increases in growth of macrofauna, especially bottom dwellers and detritivores, in the presence of grass carp. Kirkagac and Demir (2004) observed a three fold increase in benthic fauna abundance after elimination of aquatic macrophytes by grass carp. In contrast, Crisman and Kooijman (1981) observed no change in either the density of individuals or the number of species encountered between pre and post stocking periods.

While it is clear that grass carp do not influence zoobenthos directly, indirect changes at the primary level may (Terrell and Terrell 1975). Better mixing of the water body and increased oxygen production due to greater phytoplankton abundance allow for fewer anoxic periods and therefore depending on the trophic state of the system may make the water body more tolerable for benthic fauna (Aliev 1976).

### **Determining Stocking Density**

Many factors go into determining appropriate stocking density including climate, fish size, expected weed growth and aquatic plant management goals (van Zon et al. 1976). "Overstocking" results in elimination of all aquatic vegetation while "understocking" results in either selective reduction of vegetation (Blackwell and Murphy 1996; Bonar et al. 2002) or in no control at all (Bonar et al. 2002). It has been shown that low stocking densities can be used to achieve intermediate levels of control, but it is important to remember that this opens a niche available to be filled and plants rejected by the grass carp are left and may grow vigorously (van Zon 1977). Unfortunately, stochastic climatic events that lower water levels or increase color can eliminate submerged plants, an undesirable outcome, and stocked grass carp, even at low levels, may delay plant recovery (Leslie et al. 1993).

Cassani et al. (1995) assessed triploid grass carp stocking densities based on amount of vegetation and found that a stocking rate of 3 grass carp per metric ton of vegetation (wet weight) significantly reduced macrophyte biomass but did not result in complete elimination in the 4 or 5 years they were monitored after stocking. Stocking rates of 4-8.4 grass carp per metric ton of vegetation wet weight reduced macrophyte biomass to zero in 8-17 months. Leslie et al. (1987) observed that reduction of high aquatic plant biomass through herbicide treatment and low stocking densities of grass carp (1-2 fish per metric ton of vegetation) had the potential to reduce damage to native aquatic flora while still controlling nuisance aquatic species.

Most studies have used stocking rates determined not by weight of macrophyte biomass but by coverage area; however, results using this method have been highly variable. Bonar et al. (2002) studied 31 lakes in Washington State using a wide range of stocking densities from 5-174 fish per vegetated acre. In their study, after 19 months, intermediate control was achieved for seven lakes with a median stocking density of 24 fish per vegetated acre; however, stocking rates as low as 8 fish per vegetated acre produced complete eradication whereas rates as high as 74 fish per vegetated acre produced no control. Osborne and Sassic (1979) and Kirk and Henderson (2006) both observed complete eradication of macrophytes in approximately 2 years after grass carp were stocked at 30 and 16 fish per vegetated acre respectively. In the Santee Cooper Reservoirs Kirk and Henderson (2006) studied, control has been maintained with an estimated 1 fish for every 7 formerly vegetated acres for 12 years with no restocking since the initial introduction. Leslie et al. (1994) stocked Lake Conway Florida at an average of 10.4 female fish (approximately 20 fish per hectare and 1.6 fish per metric ton of vegetation). Two years after stocking, hydrilla was greatly reduced with no discernable effect on frequency or occurrence of native aquatic plants. An additional 2.4 and 1.5 triploid grass carp per hectare were stocked 9 and 11 years later, respectively, with similar satisfactory result that the authors noted had not been duplicated in other Florida lakes over the long term. Shireman and Hoyer (1986) observed elimination of aquatic vegetation in Lake Baldwin Florida with a stocking density of 9 fish per acre. Hanlon et al. (2000) evaluated macrophyte control in 38 Florida lakes using triploid grass carp and found that a stocking density of 10-12 fish per hectare of water or 25-30 fish per hectare of vegetation is the range between which intermediate control is possible, though not guaranteed. Hanlon et al. (2000) also suggests that with low density stocking below 25 grass carp per hectare vegetation the growth rates of aquatic macrophytes are often greater than the consumption rates of grass carp and aquatic plants may continue to expand, though such low density stocking may impact plant species composition due to grass carps selective feeding habits. Leslie et al. (1993) note that plant removal is slow when using grass carp and patience or supplemental management methods are required. They also suggest that managers should underestimate the stocking rate, provide supplemental plant control, and/or slowly increase the stocking rate until plant management objectives are met.

All aforementioned studies used grass carp ranging from 20-30 cm in total length and introduced the grass carp during either seasonally low vegetation levels or after reduction

by herbicides (Osborne and Sassic 1979; Shireman and Hoyer 1986; Leslie et al. 1987; Cassani et al. 1995; Hanlon et al. 2000; Bonar et al. 2002; Kirk and Henderson 2006).

Several computer models have also been developed by various government agencies (Miller and Decell 1984; Wiley et al. 1984; Swanson and Bergersen 1988; Boyd and Stewart 1990; Santha et al. 1991). The most comprehensive to date is the AMUR/STOCK model developed by Boyd and Stewart (1990). The AMUR/STOCK model takes into consideration environmental and physiological factors which affect grass carp feeding rates, plant palatability, assimilation efficiency of ingested material to estimate fish growth and desired management goals and provides the user with a year 1 stocking rate (fish/ vegetated hectare) needed to attain the desired level of control. The model can also provide estimates of plant biomass density, number of stocked fish remaining at the end of each post stocking year, average size of the remaining fish at the end of each post stocking year and number of hectares of plant growth controlled each year of the stocking period. It is important to remember; however, that such models are simplified versions of complex systems and their output data is only as accurate as the beginning parameters provided by the user.

### **Barriers to Movement**

The following barrier information was summarized from report prepared for the Minnesota Department of Natural Resources in cooperation with the Wisconsin Department of Natural Resources and the U.S. Fish and Wildlife Service (FishPro 2004).

Two barrier types have been considered or implemented to constrain the movement of fish: behavioral or physical. Behavioral systems include strobe lights, air bubble curtains, acoustic, electrical fields, hydrodynamic louver screens, and combinations of any of the methods listed. Physical barriers include rotating drum or traveling screens, floating curtains, vertical drops or velocity barriers. Each method is briefly discussed.

**Strobe light:** A method that has been extensively evaluated in laboratory and field situations. Deterrence has been achieved for a number of species, especially juvenile American shad. Combining strobe lights and bubble curtains have been found to successfully divert or deter 77 to 80 percent of all fish that approach hydroelectric turbine intakes. Water turbidity levels diminish deterrence. Flash intensity and duration affects fish response. Flash duration can appear as a constant light source due to light attenuation over distance and will attract fish. However, strobe lights were ineffective as a barrier to grass carp movement in South Florida agricultural canals (John Cassani, personal communication).

**Bubble curtain:** Effective in laboratory tests up to 98 percent but declining in effectiveness to 51 to 80 percent in darkness or high turbidity. Deterrence is also negatively affected by boat traffic or current flow.

**Acoustic:** Two methods are in use: 1) an array of underwater loudspeakers or sound projectors that produce a diffuse field of sound or 2) sound sources linked to a bubble curtain to produce a wall of sound. Proprietary designs, Sound Projector Array and Bio-

Acoustic Fish Fence, implement either focused sound (the Array) or sound integrated with a sheet of bubbles (the Fence). The Illinois Natural History Survey tested a combination of these proprietary systems to achieve 57 percent deterrence of a mix of bighead and silver carp. Repeated exposure improved deterrence but fish crossed the combined barriers when frightened. Fish vary in their sound sensitivity as related to body size, presence or absence of a swim bladder, and anatomical differences that influence sound conductance from the swim bladder to the inner ear. Resident fish may become habituated to sound. Environmental conditions also influence effectiveness: site morphology, current patterns, seasonal stratification or turbidity.

Electrical: Two methods have been implemented: 1) graduated and 2) pulse. Graduated fields have been used effectively to prevent upstream movement where a minimum .6 to .9 m/s downstream current is present to carry fish out of the field. Field effectiveness is influenced by the fish length. Longer fish are repelled earlier in the field than short fish. Pulse fields are parallel connected in series to be effective in deeper and wider locations (i.e., large rivers). Factors that influence effectiveness include the availability of sufficient electricity, human safety risk level, and locations where silt and debris accumulation would be minimal.

Hydrodynamic louver screens: A physical structure is placed across a channel that increases current velocity to repel fish. Drawbacks include the louvers create a damming effect, structures can only be constructed in a uniform channel, debris can accumulate and block/restrict flow, variable flow patterns influence effectiveness, and the structure will block navigation.

Physical barriers may be negatively affected by debris, variable flow velocity and will effect nontarget species movement, navigation or flood control management. Vertical drops must create a hydraulic drop higher than the leaping ability of the target species. Rotating drum screens and traveling screens are designed to handle debris but will impede navigation and nontarget species movement. Floating barriers (a piling or float supported cable that suspends nets or chains) are affected by debris and impede navigation. Velocity barriers increase current velocity by funneling currents. The velocities generated must exceed the burst or sustained swimming speeds of the target species.

Maceina et al. (1999) examined the effectiveness of three barrier types installed in Lake Seminole (Georgia-Florida) embayments to prevent triploid grass carp escape. Barrier designs were selected to allow recreational boat passage: weighted, swinging gate, tandem V-shaped funnel weirs, and low-voltage electric field (3-4 volt DC field at a peak current output of 12 amps at 10-ms pulse over a 500-ms duty cycle for an average current of 1.44 amps) in combination with a V-shaped funnel weir. The annual escape rate for the V-shaped weir and swinging gate were 42 percent and 35 percent, respectively. No verified escapes occurred from the embayment that included the electric field combined with a V-shaped funnel weir. They estimated the average annual cost to manage submersed vegetation in Lake Seminole to be \$720 per hectare for herbicides, \$5,500 per hectare for mechanical harvesting, and \$100 per hectare for barrier installation, grass carp

stocking, supplemental stocking, and barrier maintenance. Leslie et al. (1993) noted that stocking grass carp, without containment, upstream of wildlife refuges or estuaries “can only be viewed as environmentally irresponsible (page 15).”

### **Methods of Grass Carp Removal**

Santha et al. (1991) devised a model that involves alternately stocking and harvesting grass carp in order to achieve 40 percent of aquatic plant cover in 1.5 years with respect to the initial carrying capacity of the environment. However, they determined that due to unreasonable costs and the general ineffectiveness of grass carp removal techniques their model was not a viable option for maintaining intermediate plant control. Schramm and Jirka (1986) conducted tests of 11 methods of grass carp capture in agricultural canals and found most of them to be unsuccessful. Attracting grass carp to traps baited with aquatic vegetation appears to be a useful technique for capturing small numbers of grass carp over a long period of time when the area contains no consumable vegetation. Otherwise, the most successful method was to bait an unobstructed area and after the fish were attracted containment by placement of a barrier and collection with rotenone as described by Colle et al. (1978). Bonar et al. (1993) also conducted a survey of methods for grass carp capture and found that herding was consistently the most effective method they tested (.17 - .56 fish/man hour) followed by angling (0 to .14 fish/man hour).

Rotenone collection has been successful in studies but is highly variable in different study areas and requires precise planning and implementation. Differences in water quality on the toxicity of rotenone to fish mandates field bioassays within the specific water body before determining the feasibility of selectively removing grass carp (Colle et al. 1978). Colle et al. (1978) also found that while it is possible for rotenone to be highly selective for grass carp the time of year of removal may greatly affect the selectivity of the treatment since fry and juvenile fish are more susceptible than adults. They also found that water temperatures must be above 20° C to insure rotenone effectiveness. Therefore, early fall is suggested as an ideal time for rotenone treatment since water temperatures are still warm but most spawning and hatching has ceased (Colle et al. 1978).

Willis (2002) utilized operant conditioning to train grass carp to come to sound and then Duncan (2002) followed that study by assessing the utility of using sound to recapture released grass carp. Duncan (2002) achieved a 61 percent recovery rate in his first trial. While these are promising initial results, more study is warranted on this topic to investigate whether fish would respond in field systems where vegetation is available in addition to the bait feeding and how effective the sound transmission could be in larger systems. The cost effectiveness of the time required to train the fish is also not considered. Another new method of grass carp removal is the use of biodegradable capsules containing fish toxicant which would be implanted in the carp and used to euthanize the fish after a given period of time (Thomas 2004).

## **Risk Analysis**

### **Introduction**

*The Generic Nonindigenous Aquatic Organisms Risk Analysis Review Process* (hereafter “Generic Analysis”) is comprised of a pathway risk analysis or an organism risk analysis. The steps for both are, first, “Initiation” consisting of a request to evaluate a pathway or a single organism. The second step is “Risk Assessment” that consists of: 1) identification of interested parties and solicitation of input; 2) list of nonindigenous organisms of concern, 3) information collection on the pathway or organism and associated organisms (literature and investigation); 4) assessing the risks associated with the a) probability of establishment by examining the probability that an organism is in a pathway and the potential for entry, colonization and spread, and b) consequences of establishment by examining the potential for economic, environmental and perceived (social and political) effects; and 4) recommendations. And the third and final step is “Risk Management” that has the objectives of developing: 1) a risk mitigation matrix consisting of appropriate policies, regulations and operational measures and 2) an iterative and realistic risk management operational plan that includes a monitoring system to revise and update mitigation over time (i.e., adaptive management) (ANSTF 1996).

During the second workshop, members addressed and discounted the risk of whether triploid grass carp would become established because in Florida this fish is purposefully stocked to control aquatic vegetation to achieve certain recreational, ecological, or flood control goals. To assess the consequences of stocking triploid grass carp within open water systems, members created a risk rating and utilized the uncertainty ratings from the Generic Analysis. Risk was conceptualized as being from 10 (high risk of occurrence) to 1 (low risk) and the uncertainty ratings were:

Uncertainty ratings:

Very Certain	VC	Sufficient data to support certainty
Reasonably Certain	RC	Good but not extensive data or experience
Moderately Certain	MC	Limited data or experience
Reasonably Uncertain	RU	Very limited data or marginally applicable data
Very Uncertain	VU	A guess

**Consequences of Stocking**

Considerable and strong reservations were expressed by the workshop members concerning the stocking and management of triploid grass carp in large, unconfined water bodies (>500 acres). Members agreed that each lake is different. Attempting to generalize Florida lake ecology simplifies dynamic, sometimes unpredictable systems that are influenced by adjacent land uses, soils, and subject to stochastic weather-driven events. A specific management plan should be developed that considers lake morphometry, hydrology, sediments, water chemistry, surrounding land uses, resource and recreational management goals, and seasonality. In addition, logistical constraints (cost and manpower) prevents the effective monitoring of triploid grass carp in large water bodies and the members were uncertain whether barriers to triploid grass carp

movement are effective in open water situations or practical to use in Florida situations.<sup>2</sup> Some lakes may not recover from vegetation removal because of certain morphological characteristics (size and depth). Triploid grass carp are also recognized as being vulnerable to predation natural mortality may not be predictable.<sup>3</sup> The downstream effects of triploid grass carp cannot be predicted as well as their effects on the target water body or connected water body if stochastic weather events eliminate vegetation through increased turbidity or reduced water clarity. The members also recognized that Floridians have not demonstrated the patience required to allow triploid grass carp a five to seven year time frame to reduce vegetation coverage and that current vegetation management models are not robust enough to predict outcomes without considerable public investment into ecosystem monitoring (i.e., flora, fauna, and water chemistry).

Members recognized and accepted these critical reservations and considered two general scenarios as being unacceptable outcomes to the consequence of establishment (i.e., stocking triploid grass carp) in open waters: 1) complete vegetation removal for an extended period of time and 2) no vegetation control.

Each scenario was discussed in-depth relative to four hydrilla management options identified during the Florida LAKEWATCH sponsored workshop (Hoyer et al. 2005) and a fifth management option added during the workshop. Members refined the management options by identifying stocking rates, period of time, or percentage of vegetation coverage.

- 1) Implement high stocking rates to eliminate hydrilla, rapidly followed by efforts to reduce and manage triploid grass carp populations (i.e., removal techniques, barriers, and controlled mortality) to achieve hydrilla maintenance control. The suggested stocking rates range from 10 to 20 fish per water body surface acre with complete hydrilla removal within 18 months.
- 2) Implement stocking rates at low levels to achieve hydrilla management objectives over a five-year period and the methodologies to assess and adjust stocking to maintain aquatic plant coverage objectives. Stocking rates range from 0.5 to 5 fish per water body surface acre with a goal of maintaining 25 to 50 percent vegetation coverage within five years. Supplemental stocking may be required.
- 3) Herbicide application to achieve hydrilla management objectives, followed by stocking low levels of triploid grass carp to maintain hydrilla management objectives. Initial herbicide treatment to achieve a 20 percent vegetation coverage followed by stocking rates of 0.5 to 3 fish per water body surface acre with a final vegetation coverage objective of 20 to 50 percent maintained through herbicide treatments. No restocking during the five-year period.
- 4) Integrated hydrilla management: triploid grass carp, chemical, water level, mechanical, and other biological control (i.e., insects).

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<sup>2</sup> Members knew of the grass carp barrier efforts on Lake Seminole and requested that the literature review include a section with information specific to barrier implementation on the lake.

<sup>3</sup> Mortality rates are fairly well worked out; about 20 percent annually over the long-term (>10 years) and 5 percent annually over the short-term (<5 years) but individual water bodies may vary (B. Jagers, personal communication).

- 5) Ultra-low triploid grass carp stocking to control an incipient hydrilla population. Stocking rate of less than 0.5 fish per water body surface acre.

**Complete Vegetation Removal**

An unacceptable outcome to stocking triploid grass carp is the complete removal of all vegetation due to the importance of macrophytes for aquatic communities, waterfowl, wading birds, and water clarity. However, vegetation recovery within a defined time period was considered acceptable. The workshop members were polled as to what would be an unacceptable period during which there was no vegetation present for time periods from one to 10 years and a table was constructed to record the responses.

Complete vegetation removal	Years				
	1	2	3	6	10
Fisheries	A	A	A	U	U
Waterfowl	A	A	A	U	U
General Recreation	A/WQ	A/WQ	A/WQ	A/WQ	A/WQ
Riparian Land Owners	A/WQ	A/WQ	A/WQ	A/WQ	A/WQ
Key: A – acceptable U – unacceptable A/WQ – acceptable as long as water quality (clarity, appearance) does not change.					

Fishery and waterfowl managers were willing to accept three years of no vegetation with the realization that two year classes of fish can be “lost” and fish populations can recover. A maximum of three years with no vegetation on an individual water body would be acceptable to waterfowl because they are mobile and can move to other habitat. An extended vegetation-free time period increases the risk of the birds not returning to that water body after the vegetation returns. Waterfowl tend to return to the same water bodies year-after-year, as long as the habitat remains suitable, but when the habitat is absent for extended periods, those “traditions” can be lost. General recreation users (boating, skiing, personal water craft, etc.) as well as riparian land owners generally welcome the lack of vegetation as long as lake water quality (clarity, odor, appearance) are not negatively affected.

**Unacceptable Risk Scenario I: Complete vegetation removal for three or more years**

Members weighed whether each management option would result in complete vegetation removal for three or more years with the understanding that associated with each management option appropriate lake management occurs (flora, fauna and water quality monitoring), resources are available (manpower, equipment and funding), current technology is employed, and the lake is subject to stochastic events that may significantly influence vegetation abundance.

Management Options	Risk Rating: Vegetation Removal for 3+ Years									
	High			Medium				Low		
	10	9	8	7	6	5	4	3	2	1
1) High TGC stocking	RC - 10									

2) Low TGC stocking	RU - {8 to 5}
3) Herbicide with low TGC stocking	RC - {3 to 2}
4) Integrated hydrilla management	RC - {3 to 2}
5) Ultra-low stocking	VC - 1
Note: numbers represent a consensus numerical risk rating or range of responses. VC – Very certain RC – Reasonably certain RU – Reasonably uncertain	

### Management Option Discussion

- 1) Implement high stocking rates to eliminate hydrilla rapidly followed by efforts to reduce and manage triploid grass carp populations (i.e., removal techniques, barriers, and controlled mortality) to achieve hydrilla maintenance control. The suggested stocking rates range from 10 to 20 fish per water body surface acre with complete hydrilla removal within 18 months.

Members were reasonably certain that there was a high risk that vegetation would be completely eliminated for three years or more because triploid grass carp removal from a large system is logistically impossible or impractical and it is not logistically feasible to monitor the triploid grass carp population and determine how many remain within the water body. The unpredictable vegetation response linked to public impatience with a triploid grass carp only stocking would probably result in the adding of triploid grass carp which could lead to a stocking rate in excess of the stated objective. Uncertainty exists concerning: 1) whether or not the fish would leave after the hydrilla is eradicated and 2) how long the hydrilla would be removed because there is a chance the carp would leave and vegetation would recover.

- 2) Implement stocking rates at low levels to achieve hydrilla management objectives over a five-year period and the methodologies to assess and adjust stocking to maintain aquatic plant coverage objectives. Stocking rates range from 0.5 to 5 fish per water body surface acre with a goal of maintaining 25 to 50 percent vegetation coverage within five years. Supplemental stocking may be required.

This management option has been achieved in small, closed systems. The initial low stocking rate can be calculated but the annual adjustment will be difficult without costly annual monitoring for the five-year period. Public impatience with low stocking may result in incremental stockings without sufficient time allowed for a vegetation response (at least three years of monitoring) that would result in overstocking and vegetation eradication. Members were reasonably uncertain and identified a wide risk range (8 to 5) subject to whether there were present high residual hydrilla tubers in the sediments or initially small amounts of hydrilla. The longer term, five-year time period also increases the potential for stochastic events to play a larger role in influencing vegetation coverage, but risk would be reduced with periodic vegetation assessment.

- 3) Herbicide application to achieve hydrilla management objectives followed by stocking low levels of triploid grass carp to maintain hydrilla management objectives. Initial herbicide treatment to achieve a 20 percent vegetation coverage

followed by stocking rates of 0.5 to 3 fish per water body surface acre with a final vegetation coverage objective of 20 to 50 percent maintained through herbicide treatments. No restocking during the five-year period.

Members were reasonably certain this management option yielded a lower risk of producing no vegetation for three or more years. Herbicide application reduces initial coverage to 20 percent coverage, low level triploid grass carp stockings can be fine tuned to lake physical, chemical and biological characteristics, and the vegetation management objective will be maintained with periodic herbicide applications.

- 4) Integrated hydrilla management: triploid grass carp, chemical, water level, mechanical, and other biological control (i.e., insects).

Integrating a variety of hydrilla management practices was considered a desirable option. However, for large water bodies lowering water levels is impossible or impractical, mechanical removal is expensive and is implemented to create boat trails, no insects have been found that are effective for controlling hydrilla, and federal, state, and local agencies working on large water bodies have an unfortunate history of not communicating and coordinating their intentions and activities. To develop an integrated approach would require greater management intensity. However, the members were reasonably certain that the risk was low that vegetation would be eliminated for three or more years primarily based upon the realization that the only effective components of an integrated approach that can be implemented are herbicide applications and triploid grass carp stockings.

- 5) Ultra-low triploid grass carp stocking to control an incipient hydrilla population. Stocking rate of less than 0.5 fish per water body acre.

Members identified Lake Miccosukee Florida (Jefferson/Leon counties) as the only instance where a low level stocking has been practiced to control hydrilla.<sup>4</sup> The lake is large and shallow with 90 percent coverage in emergent native vegetation and a low dam at the outfall maintains a static water level. Hydrilla has appeared in the limited open areas and boat trails and has been effectively controlled with a very low triploid grass carp stocking rate. Based upon this water body, members were very certain that the risk of eliminating vegetation for three or more years was the lowest of the five options considered.

**Unacceptable Risk Scenario II: No vegetation control**

No vegetation control	Years				
	1	2	3	6	10
Fisheries (vegetation target 25-50%)	A	A	U	U	U
Waterfowl	A	A	A	A	A

<sup>4</sup> There are a number of examples of smaller Florida water bodies (<500 surface acres) where this approach has worked well: Cherry Lake, Madison County; Peacock Lake, Suwannee County; Lake Willis, Orange County; Lake Alma, Orange County, etc. (B. Jagers, personal communication).

General Recreation	U	U	U	U	U
Riparian Land Owners (flood control)	U	U	U	U	U
Key: A – acceptable U – unacceptable					

No vegetation control was defined as coverage exceeding 50 percent of the water body for a five-year period. Vegetation coverage of greater than 50 percent coverage is acceptable for fisheries for two year classes and acceptable for any length of time for waterfowl that consume and thrive on hydrilla-covered lakes when boat trails are maintained to allow access, but is an unacceptable condition for general recreational users and riparian land owners. No vegetation control is unacceptable for water bodies subject to flood control management. The risk to water control structures becoming clogged with hydrilla is very high. Similar to Risk Scenario I, five management options were considered with the understanding that lake management occurs (flora, fauna, and water quality monitoring), resources are available (manpower, equipment, and funding), current technology is employed, and the lake is subject to stochastic events.

Management Options	Risk Rating: No Vegetation Removal									
	High			Medium				Low		
	10	9	8	7	6	5	4	3	2	1
1) High TGC stocking	RC – {3 to 1}									
2) Low TGC stocking	RU – {5 to 3}									
3) Herbicide with low TGC stocking	RC – {3 to 1}									
4) Integrated hydrilla management	RC – {3 to 1}									
5) Ultra-low stocking	Not applicable									
Note: Numbers represent a consensus numerical risk rating or range of responses. RC – Reasonably certain RU – Reasonably uncertain										

### Management Option Discussion

- 1) Implement high stocking rates to eliminate hydrilla rapidly followed by efforts to reduce and manage triploid grass carp populations (i.e., removal techniques, barriers, and controlled mortality) to achieve hydrilla maintenance control. The suggested stocking rates range from 10 to 20 fish per water body surface acre with complete hydrilla removal within 18 months.

Members were reasonably certain that with high stocking rates there is a low risk that no vegetation control would occur. The potential for triploid grass carp to leave the water body and mortality rate contributes to the risk rating range.

- 2) Implement stocking rates at low levels to achieve hydrilla management objectives over a five-year period and the methodologies to assess and adjust stocking to maintain aquatic plant coverage objectives. Stocking rates range from 0.5 to 5 fish per water body surface acre with a goal of maintaining 25 to 50 percent vegetation coverage within five years. Supplemental stocking may be required.

The members were reasonably uncertain of no vegetation control and identified a low-medium risk rating range (numerical range of 3 to 5) because: 1) low stocking rates increase the risk of no vegetation control, and 2) public impatience would trigger additional stockings without sufficient vegetation response time (i.e., three years) would reduce of the risk of no vegetation control but could result in the unacceptable outcome of eliminating vegetation (Risk Scenario I).

- 3) Herbicide application to achieve hydrilla management objectives followed by stocking low levels of triploid grass carp to maintain hydrilla management objectives. Initial herbicide treatment to achieve a 20 percent vegetation coverage followed by stocking rates of 0.5 to 3 fish per water body surface acre with a final vegetation coverage objective of 20 to 50 percent maintained through herbicide treatments. No restocking during the five-year period.

The initial herbicide treatment and subsequent treatments reduce the risk and uncertainty of no vegetation control as an outcome.

- 4) Integrated hydrilla management: triploid grass carp, chemical, water level, mechanical, and other biological control (i.e., insects).

Similar to Risk Scenario I, integrating a variety of hydrilla management practices is a desirable, if impractical, option. For large water bodies lowering water levels is impossible or impractical, mechanical removal is expensive and is implemented to create boat trails, no insects have been found that are effective for controlling hydrilla, and federal, state, and local agencies that may be working on large water bodies have an unfortunate history of not communicating and coordinating their intentions and activities. To develop an integrated approach would require greater management intensity. However, the members were reasonably certain that the risk was low that no vegetation control would be a result primarily based upon the reality that the only components of an integrated approach that can be implemented are herbicide and grass carp.

- 5) Ultra-low triploid grass carp stocking to control an incipient hydrilla population. Stocking rate of less than 0.5 fish per water body surface acre.

This management option is not applicable because the initial very low levels of hydrilla are below the 50 percent vegetation coverage.

### **A Potential Management Option**

The risk analysis discussion for each of five management options resulted in recognition that the third option, an initial herbicide application to achieve hydrilla management objectives followed by stocking low levels of triploid grass carp to maintain hydrilla management objectives, offered the lowest risks for either of the unacceptable outcomes: 1) all vegetation eliminated for three years or more or 2) no vegetation control with coverage exceeding 50 percent of a water body. The members then identified the management requirements that must be included to implement the practice and the lake or vegetation conditions under which this management option might be considered.

A management plan must be developed on an individual lake basis to adequately consider limnological, morphometric, surrounding land use conditions, and two complex challenges specifically associated with herbicides and triploid grass carp. The primary challenges to open water herbicide application are: 1) current high costs of available herbicides that may not specifically target hydrilla, 2) water outflow rates may nullify or mitigate herbicide effectiveness, 3) unknown downstream herbicide effects on non-target vegetation, and 4) herbicide effects associated with water withdrawn for irrigation or human consumption. Grass carp associated concerns include: 1) low level grass carp stocking may be impractical to maintain due to emigration if barriers can not be implemented to retain fish in the water body, and 2) managers must carefully calculate stocking rates based on vegetation biomass to avoid overstocking.

Specific lake conditions must be considered before implementing the herbicide and carp stocking management option. Lake characteristics favorable to this management scheme include: shallow, eutrophic lakes or lakes with sandy or low organic containing sediments and good light penetration are favorable to re-establishment of native vegetation. Unfavorable lake characteristics include: 1) smaller or shallow lakes, with a tendency to dry out, can concentrate carp that will consume all vegetation, 2) high flow will trigger fish to leave the water body, 3) highly flocculated sediments, in lakes with enough fetch, will result in wind-mixed turbidity that will independently and negatively affect vegetation coverage over an extended period of time, and 4) surface water inflow from surrounding lands can introduce long-term water chemistry changes (i.e., color) that will inhibit plant growth or recovery.

Specific macrophyte conditions favorable to this management option include lakes, with a mix of hydrilla and native plants for two reasons. Hydrilla can be controlled by herbicide to allow the native plants to re-vegetate or if native plants are unpalatable to the triploid grass carp, the unpalatable native plants will provide structure to support fisheries and aquatic communities (note: hydrilla resistance to fluridone herbicides upsets this strategy). Unfavorable macrophyte conditions include: lakes dominated by native species that are highly preferable to grass carp, the presence or colonization by unpalatable exotic plants may result in the replacement of hydrilla with a species more difficult or costly to manage (e.g., *Hygrophila* in South Florida), lakes dominated by hydrilla or that contain waterfowl-limiting forage are difficult to manage with this management option to yield plant coverage to benefit waterfowl.

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## Appendix A

### **Angling:**

Chris Horton, Associate Director  
BASS  
P.O. Box 10000  
Lake Buena Vista, FL 32830  
407-566-2217  
[Christopher.m.Horton@bassmaster.com](mailto:Christopher.m.Horton@bassmaster.com)

### **Fishery management:**

Tom Champeau  
Florida Fish and Wildlife Conservation Commission  
Southwest Regional Office  
3900 Drane Field Road  
Lakeland, FL 33811-1299  
863-648-3202  
[TOM.CHAMPEAU@FWC.STATE.FL.US](mailto:TOM.CHAMPEAU@FWC.STATE.FL.US)

Bruce V. Jagers  
Florida Fish and Wildlife Conservation Commission  
601 W. Woodward Ave.  
Eustis FL 32726  
352-742-6438  
[Bruce.jagers@myfwc.com](mailto:Bruce.jagers@myfwc.com)

### **Florida Dept. of Environmental Protection**

Andrew (Drew) Leslie  
DEP-Invasive Plant Management  
3900 Commonwealth Blvd, MS 705  
Tallahassee, FL 32399  
850-245-2809 ext 2822  
[drew.leslie@dep.state.fl.us](mailto:drew.leslie@dep.state.fl.us)

### **Florida Fish and Wildlife Conservation Commission:**

Scott Hardin  
Florida Fish and Wildlife Conservation Commission  
620 South Meridian Street  
Tallahassee, FL 32399  
850-488-4068  
[scott.hardin@fwc.state.fl.us](mailto:scott.hardin@fwc.state.fl.us)

### **Grass carp production:**

Mike Freeze  
Keo Fish Farm  
PO Box 166  
Keo, AR 72083

501-842-2872  
[keoff@futura.net](mailto:keoff@futura.net)

**Pond management:**

Dr. Charles Cichra  
University of Florida, Dept of Fisheries and Aquatic Sciences  
7922 Northwest 71<sup>st</sup> Street  
Gainesville, FL 32606  
352-392-9617 ext 249  
[cecichra@ufl.edu](mailto:cecichra@ufl.edu)

**University of Florida:**

Dr. Mark Hoyer  
University of Florida, Dept of Fisheries and Aquatic Sciences  
7922 Northwest 71<sup>st</sup> Street  
Gainesville, FL 32606  
352-392-9617 ext 227  
[mvhoyer@ufl.edu](mailto:mvhoyer@ufl.edu)

**U.S. Fish and Wildlife Service:**

Vince Mudrak, Center Director,  
Warm Springs Fish Technology Center  
5308 Spring Street  
Warm Springs, Georgia 31830  
706-655-3382  
[Vincent\\_Mudrak@fws.gov](mailto:Vincent_Mudrak@fws.gov)

**Waterfowl hunting:**

Diane Eggeman  
Florida Fish and Wildlife Conservation Commission  
8932 South Apalachee Parkway  
Tallahassee, FL 32311  
850-488-5878  
[diane.eggeman@fwc.state.fl.us](mailto:diane.eggeman@fwc.state.fl.us)

**Water Management District:**

Dan Thayer  
Division of Vegetation Management  
South Florida Water Management District  
P.O. Box 24680  
3301 Gun Club Road  
West Palm Beach, FL 33416  
561-682-6129  
[dthayer@sfwmd.gov](mailto:dthayer@sfwmd.gov)

Ellen Donlan

Division of Vegetation Management  
South Florida Water Management District  
P.O. Box 24680  
3301 Gun Club Road  
West Palm Beach, FL 33416  
561-682-6129

**Lee County:**  
John Cassani  
Lee County Hyacinth Control District  
P.O. Box 60005  
Ft. Myers, FL 33906  
239-694-2174  
[cassani@lchcd.org](mailto:cassani@lchcd.org)

**Invited Guest**  
Patty Eklund\*  
Graduate Student  
University of Arkansas at Pine Bluff  
Department of Aquaculture and Fisheries  
1200 North University Drive  
Mail Slot 4912  
Pine Bluff, AR 71601  
870-575-8116  
[peklund@uaex.edu](mailto:peklund@uaex.edu)

\*Ms. Eklund's thesis concerns the economic value of triploid grass carp to control aquatic plants in Arkansas.

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