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Atlantic States Marine Fisheries Commission

Healthy, self-sustaining populations for all Atlantic coast fish species or successful restoration well in progress by the year 2015

Guidelines for Stocking Cultured Atlantic Sturgeon for Supplementation or Reintroduction

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SUMMARY OF RECOMMENDATIONS

I. PLANNING, EVALUATION AND TAGGING REQUIREMENTS

Recommendation 1: Planning, Monitoring and Reporting

Management jurisdictions involved in stocking programs for Atlantic sturgeon should provide a detailed proposal to the ASMFC Sturgeon Technical Committee for review and recommendation to the Board. Such plan will include goals and objectives, population surveys, broodstock sources and selection criteria, numbers, sizes and locations to be stocked, and timelines. The plan should also include annual monitoring of the status of their population, the effects of stocking, and possible interactions with shortnose sturgeon if they co-exist. Stocking and monitoring results should be reported to ASMFC each year by October 1 through annual state compliance reports.

Recommendation 2: Habitat Quality and Population Surveys

Prior to initiation of a large-scale sturgeon stocking program, areas targeted for stocking will be evaluated for presence-absence of remnant populations, determination of the relative quality and quantity of available habitat (e.g., water quality, substrate, flow characteristics, food availability) and possible human impacts on these environments (dams, dredging, water withdrawals, etc.). Small- scale releases of marked cultured sturgeons may be useful a component of these evaluations.

Recommendation 3: Tagging

All stocked cultured sturgeon including released broodfish should be marked. The Atlantic Sturgeon Technical Committee or a special subcommittee should examine the entire range of tagging options to include wild-caught and hatchery origin fish of all sizes and in all locations. A comprehensive tagging protocol should be developed for ASMFC states and become part of these stocking guidelines, when available.

II. BROODSTOCK SELECTION AND MINIMUM NUMBERS

Recommendation 4: Source of Broodstock

Wherever possible, broodstock should be taken from the same river in which stocking will occur. When such fish are not available, broodstock used to produce fish for stocking should be taken from a nearby source(s) which will allow maintenance of sturgeon abundance. Such sourcing of broodstock will consider the genetic as well as the logistical (fish availability) implications associated with the stocking plan.

Recommendation 5: Number of Spawners

The stocking plan will incorporate broodstock collection and progeny production components that meet genetic criteria for maximizing effective population size of broodstock while achieving an inbreeding rate of less than 1%, preferably 0.5%. Plans may use various genetic and temporal approaches including cautious application of unbalanced sex ratios, egg or sperm splitting, and mixing of various genetic stocks. In other than small-scale research stocking efforts, proponents may have to commit to long-term stocking efforts.

III. FATE OF POST-SPAWN BROODSTOCK AND PROGENY

Recommendation 6: Fate of Post-Spawn Broodstock

Broodstock should typically be spawned only once unless there is genetic justification to reuse them. After use, they should be marked and returned to their river of origin whenever feasible.

Recommendation 7: Fate of Progeny

If progeny produced are excess to ASMFC approved plan needs, they may be used for research purposes, educational exhibits, or provided to private aquaculture interests. Any excess progeny released into the wild for research or study purpose must be specifically approved in advance by ASMFC. If there is no need for these fish they should be properly euthanized.

GUIDELINES FOR STOCKING CULTURED ATLANTIC STURGEON FOR SUPPLEMENTATION OR REINTRODUCTION

Atlantic Sturgeon Culture and Stocking Committee
Atlantic States Marine Fisheries Commission
Washington, D.C.

INTRODUCTION

Atlantic sturgeon populations on the East Coast of the United States are severely depressed. Implementation of a harvesting ban in U. S. waters has been enacted but abundance is low in many populations and several are believed to be extirpated. A variety of reasons are suspected for the sturgeon population decline including loss of access to spawning grounds, loss and degradation of critical nursery and sub-adult habitats, and mortality related to incidental captures in other fisheries as well as previous directed fisheries.

In 1992, the Atlantic States Marine Fisheries Commission (ASMFC) Policy Board accepted recommendations from the Atlantic Sturgeon Aquaculture and Stocking Committee which included preparation of a discussion paper to address (1) possible inter-basin transfer of broodstock and/or hatchery produced progeny to supplement/restore depleted populations, and, (2) other interjurisdictional issues related to stocking of sturgeon (ASMFC, 1992). This document was revised and expanded and a breeding and stocking protocol for cultured Atlantic sturgeon was accepted by the species board (ASMFC, 1996).

Substantial new information has been obtained in the past decade including broodfish collection, rearing, and spawning techniques; production of juveniles and marking techniques; sturgeon species interactions; population differentiation; and the degree of natal stream fidelity. The purpose of this revision of the protocol is to provide guidance relative to the production of fish for release, to collect biological and behavioral data, and for use in restoration or enhancement efforts. These recommendations replace those of the 1996 protocol.

BACKGROUND

Following several years of development ASMFC adopted a fishery management plan (FMP) for Atlantic sturgeon (ASMFC, 1990) which had a goal of rebuilding sturgeon populations to levels that would support fisheries capable of producing sustained harvest of 700,000 pounds, about 10% of reported landings from the 1890s. A major feature of that plan was establishment of a 7-foot minimum size limit to allow most females an opportunity to spawn at least once prior to harvest. Some states petitioned for, and received, approval of a smaller fish size limit under the "conservation equivalency" provision of the plan. By the late 1990s, it was apparent to sturgeon managers that the FMP restrictions were insufficient to protect the stocks and Amendment 1 was adopted in 1998. That amendment closed all sturgeon fisheries on the Atlantic Coast of the U.S. (inland and territorial sea) until 20 year-classes of females are established in each spawning stock (ASMFC 1998). One year later, NMFS placed a moratorium on all sturgeon harvest and possession in the Exclusive Economic Zone (EEZ). At an average maturity of 15-20 years for females, this moratorium is expected to remain in place for up to 40 years in some locations. Another important provision of the Amendment is a recommendation to assess, and where possible, minimize or eliminate bycatch mortality of sturgeon in other fisheries.

In addition to strict harvest controls, the original FMP and the Amendment included numerous recommendations focused on rebuilding sturgeon stocks through identification and protection of essential habitats, basic life history research, population identification, and defining the role of stocked cultured fish in restoration. Three recommendations specifically addressed the latter need:

- The ASMFC should encourage an expanded culture effort to develop techniques to rear Atlantic sturgeon and evaluate fish for stock restoration.

- The ASMFC should encourage culture research to identify and control early life stage diseases, synchronize spawning times of males and females, and reduce handling stress problems.

- The ASMFC should establish a culture and stocking committee to provide guidelines for culture and restoration stocking of sturgeon.

With this identified need to evaluate the role of artificial propagation in Atlantic sturgeon recovery ASMFC established an Atlantic Sturgeon Culture and Stocking Committee. That committee, comprised of state and federal biologists, defined six broad problem areas and developed numerous recommendations to address them (ASMFC, 1992). Many of the culture recommendations in the report encouraged agencies to develop techniques for broodfish collection and holding, induced spawning and sperm preservation, incubation, hatching, and rearing of Atlantic sturgeon. However, recommendations related to stocking of the cultured progeny were necessarily cautious and included:

- Determine the extent to which Atlantic sturgeon are genetically differentiable among rivers.

- If management units are defined by river then genetic integrity of stocks within river basins should be maintained by stocking only progeny of native broodstock.

- If native broodstock no longer exist, or are in such low abundance as to preclude effective collection, priority should be given to stocking fish from adjacent, hydrologically similar river systems.

- Broodstock should be collected at times and in numbers that do not unduly stress the native population yet adequately represent the inherent variation of that stock.

- An adequate effective breeding population size should be maintained to the extent possible in culturing Atlantic sturgeon for restoration purposes so that genetic integrity of the local recipient stock is maintained.

REASONS FOR CULTURE AND STOCKING

There is substantial uncertainty when considering restoration efforts. Hard data on population abundance is lacking for most systems but consensus is that natural stock rebuilding has not occurred and most populations are at depressed levels. Further, there is concern that additional decreases in remnant populations are possible if no actions are taken. Fundamental to the restoration issue is the reason(s) for the declining populations and whether stocking fish can alleviate the situation and result in self-sustaining populations. In spite of this issue being on the forefront of fishery managers and researchers priority needs lists, in general little new information has been provided in the past decade other than new genetic findings and the identification of possible impacts of incidental captures and mortalities on population recovery.

Unlike continuous monitoring programs that are necessary and important but often not funded, stocking fish is a tangible and highly visible activity that often receives public support. Thus, the issue will be to use this tool in a responsible fashion to collect new information on populations as well as to validate the tool's usefulness in restoration programs (Blankenship and Leber 1995).

Atlantic sturgeon stocks appear to be at extremely low levels throughout most of their range, and the species is highly susceptible to fishing and human-induced habitat perturbations (Smith and Clugston 1994; Boreman 1997; and Gross *et al*. 2002). These low levels prompted a petition in 1997 to list the species as threatened or endangered under the Endangered Species Act (ESA). In response to the petition, the National Marine Fisheries Service (NMFS) and the U.S. Fish and Wildlife Service (USFWS) conducted a status review of Atlantic sturgeon. In 1998 they concluded that the species was not threatened or endangered, but NMFS added it to its candidate species list in order to provide impetus for future reevaluation of the species' status (NMFS and USFWS 1998).

Despite the ASMFC and NOAA moratoria on harvest and possession, recent information indicates that some populations continue to decline (Kahnle *et al*. 2004a). Inconsistency in reporting among the states makes it difficult to accurately estimate bycatch mortality in river systems, but bycatch in gill nets is a substantial cause of mortality for both Atlantic and shortnose sturgeon (Collins *et al*. 1996; Kynard 1997). Marine bycatch mortality is likely to be relatively high, especially in sink gill nets and drift gill nets in goosefish, Atlantic cod, summer flounder and scup fisheries along the northeast coast of the United States (Stein *et al*. 2004). Even without bycatch mortality, the life history of this species (long lived, late maturing, intermittent spawning) suggests that it will take at least several decades to restore Atlantic sturgeon to levels that could sustain fisheries.

During the past century, most sturgeon populations have suffered substantial declines in abundance with some populations having become extirpated. Worldwide, there has been unity in the interest to preserve and restore sturgeon populations as reflected in discussions at the five International Sturgeon Symposiums and by the recent establishment of the World Sturgeon Conservation Society. For many species, stocking appears to be an important approach to maintaining and rebuilding populations especially when combined with harvest restrictions. In addition, aquaculture of sturgeons has been viewed as a means of meeting market demand for sturgeon products and for reducing take of wild fishes. As such, aquaculture operations are underway in many areas of the world including Asia, Europe and North and South America.

Stocking of Atlantic sturgeon has the potential to meet basic needs related to conservation of the species. A primary purpose may be to provide a means of reintroducing this species to waters that historically supported Atlantic sturgeon, but where the natural stock is extirpated. Stocking can also provide fish for supplementation when natural stocks are in extremely low abundance, and can provide the opportunity to control parentage, mark progeny, and know how many fish entered the system being restored (Waldman and Wirgin 1998). Culture programs may also serve to maintain refugia populations for nearly extinct populations on a temporary basis until threats to the habitat are alleviated, until necessary habitat modifications are completed, or when potentially catastrophic events occur (USFWS and NMFS 2000). Small-scale stocking can be used for various research studies (*e.g.*, environmental tolerance, toxicity evaluation, behavior, tag retention, *etc*.) to improve management of the species by elucidating life history and ecological characteristics. Stocking may also be a useful tool for obtaining information on possible bottlenecks and the potential for a population to recover.

Grogan and Boreman (1998) suggest a method to assess population extinction based on statistical analysis of incidental captures over time. Also, NMFS approved a field survey protocol to determine whether sturgeon are extirpated from a particular river before embarking on a reintroduction program (Moser *et al*. 2000). Information obtained from these methodologies can help managers assess the likelihood that a population has been extirpated, which may be an important consideration for a stocking program.

Federal Management Considerations

The federal policy regarding controlled propagation of ESA listed species is aimed specifically at coordinating such culture and stocking activities of the U. S. Fish and Wildlife Service and National Marine Fisheries Service. It recommends that the potential benefits and risks of stocking should be assessed and alternatives requiring less intervention objectively evaluated (USFWS and NMFS 2000). It further recommends for listed species that artificial propagation be used for population enhancement or restoration purposes "only when other measures used to maintain or improve the species' status in the wild have failed, are determined to be likely to fail, are shown to be ineffective in overcoming extant factors limiting recovery, or would be insufficient to achieve full recovery". Also, "all reasonable effort should be made to accomplish conservation measures that enable the species to recover in the wild, with or without intervention, before implementing stocking for reintroduction or supplementation" (USFWS and NMFS 2000). Sturgeon stocking project supporters should provide for the disposition of cultured fish that are surplus to program needs or unfit for introduction into the wild.

Regardless of language in this policy it is recognized that, for species listed under ESA, an approved recovery plan will identify the details of any culture-based population enhancement effort. As of this writing the Atlantic sturgeon is not listed as threatened or endangered. However, NMFS and USFWS have begun another status review and the result of that review could lead to listing.

HISTORY OF ATLANTIC STURGEON CULTURE

Near the end of the 19th century, fishery managers realized that most sturgeon fisheries had experienced substantial declines and agreement was reached to rehabilitate the various stocks through artificial propagation programs (Ryder 1890; Cobb 1900; Stone 1900). The first successful spawning of a North American sturgeon was an Atlantic sturgeon. In 1875, working with commercial fishermen on the Hudson River, Seth Green and Aaron Marks of the New York State Fish Commission produced about 100,000 fry but reported great difficulty in obtaining simultaneously ripe fish of both sexes (Green 1879). John Ryder (1890) studied the sturgeon industry on the east coast of the U.S. and in 1888 initiated culture experiments on the Delaware River at the suggestion of the U.S. Commissioner of Fish and Fisheries. Ryder described in great detail the process of obtaining gametes and fertilizing and incubating Atlantic sturgeon eggs. Eggs were incubated in floating wooden boxes near the eastern end of the Chesapeake and Delaware Canal and success was limited by severe fungal infestation. Bashford Dean, an instructor in biology at Columbia University, incubated Delaware River sturgeon eggs in a floating cage containing parallel screen-covered trays and placed the cages at different locations across the river channel. He found that fungus problems reported by Ryder could be overcome by incubating eggs in strong currents and in waters with higher salinity and less silt (Dean 1894). No account of the number of fry hatched or their fate was given but collection of running ripe females was problematic.

Nearly 100 years later, the South Carolina Department of Marine Resources performed hormone-induced spawning and culture with Atlantic sturgeon captured in the Atlantic Ocean off the north Georgetown jetty (Smith *et al.* 1980). Broodstock were transported to the USFWS Orangeburg National Fish Hatchery and injected with sturgeon pituitary extract to induce gonad maturity and enable collection of viable gametes. Attempts to manually strip eggs were not successful, but 20,000 to 30,000 eggs were obtained through an abdominal incision. Diatomaceous earth was highly efficient in preventing egg clumping and eggs were incubated in McDonald hatching jars. Despite these improvements over early culture attempts, fungus infections occurred within three days and thereafter formalin treatments were administered. Hatching was completed by 140 hours and resulted in the production of about 100 fry, some of which survived for 130 days. The next year approximately 11,000 fry were produced (Smith *et al.* 1981) with most being placed into an earthen pond for culture. High pH levels related to a phytoplankton bloom led to total mortality. Fish that were not stocked in the pond survived for 204 days and reached lengths of about 18 centimeters. The importance of this work in SC was the demonstration that ripe females could be induced to ovulate using hormones and that small juveniles could be produced.

In 1991, the U.S. Fish and Wildlife Service Northeast Fishery Center at Lamar, PA (NEFC) embarked upon a program to further develop the culture technology for Atlantic sturgeon in response to recommendations by ASMFC. Initial efforts were focused on the capture and transport of potential broodfish from the Hudson River commercial fishery. The first successful spawn was achieved in 1993 following techniques used for white sturgeon (Conte *et al.* 1988). These fish had been transported six hours by truck to NEFC's facility where they received injections of luteinizing hormone releasing hormone analog (LHRHα). Experiments were performed on the effects of incubation temperature and egg disinfection techniques and about

13,000 fry were hatched using McDonald-style hatching jars. Later, research was expanded to identify suitable diets for first-feeding fry and fingerlings (Mohler *et al.* 1996). Subsequent attempts in 1994-1996 were also successful with as many as 160,000 fry being produced in one year. Over the years NEFC provided over 29,000 propagated juveniles to 18 different organizations including federal and state agencies, universities, public aquaria, and independent researchers. During 12 years of work, NEFC researchers demonstrated the feasibility of capturing, holding, and spawning wild Atlantic sturgeon and identified predictable techniques for the production of juveniles. This work culminated in the preparation and publication of a culture manual for the production of Atlantic sturgeon (Mohler 2004).

Of particular significance during this period was the use of the propagated juveniles to provide field data useful for the management of this species. Due to NEFC's propagation success, over 8,000 juveniles were made available for use in research stocking efforts focused on collecting information on movements, growth, and survival. The first release occurred in the Hudson River (NY) in October 1994 when 4,900 three-month old fish were marked and released while the second stocking occurred in the Nanticoke River (MD) in July, 1996 when 3,300 yearlings were tagged and released (St. Pierre 1999). Substantial information was collected from these two efforts including the demonstration that stocked cultured fish exhibited high survival rates (based upon high recapture rates) (Secor *et al*. 2000b; Welsh *et al*. 2002) and that marked cultured fish could be used for population assessment (Peterson *et al*. 2000).

Based in part on these successes, there has been recent interest in development of private Atlantic sturgeon aquaculture and in conducting additional restoration efforts. Examples include: (1) the University of New Brunswick and Canadian Caviar Company both successfully induced spawning of Saint John River broodstock using LHRHα; (2) experimental and commercial rearing attempts are underway in Florida at several sites (and planned in North Carolina) using Canadian source Atlantic sturgeon fry; (3) several hundred 8-11 year old subadult sturgeon reared at NEFC from Hudson River parents were specially-marked and stocked back into the Hudson River to investigate in-river movements, timing of exodus to the ocean, at-sea migrations, and perhaps future homing tendencies for mature fish (Kahnle *et al*. 2004b); and, (4) Maryland DNR and Mirant Corporation are attempting broodstock development using Hudson River origin subadults from earlier NEFC culture efforts, and wild fish from Chesapeake and Delaware bays. Success in this latter effort could result in future production of juveniles for possible experimental stocking in Maryland waters of Chesapeake Bay.

STURGEON SPAWNING CHARACTERISTICS AND JUVENILE PRODUCTION

Maturation and Spawning

Although there is delayed maturity, Atlantic sturgeon grow to advanced ages (up to 60 years - Magnin 1964) and thus can spawn on multiple occasions over a protracted period. Populations show clinal variation in maturation age with estimated age at maturity of females being 7-19 years in SC (Smith *et al*. 1984), 15-30 in the Hudson River (Van Eenennaam *et al.* 1996) and 27-28 years in the St. Lawrence River (Scott and Crossman 1973). Males typically mature at a smaller size and at an age several years younger than females. In the Hudson River during 1992- 1995, Stevenson and Secor (1999) reported that average age of females was 19 (maximum age 42) years while that of males was 15 years (maximum 36 years). Van Eenennaam *et al.* (1996) concluded that males may spawn annually once mature, but females spawn at lower frequency. In SC, spawning occurs at irregular intervals and was estimated at 3-5 years for females and 1-5 years for males (Smith 1985). Spawning behavior is suspected to mimic other anadromous species (e.g. striped bass, shortnose sturgeon) with several males participating with a single female.

Availability of Broodstock

Based on recent research and commercial fisheries data, the largest U. S. breeding population appears to occur in the Hudson River. However, Atlantic sturgeon are currently present in 32 rivers with spawning believed to be occurring in at least 14 of these rivers based on presence of sexually mature adults and/or juveniles ≤ age 1 (NMFS and USFWS 1998). Of these, only four spawning rivers occur north of North Carolina and recent genetic information suggests that many of the fish in upper Delaware Bay may be migrants from a mixture of other systems, particularly the Hudson and southern rivers.

Acquisition of Juveniles

There are four basic approaches for obtaining juveniles for use in stocking efforts. They are: (1) spawn recently captured ripe adults collected on or near spawning grounds; (2) collect non-spawning adults and condition them in captivity for spawning; (3) collect juveniles and rear to adult size in captivity for spawning; and, (4) purchase juveniles from a commercial producer. The various approaches are discussed below.

1. *Spawn recently captured ripe adults collected on or near spawning grounds*

This approach was utilized during initial spawning trials around the turn of the century and more recently in SC and PA. Although appearing to be the most efficient approach, many logistical considerations have shown this to be a difficult endeavor. Over the range of the species, riverine spawning areas are generally unknown or only poorly defined (except the Hudson River) yet these are the best areas to collect ripe broodstock. Further, with several exceptions, population abundance appears to be depressed and thus opportunities to capture simultaneous ripe males and females are limited. Experience in NY and SC indicates that the use of former commercial sturgeon fishermen can be highly beneficial in efforts to collect ripe adults. However, even using this approach does not assure large numbers of fish in spawning condition. For example, in SC, a project focused on capture of adults in the Edisto and Combahee rivers during 1998-1999 using three ex-commercial fishermen (Collins *et al*. 2000). A total of 39 adults were captured, 28 for which sex was determined: Twenty-one were males (139-195 cm TL) and 7 were females (180-234 cm TL), including ripe and spent individuals. Sturgeon fishermen were also used by the USFWS during 1997-1998 to collect adults from the Hudson River for hatchery use. These efforts yielded only three ripe females of 143 adults captured and only one female was successfully spawned (J. Mohler, pers. comm.). This approach requires substantial field collection efforts during the natural spawning season but avoids the costs associated with facilities for long term holding of the large broodstock.

2. *Collect non-spawning adults and condition them in captivity for spawning*

This approach was demonstrated in PA by USFWS using Hudson River broodfish and requires collection of adults and holding them in culture facilities. Timing of capture is less critical and fish can be accumulated over months or years. Large tanks are required and basic water quality control is necessary. Use of photo-thermal conditioning can be useful for controlling maturation and improving predictability of spawning success. This approach requires substantial costs and multi-year operations. Use of hormonal therapy to induce oocyte maturation and ovulation and sperm cryopreservation techniques will substantially improve the practicality of this approach.

3. *Collect juveniles and rear to adult size in captivity for spawning*

This approach is no doubt the most time consuming and costly method as a long time commitment is required. However, it does offer more flexibility in collection of animals, as subadults can be collected nearly all year. Further, substantial numbers of animals can be collected and genetically screened so that only the most desirable fish will be reared to maturity. Although no one to date has successfully employed this approach to produce broodstock (MD DNR is now working to that end), USFWS showed that 8-year-old cultured fish were pre-vitelogenic (Mohler 2004). Use of cryopreservation techniques to store sperm may be useful to reduce the need to hold large number of males.

4. *Purchase juveniles from a commercial producer*

Currently there are no commercial producers of Atlantic sturgeon juveniles in the U.S. although commercial grow-out trials have been permitted in FL. However, there is at least one Canadian company that does produce juvenile Atlantic sturgeon when there is demand. This option may be viable if the source broodstock meet the genetic criteria needed for the planned stocking efforts.

Summary

Problematic considerations will influence the ability to collect broodstock and produce the most desirable animals for use in restoration efforts. Many of the considerations were previously identified (ASMFC 1996) and the situation may still be challenging, as broad-based natural population rebuilding has not occurred in most systems. However, evidence of numbers of young juveniles and adults in systems in SC and GA and perhaps elsewhere indicate that adults and juveniles can be collected (ASMFC 2004).

Considerations impacting collection of adults and production of juveniles include:

- Low numbers of adults available, especially in systems where restoration is being considered
- Spawning areas are poorly defined in most systems
- Adult sized fish captured in a system are not necessarily native to that system
- Most adult fish collected cannot be immediately spawned
- Ripe fish of both sexes are often not available at the same time
- Spawning and culture techniques need refinements to be fully predictable
- Special (and expensive) hauling equipment is needed
- Development of captive broodstock populations may require 10 or more years
- Maintaining large numbers of broodfish requires large and costly holding facilities
- Availability of ex-sturgeon fishermen to assist in collections is decreasing with time

POPULATION STRUCTURE OF ATLANTIC STURGEON

For the purpose of this guideline, an Atlantic sturgeon "population" is defined to be a genetically distinct natural breeding stock. Like other true anadromous fishes, there are many separate populations throughout the range of the species. Mixed aggregations of sturgeon occur at sea and in large estuaries sometimes confounding genetic interpretations. Ideally, genetic conclusions should be based on samples from mature adults collected from spawning grounds or from eggs, larvae or small juveniles (less than one year old) from the presumptive natal river. For Atlantic sturgeon such animals are rarely available in large numbers and thus geneticists have had to rely on available specimens to perform their analyses. In spite of this limitation, substantial information has been obtained on various populations of Atlantic sturgeon.

Based on mitochondrial DNA (mtDNA) haplotype frequencies revealed by restriction endonuclease analysis, Waldman *et al.* (1996) demonstrated geographic heterogeneity ($P < 0.05$) among collections of Atlantic sturgeon specimens from the St. John River (New Brunswick), Hudson River, and several rivers in Georgia. Wirgin *et al.* (2000) surveyed mtDNA control region variation using sequencing among 322 Atlantic sturgeon from 11 rivers between the St. Lawrence and Satilla (Georgia). Numbers of individual haplotypes in each system showed a pronounced latitudinal cline that ranged between monomorphism for collections from two Canadian rivers to as many as 17 haplotypes observed in the Savannah River collection. Of the 39 haplotypes revealed, 64% were unique to particular rivers, indicating strong population subdivision.

Waldman *et al.* (2002) expanded the numbers of specimens sampled to 477 and rivers to 13 from Wirgin *et al.* (2000). One haplotype was numerically dominant (43.8% of all specimens) and was seen in every population except that from the Satilla. But among the 40 haplotypes revealed, only 10% were unique to the previously glaciated systems (Hudson River and northward) whereas 87.5% were unique to the nonglaciated region (James River and southward), which indicated recent (post-Pleistocene) recolonization of those northern rivers. Significant differences ($P < 0.05$) were observed between all nearest-neighbor locations except from two South Carolina rivers, the Edisto and Combahee. When collections from these proximal rivers were treated as a single stock, they were significantly different from all others, with the exception of the nearby Ogeechee.

Population-level variation in Atlantic sturgeon also was examined by King *et al.* (2001) who characterized microsatellite nuclear DNA (nDNA) among 202 individuals from five rivers where they presumably originated. A variety of statistical analyses indicated that all five populations were strongly discrete. These diploid data were examined in tandem with the haploid data from Waldman *et al*. (2002) in the study by Wirgin *et al*. (2002). Both approaches showed moderate to high levels of genetic diversity and a high degree of population structure, although no latitudinal cline in genetic diversity was evident for nDNA.

These DNA studies also analyzed some possible mixed aggregations of Atlantic sturgeon. Waldman *et al*. (1996) used mtDNA haplotype frequencies of 112 Atlantic sturgeon from the New York Bight in a mixed-stock analysis and found that the Hudson River contributed approximately 99% of this aggregation. They also surveyed subadults from the Delaware River estuary and concluded that they either were a mixture of both the Hudson River stock and southeastern populations or of the Hudson stock and a relict Delaware River stock. Subadults (N = 103) also were sampled from the nearby Chesapeake Bay and their nDNA, analyzed by King *et al.* (2001), suggested they were made up of individuals from both the Chesapeake Bay and other geographic locations. Later evidence for an extant localized Chesapeake Bay population was found by Waldman *et al.* (2002) in Virginia's James River.

In summary, several mtDNA and nDNA studies, taken together, indicate strong geographic population structuring for Atlantic sturgeon---at the level of individual rivers, with the possible exception of some systems in South Carolina and Georgia. Table 1 below shows the current status of sturgeon populations along the Eastern seaboard.

Table 1. Contemporary Status of Atlantic Sturgeon Populations in Eastern U.S. Rivers (after Waldman & Wirgin 1998, with new information added). YOY = young-of-the-year.

River	Status	Source
Kennebec/ Androscoggin	Ripe adults and subadults surveyed during 1990s	T. Squiers
Penobscot	Extremely scarce	T. Squiers
Merrimack	No recent evidence of spawning; seasonally inhabited by adults	Kieffer & Kynard 1993
Connecticut	No recent evidence of spawning; only subadults seen in recent years	T. Savoy
Hudson	Recent recruitment documented	J. Mohler
Delaware	Lower river used seasonally by subadults of uncertain stock origin, young-of-year encountered rarely.	C. Shirey
	Unpublished mitochondrial DNA suggests a relict stock	I. Wirgin
Maryland waters of Chesapeake Bay	No evidence of spawning over past 25 years	D. Secor
	Reward program resulted in no capture of wild yearlings, but > 300 hatchery yearlings	Secor *et al*. 2000b Welsh *et al*. 2002
Virginia waters of Chesapeake Bay	Considered rare until 1997 when targeted programs found numerous subadults and possible YOY in the James River	J. A. Musick, A. Spells
Roanoke	No information available; subadults of unknown stock origin common in Albemarle Sound	W. Laney
Pamlico	Listed as Atlantic sturgeon river	Van Den Avyle 1984
Neuse	Listed as Atlantic sturgeon river	Van Den Avyle 1984
Cape Fear	Moderately abundant subadults; some YOY caught in 1997	Moser & Ross 1995
Winyah Bay Drainage	Small- to moderate-sized population	Collins *et al*. 1996
Santee	Some adults seen in lower river	M. Collins
Ashepoo/Cooper/ Edisto	Reproduction occurring; numerous YOY	M. Collins

Savannah	Small to moderate-sized population	Collins *et al*. 1996
Ogeechee	Surveys in 1990s indicated small population	Rogers *et al*. 1994
Altamaha	Moderate-sized population	Rogers *et al*. 1994
Satilla	Little is known; rare—1995 survey captured two individuals	J. L. Music *et al*. 1995
St. Marys	Commercially fished until mid 1980s; now rare to absent; 1995-1996 surveys caught none	J. L. Music
St. Johns	Historically and presently very rare; occurrences may be winter migrants from populations to north	Gilbert 1992

<u>Sources</u>

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D. Secor - Chesapeake Biological Laboratory
J. A. Musick – Virginia Institute of Marine Sciences
J. L. Music – Georgia Department of Natural Resources
A. Spells – U.S. Fish and Wildlife Service
W. Laney – U.S. Fish and Wildlife Service
M. Moser – National Marine Fisheries Service
M. Collins – South Carolina Marine Resources

GENETIC CONSIDERATIONS

Captive broodstock in fisheries management can be used to achieve one or a combination of the following goals: introduction of a non-native species into a new habitat, supplementation of weak existing populations, restoration of locally extinct populations, or conservation of a threatened or endangered population in a hatchery environment. When one of the results of a culture and stocking program is the establishment of naturally reproducing or self-sustaining populations, consideration of the genetic diversity of the population being stocked, as well as the potential genetic interactions with any existing population that could breed with the stocked individuals, should also be included in broodstock management. General genetic concepts important to broodstock management include minimizing artificial selection, maximizing the number of broodstock spawned, maintaining natural spawning sex ratios, and reducing the potential for inbreeding and negative genetic impacts of the stocked fish onto existing populations.

The establishment of captive broodstocks for the restoration of Atlantic sturgeon is a potential management strategy for population reintroduction and supplementation. However, due to certain life history characteristics of Atlantic sturgeon (e.g., longevity, age and size at maturity, intermittent spawning), it is increasingly important to incorporate genetic concepts into broodstock management. Extreme care must be taken in culture and stocking programs to insure against excessive inbreeding, loss of genetic diversity, or diminished survival, yield, or reproduction of remnant wild stocks. Kapuscinski and Jacobson (1987) point out that the fitness of fish used as broodstock or for production of progeny for stocking is extremely important because the fitness of future generations depends upon genetic characteristics of the present generation.

The knowledge of genetic characteristics of existing populations is also important for supplementation programs. Genetic characterization of Atlantic sturgeon sampled from rivers along the Atlantic coast of North America using mitochondrial DNA and nuclear DNA analyses have identified populations defined by drainage (see previous section). Due to the significant differences between Atlantic sturgeon populations, Waldman and Wirgin (1998) recommended against the transfer of sturgeon (via stocking) between basins to prevent the loss of genetic differences between populations and any subsequent loss of drainage-specific genetic adaptations, and, that genetic guidance be incorporated into restoration stocking efforts. Therefore, the goal of this section is to provide the theoretical background to the genetic concepts important to broodstock management, and to discuss how these concepts apply specifically to Atlantic sturgeon.

Effective Population Size and Sex Ratios

Effective population size (N_e) is the number of individuals in a population that successfully reproduce and contribute offspring to the next generation. Estimates of effective population size are often less than the census population size because not all individuals in a population are sexually mature or successfully reproduce in a given breeding season. Estimates of effective population size can be calculated based on the number of females (N_f) and males (N_m) in a population (Wright 1931) such that:

$$N_e = \frac{4N_f N_m}{N_f + N_m}$$

Table 2 describes the effective population number based on small numbers of males and females spawned (Kincaid 1993). The effective population size derived from a captive breeding program is maximized when equal numbers of males and females are used. When the sex ratio is skewed to one sex or the other, estimates of N_e are reduced compared to equal sex ratios.

Table 2. Effective population number based on the actual number of males and females used to produce the progeny generation for one year-class. Identify the number of females in columns and the number of males in rows; the calculated effective breeding number for this combination can be read at the column and row intersection.

Number Male Parents	Number of Female Parents											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	2.0	2.7	3.0	3.2	3.3	3.4	3.5	3.6	3.6	3.6	3.7	3.7
2	2.7	4.0	4.8	5.3	5.7	6.0	6.2	6.4	6.5	6.7	6.8	6.9
3	3.0	4.8	6.0	6.9	7.5	8.0	8.4	8.7	9.0	9.2	9.4	9.6
4	3.2	5.3	6.9	8.0	8.9	9.6	10.2	10.7	11.1	11.4	11.7	12.0
5	3.3	5.7	7.5	8.9	10.0	10.9	11.7	12.3	12.9	13.3	13.8	14.1
6	3.4	6.0	8.0	9.6	10.9	12.0	12.9	13.7	14.4	15.0	15.5	16.0
7	3.5	6.2	8.4	10.2	11.7	12.9	14.0	14.9	15.7	16.5	17.1	17.7
8	3.6	6.4	8.7	10.7	12.3	13.7	14.9	16.0	16.9	17.8	18.5	19.1
9	3.6	6.5	9.0	11.1	12.9	14.4	15.7	16.9	18.0	19.0	19.8	20.6
10	3.6	6.7	9.2	11.4	13.3	15.0	16.5	17.8	19.0	20.0	21.0	21.8
11	3.7	6.8	9.4	11.7	13.8	15.5	17.1	18.5	19.8	20.6	22.0	23.0
12	3.7	6.9	9.5	12.0	14.1	16.0	17.7	19.1	20.6	21.8	23.0	24.0

Besides skewed sex ratios, other mechanisms can also bias estimates of N_e, such as variance in reproductive success and mating strategy. When mating is limited to one male and one female per spawning pair (and males are not re-used), then estimates of N_e based on the number of males and females spawned is appropriate. However, hatchery spawning strategies have historically pooled gametes from multiple individuals during spawning (*e.g.* an "insurance" male

is used when applying milt to eggs to ensure that viable sperm is used to fertilize eggs). When gametes from more than one individual are pooled (such as pooling milt from males), or when males are used to fertilize eggs from multiple females, variance in reproductive success can bias estimates of effective population size. Pooling of gametes increases the potential for reproductive variance primarily due to unequal fertilization among males (via sperm competition), and therefore can decrease the effective population size (Withler 1988). The sex ratio-based equation is solely calculated using the number of males and females in a population and does not account for variation in reproductive success.

Lande and Barrowclough (1987) developed equations to incorporate both male and female reproductive variance into estimates of effective population size. Estimates of N_e that incorporate reproductive variance can be much lower than estimates of sex-ratio based N_e, but more accurately reflect the number of individuals contributing to the next generation. To obtain estimates of variance N_e when gametes are being pooled, parentage analysis of the offspring can be assessed using molecular genetic techniques to determine the mean and variance of individual parental contribution.

Application to Atlantic sturgeon broodstock management

Application of the genetic concept of effective population size is of particular importance to Atlantic sturgeon captive broodstock management. The number of Atlantic sturgeon able to be captured and incorporated into a captive broodstock is often limited, either because very few individuals are collected, or because not all individuals mature at the same time. Space in a hatchery facility may be limiting due to the size of mature adults, and therefore adequate numbers of broodstock may not be able to be maintained. Additionally, Atlantic sturgeon adults are highly fecund. In practice, the high fecundity reduces the need to maintain large numbers of adults in captivity to achieve production/stocking goals, if the goal of the hatchery program is strictly production. However, most Atlantic sturgeon captive broodstock programs would serve as reintroduction or supplementation programs.

Maintaining relatively few adults for spawning reduces the effective population size, resulting in a reduction in the amount of genetic diversity passed on to subsequent generations. One option to minimize the number of adult sturgeon maintained in a hatchery is to use cryopreservation techniques to preserve sperm, so that hatchery space can mainly be focused on rearing females. If numbers of ripe females are limited (1-3), then egg lots could be split and fertilized with the milt of multiple males. However, these males should not be used to fertilize the eggs of other females, nor should they be used in consecutive years. The concern is the increased relatedness among the offspring through the creation of both full and half siblings.

Recommendations:

- Use as many adults for reproduction as possible.
- Minimize re-use of spawning adults during a season and, if possible, avoid splitting egg lots and fertilizing with multiple males or pooling gametes.
- Spawn different adults each year.
- Incorporate cryopreservation techniques to ensure and maximize the number of males that are available when females are ready to spawn. To ensure viability, cryopreserved sperm should be used the same year it is collected.

Stocking proportions

Another component to broodstock management and stocking programs is the N_e of the population being stocked. The primary goal of a stocking program when the stocked population is small is to increase the census population size. From a conservation genetic perspective, the goal would be an increase in the N_e of the overall population. However, changes to the overall N_e depend on the interaction between the effective population sizes of the captive population (N_c) and the wild population (N_w), and the proportional contribution of the captive population (x) to the overall population. Ryman and Laikre (1991) described the relationship of the stocking rate and the various effective population sizes as:

$$\frac{1}{N_e} = \frac{x^2}{N_c} + \frac{(1-x)^2}{N_w}$$

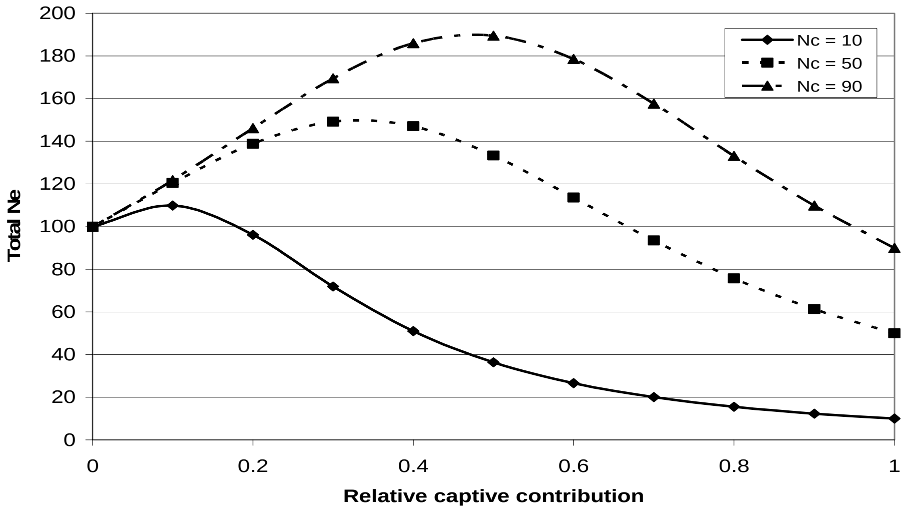
Figure 1 depicts the N_e of the total (combined) population N_e given varying levels of relative captive contribution, two different effective sizes of a wild population, and various effective sizes of a captive population. Relative contribution of the captive population ranges from 0 (not stocked, no captive contribution) to 1 (all individuals in the population are from the captive population). Conclusions from Figure 1 related to broodstock management include:

- When N_c is much smaller than N_w, the total N_e decreases from the initial value (N_w), even with a very small contribution of the captive population.
- As the contribution of the captive population increases, the total N_e decreases until it reaches the N_c when the contribution of the captive population is 1.
- The more similar N_c is to N_w, the greater the increase is to the total N_e.
- The relative contribution of the captive population can greatly impact the total N_e regardless of the N_w or N_c.

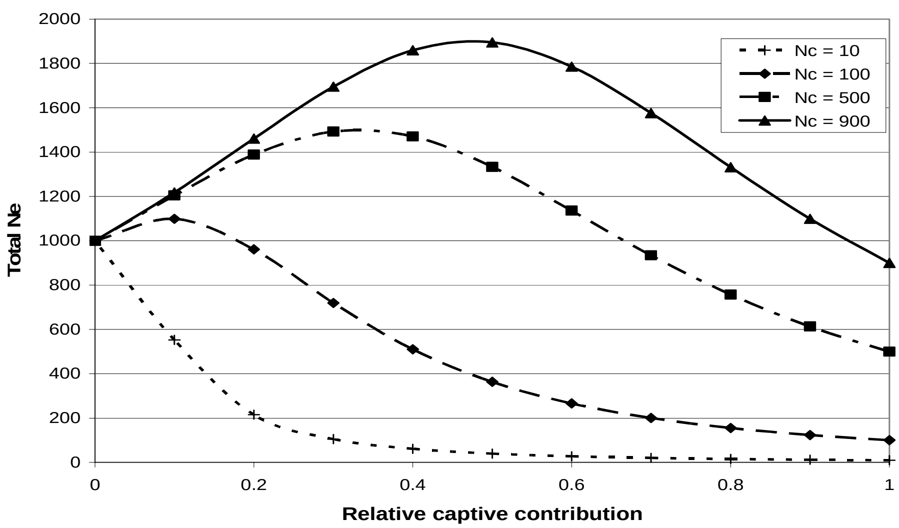
Consideration of the captive and wild effective population sizes, along with the relative contribution of the captive population can be useful in guiding broodstock and stocking programs. In situations when the effective population sizes are not known, applying these concepts to an estimated census population of spawning adults can be used for management.

Figure 1. Two examples describing changes in total effective population size given varying amounts of stocking a captive population into a wild population. Examples are provided when the N_e of the wild populations equal 100 (a) and 1000 (b). Based on the calculations of Ryman and Laikre (1991).

a) Wild N_e (the N_e of the population being stocked) = 100



b) Wild N_e (the N_e of the population being stocked) = 1000



Application to Atlantic sturgeon broodstock management

Small hatchery effective population size results in limiting the amount of genetic diversity maintained in a population over time. Spawning few adults also increases the relatedness of offspring. Consequently, the potential for inbreeding among the stocked offspring increases. The potential for inbreeding is also a function of the relative abundance of hatchery individuals in the wild population. Understanding the proportional contribution of the wild population that is being stocked is critical, especially when the effective population size of most Atlantic sturgeon captive broodstocks will be small. Otherwise, if the proportional contribution of hatchery sturgeon to a wild population is large, the total effective population size will be reduced with a small hatchery effective population size.

Recommendations:

- Maximize the effective population size of the hatchery broodstock.
- Obtain spawning population estimates for the wild population being stocked.
- Minimize the relative stocking rate when large differences exist between the hatchery and wild effective population sizes.

Inbreeding

Inbreeding is one of the most important genetic concerns in hatchery management, resulting in the loss of genetic diversity (Ryman 1991; Busak and Currens 1995). Inbreeding depression occurs when closely related individuals breed, and can be correlated to decreased fitness in future generations (Lynch and O'Hely 2001). The rate of inbreeding in a population depends on the effective size of the population such that:

$$\Delta F = \frac{1}{2N_e}$$

where ΔF is the rate of inbreeding per generation and N_e is the effective population size. In a captive broodstock, the principal factors affecting inbreeding are the number of broodfish spawned, sex ratio, mating strategy, reproductive variance, and in the case of long-term captive broodstock with little to no addition of individuals, the size of the founding population. When the sex ratio is unequal, the rate of inbreeding can be calculated:

$$\Delta F = \frac{1}{2N_e} = \frac{1}{8N_m} + \frac{1}{8N_f}$$

Some sturgeon populations may show a preponderance of males on the spawning grounds (Doroshov and Van Eenennaam 1994). In the natural environment, sturgeon mating strategies may incorporate gametes from multiple males to fertilize the eggs from a single female. In a closed (or relatively closed) captive broodstock situation the re-use of males either in the same spawning year or in subsequent spawning years could result in an increase in the relatedness among the offspring through the creation of full or half-siblings, thereby increasing the inbreeding potential among future generations (Chesser 1991; Sugg and Chesser 1994). As described previously for variance effective population size, reproductive variation can also increase the rate of inbreeding due to a further reduction in the number of individuals contributing to subsequent generations.

In captive broodstocks that are not supplemented with wild individuals, the effects of inbreeding can increase over time. In long-lived species such as Atlantic sturgeon multiple year-classes will contribute to the same progeny generation. The generation N_e will be the sum of year-class increments (N_e, G_I) over the generational interval for the population. Generation interval (G_I) is the average age of females at first maturity, *i.e.* average age of females (in years) in the progeny (F_1) generation when they mature and begin to produce the next (F_2) generation. As a result, the generation N_e can be calculated as:

$$N_e(\text{GEN}) = \Delta F(N_{e,1} + N_{e,2} + N_{e,3} + \text{- - - - - - -}\ N_e, G_I)$$

The assumptions are that (1) individuals spawn no more than once per year, (2) matings occur randomly within each year-class, (3) survival across year-classes is equal, and (4) there is no migration, mutation, or selection. Generation N_e is very important to the discussion of artificial sturgeon breeding populations since relatively small numbers of parents mated each year are additive to future year pairings (Table 3). For example, if only four females and four males successfully mated each year over the course of a generation interval (say 15 years), the year-class N_e would only be 8 (ΔF = 6%), but the generation N_e would be 120 with ΔF = 0.42%.

In captive broodstocks, N_e should be maximized to minimize loss of genetic diversity. Although domestic animals tolerate inbreeding at a rate of about 1% per generation (N_e = 50) without showing inbreeding depression (Kapuscinski and Jacobson 1987), Kincaid (1983) suggested a minimum effective size of a breeding population of at least 100 individuals (equivalent to ΔF = 0.5%) for enhancement of wild stocks. Moreover, Kapuscinski and Lannan (1986) recommended minimum effective population sizes of more than 100 for hatchery fish, and Gharrett and Shirley (1985) support values for salmon ranging from 60 to 200. Table 3 compares the number of years it would take for a population, given various initial effective sizes of a population (N_e), to achieve an inbreeding rate of 0.5% or 1%, and the N_e(GEN) of the population when that inbreeding rate is achieved. The larger the initial N_e, the shorter the amount of time is needed for the population to achieve the desired inbreeding rate. In general, the amount of time and generational N_e to reach the specific inbreeding rate is approximately double for an inbreeding rate of 0.5% compared to 1%.

Table 3. Number of years of captive propagation it would take for a population, given an initial effective population size, to reach an inbreeding rate of 0.5% or 1%, and the effective population size (N_e(gen)) that would have been achieved over the given amount of time.

	Inbreeding rate			
	0.5%		1%	
Initial N_e	Years	N_e(gen)	Years	N_e(gen)
6	17	102	9	54
8	13	104	7	56
10	10	100	5	50
12	9	108	5	60
14	8	112	4	56
20	5	100	3	60
30	4	120	2	60
40	3	120	2	80
50	2	100	1	50

Kincaid (1983) suggested two general approaches to minimize inbreeding in captive hatchery populations: (1) random matings from large populations, and (2) rotational line crossings between year classes to minimize matings between related individuals. However, the use of rotational line crossing needs to be monitored closely as re-use of individuals increases the probability of creating related offspring. Captive breeding of sturgeon represents a unique management situation due to the high fecundity of females. The large number of eggs produced by a single female, combined with often small captive population size (due to rearing limitations and limited number of reproductively viable females per year) increases the risk of relatedness among offspring. The relative proportional contribution of captive produced sturgeon to wild populations is not known due to limited estimates of the size of most wild populations.

Application to Atlantic sturgeon broodstock management

As demonstrated in the previous section, the relationship between the relative contribution of the captive population to the wild population and the net increase to overall effective population size depends on the differences between the sizes of the captive and wild populations. Similarly, the consideration of a target rate of inbreeding in a hatchery population depends on the potential for hatchery-produced juveniles to reproduce with other hatchery individuals (and potential full or half-siblings) when they become sexually mature. If hatchery juveniles are stocked into a wild population with a large population size, the chance, when reproductively viable, of hatchery individuals spawning with each other is low. However, if hatchery juveniles are stocked into a wild population that is very small or locally extinct, there is an increased chance of the hatchery individuals spawning with other hatchery individuals when reproductively viable. Because Atlantic sturgeon are long-lived and don't become reproductively viable until an older age, it is difficult to estimate future population sizes to establish minimum effective population sizes and target rates of inbreeding for the current establishment of captive broodstocks. Given that uncertainty and in the absence of population size estimates, conservative (0.5%) inbreeding rates and maximum effective population sizes should be targeted for the establishment of captive broodstocks. For practical reasons, particularly lack of adequate numbers of ripe females, a 1% inbreeding rate may be targeted, however, efforts should be made to bring in wild caught (and not hatchery origin) individuals to add to broodstock.

Recommendations:

- Maximize the effective population size of spawning adults.
- Minimize the re-use of adults between spawning years.
- If females are not limiting, avoid splitting egg lots to fertilize with multiple males, to reduce creation of both full and half-siblings.
- Avoid stocking all offspring from the same families in the same location.

Selection Criteria

Local adaptation is often expressed as a fitness advantage of the native population relative to individuals from other populations exposed to different selection pressures. Fitness of hatchery produced or stocked fish may be less than that of the wild population native to a particular location. If the stocked fish are poorly adapted to their new environment and the numbers stocked are large compared to natural production, long-term fitness and adaptability of the population may be diminished. Krueger *et al*. (1981) suggest that the best way to insure that stocked fish will have high fitness in a particular environment is to use wild fish from the same environment as broodstock.

Alternately, outbreeding depression may occur when individuals from very genetically differentiated populations (*e.g.*, from distant waters) are bred resulting in decreased fitness of the progeny. If additional wild-caught individuals were used to supplement a captive broodstock, but were from a significantly genetically different population, negative fitness consequences in the offspring (such as decreased survival in the stocked environment) may be observed. Minimally, stocking programs that introduce individuals from a genetically differentiated population, and achieve successful reproduction of those individuals with native individuals, may experience a breakdown of locally adapted traits resulting in decreased fitness.

If wild broodstock are too scarce to achieve minimum effective population sizes, then several options can be considered. These include using broodstock only from similar or nearby environments, crossing wild x hatchery strains, mixing gametes from many populations, or maximizing genetic differences between mated pairs from the same population based on DNA analysis of individual spawners. For each of these alternatives, Krueger *et al.* (1981) recommended that mature broodstock be randomly selected from wild populations to avoid inadvertent selection for body size, spawning time, or site-specific variations, and that the hatchery rearing period should be minimized to reduce domestication.

With regard to artificial selection and hybridization programs to enhance management of fisheries, Hynes *et al.* (1981) provided the following general cautions:

- It is difficult to increase fitness of a population that is already well adapted to its environment.
- Selection programs invariably reduce the effective population size and encourage inbreeding and loss of genetic diversity.
- Artificial selection is difficult and inefficient for species with complex life histories because they cannot be maintained in a hatchery during their entire life cycle.
- Detrimental effects due to culture in an artificial environment accumulate with time.
- It is hard to obtain large selection differentials and responses to selection when spawners must be obtained from relatively small populations.

Application to Atlantic sturgeon broodstock management

Atlantic sturgeon populations along the Atlantic Coast of North America have been shown to be genetically differentiated from each other (King *et al*. 2001; Wirgin *et al*. 2000). Significant genetic differences among populations reveal that although individual sturgeon are known to travel long distances, and that the mixing of stocks occurs in the ocean and in juvenile, sub-adult, and adult rearing habitats, return to drainage of origin by adults for reproduction has resulted in separate populations. Therefore, because of distinct genetic differences between populations, transfer of stocks between basins for stocking programs is discouraged (Waldman and Wirgin 1998) due to the potential to break down locally adapted genetic diversity, and the loss of genetic differences among populations. For drainages that no longer support naturally reproducing Atlantic sturgeon populations development of broodstocks from neighboring drainages (which would have evolved under similar selection pressures) would be recommended.

Recommendations:

- Minimize intra-basin stock transfer if wild populations are present.
- If a population is extirpated, create broodstocks from neighboring drainages (e.g., use southern stocks for southern rivers).
- Avoid spawning individuals from genetically distinct populations together.
- Maintain genetic differences between populations.

Summary

Three primary concepts are important to maintenance of genetic diversity in captive broodstocks: effective population size, inbreeding and outbreeding. Establishing captive broodstocks with large numbers of males and females in equal sex ratios, and using mating strategies that minimize reproductive variance will minimize the potential for inbreeding within subsequent generations of the captive brood. Additionally, consideration of the effective population size of the captive population relative to the wild population, and the stocking rate of the captive group into the wild group will help maximize an increase in the overall effective population size. Avoidance of inbreeding in both captive and wild populations will help to sustain genetically viable populations that maintain the ability to adapt to changing conditions. Avoidance of outbreeding depression will help maintain fitness. Incorporation of genetic concepts and tools into broodstock management can be used to manage captive broodstocks that will preserve genetic characteristics of wild Atlantic sturgeon populations, and maintain genetic diversity for future generations.

POSSIBLE COMPETITIVE INTERACTIONS WITH SHORTNOSE STURGEON

The federally endangered shortnose sturgeon, *Acipenser brevirostrum*, occurs in varying abundance in many Atlantic coastal rivers that currently or historically supported Atlantic sturgeon. Unlike anadromous Atlantics, shortnose sturgeon spend their entire lives in freshwater and estuaries only rarely venturing to the marine environment. Because of their co-occurrence and overlapping distribution at various life stages, jurisdictions involved in stocking programs with Atlantic sturgeon should consider in their plans the potential competition for food and space between the species.

The spawning location and dispersal strategy of early life intervals of Atlantic and shortnose sturgeons are different, and the different strategies keep the two species spatially separate during the critical early-life period when year-class strength is established in both species. The lower-river spawning location and long-dispersal of Atlantic sturgeon are adaptations that enable the dispersing early life interval (larvae) to move within a few weeks from the spawning–egg deposition site to the freshwater-saltwater interface for rearing (Kynard and Horgan 2002). In contrast, the middle or upper river spawning location of shortnose sturgeon (usually about river km 200, Kynard 1997) is farther upstream than for Atlantic sturgeon. Further, the dispersal strategy of shortnose sturgeon early life intervals in northern rivers (*e.g.*, Connecticut River) and southern rivers (*e.g.*, Savannah River) are adaptations that insure young fish remain in fresh water until they are yearlings and have developed salinity tolerance (Kynard 1997, Kynard and Horgan 2002, E. Parker and B. Kynard unpublished data). Thus, during the first months of life when annual year-class strength and recruitment level are established (Gross *et al.* 2002), the available evidence indicates that the two species are spatially separate in most rivers and would not compete for resources critical to survival. Some overlap of earliest life phases for the two species, however, does occur in the Catskill to Kingston area of the Hudson River (Bain 1997).

Shortnose sturgeons first move downstream to the freshwater-saltwater interface, which is used by young Atlantic sturgeons, when they develop into yearlings. Examples are from the Connecticut River in the northern part of the species range (Kynard *et al*., in press), and Savannah River in the southern part (Hall *et al*. 1991). This dispersal by some shortnose sturgeon yearlings moves them to the reach used by older juvenile and adult Atlantic sturgeon for foraging. In the Hudson River, spatial habitat use during foraging overlapped between natal juveniles of the two species (Haley *et al*. 1996). This was not the case for non-natal wild Atlantic sturgeon juveniles, which did not overlap the habitat used by natal shortnose sturgeon in the Merrimack River (Kieffer and Kynard 1993). Data on habitat use of stocked and wild Atlantic sturgeon juveniles is rare. Haley *et al*. (1996) found the spatial distribution of stocked Atlantic sturgeon juveniles overlapped more with shortnose sturgeon than wild juvenile Atlantic sturgeon.

Limited studies show the diet of juvenile Atlantic and shortnose sturgeons in the estuary overlaps widely and includes a variety of benthic invertebrates (Pottle and Dadswell 1982, Carlson and Simpson 1987, Haley *et al*. 1996). The first and last cited studies found shortnose sturgeons foraged on mollusks, but Atlantic sturgeons did not. This result suggests that in areas of spatial overlap between the two species, where mollusks dominate the abundance of macroinvertebrate fauna, shortnose sturgeons could have a foraging advantage. Diets of Atlantic and shortnose sturgeon in the oligohaline and mesohaline portions of the estuary of the Edisto River, SC suggest that the diets and feeding behavior of the two species are dissimilar (M. Collins, SC Dept. of Natural Resources, pers. comm.). Based on percent occurrence of prey items, subadult Atlantic sturgeon had a diverse diet but fed primarily on spionid polychaetes. Shortnose sturgeon, on the other hand, fed on amphipods, especially gammarids which are extremely abundant in that portion of the estuary. The identifiable polychaetes utilized by the Atlantic sturgeon were infaunal (burrowing), suggesting that the fish actively sought out individual prey items and removed them from their burrows. This behavior corresponds to that noted in the Connecticut River where subadult Atlantic sturgeon fed almost exclusively on callionasid shrimp, which are also infaunal (T. Savoy, CT DEP, pers. comm.). Studies in the Hudson River concluded that the diets of the two species overlap to a large extent (Bain 1997). However, recent data from South Carolina suggest that, while subadult Atlantic sturgeon and both subadult and adult shortnose sturgeon often occupy the same habitat, their primary prey items and feeding behavior are divergent. The degree of divergence likely varies among life history stages as well as latitudinally.

Competition between juvenile Atlantic sturgeon and juvenile or adult shortnose sturgeon for limited forage has not been demonstrated in the lab or in the field. Inter-specific competition for forage could result in a slower growth rate and affect size-related life history traits (like time of sexual maturity) of the subordinate species. Laboratory studies show shortnose sturgeon (and probably Atlantic sturgeon) juveniles have a size-dominant social structure where larger fish are behaviorally superior competitors for limited forage than smaller fish (Kynard and Horgan 2002). The actual importance of body size as a factor in competition between the two sturgeon species is unknown and is likely complex. The larger maximum body size of juvenile Atlantic sturgeons could give them a competitive advantage for forage with smaller shortnose sturgeons. If inter-specific competition between Atlantic and shortnose sturgeon juveniles for limited resources exists, it likely occurs after year-class strength is established - so competition would affect growth rather than increasing mortality rate of the less competitive species.

RECOMMENDATIONS

Recommendations below are not intended to preclude states from independently moving forward with sturgeon population enhancement efforts in their waters. However, if population size and natural reproductive capability are known to be adversely affected by habitat degradation and/or excessive fishing bycatch mortality, culture initiatives should be closely tied to measures aimed at remediating those problems.

Fishery agencies that embark on sturgeon restoration efforts by stocking should recognize the desirability of either working with large numbers of broodfish to maximize N_e, or by continuing their effort with smaller numbers of broodfish for many years. Either way this will reduce the rate of inbreeding. Current status of populations suggests that large numbers of broodfish from any source are not likely to be available in the near-term. Maximizing generation N_e by using smaller numbers of broodfish each year is, for many donor waters, achievable, desirable, and less costly on an annual basis, but requires a long-term commitment.

Affected ASMFC states and federal agencies should work cooperatively in sturgeon culture, marking, stocking, evaluation, and research. Jurisdictions contemplating culture and stocking of Atlantic sturgeon will prepare stock reintroduction or restoration plans which specify measures of success. These planned performance measures may include such factors as desired numbers and source of spawning adults, expected numbers and sizes of progeny to be produced and stocked, expected number of years in the program, marking techniques employed, evaluation of growth and survival of stocked fish, relative contribution of cultured fish to future spawning classes, and any interactions between stocked and wild Atlantic sturgeon and resident shortnose sturgeon. The plan will also provide for collection of information on habitat requirements and availability, impacts of dams and pollution, and likely food sources and preferences.

Proponents of a sturgeon stocking program may be faced with a choice of working in a river with a small reproducing population (supplementation) or a river which has no remaining natural population (restoration). In the first instance, broodfish may be available from the target river which would alleviate some of the genetic concerns. However, if outside brood sources are used, extreme care will be required to avoid destroying the genetic character of the remnant stock. Also, having an extant population suggests that some essential habitat requirements are still available and functioning.

In the instance where the historic population no longer exists, a more intensive sturgeon habitat quality survey may be warranted. Inability to fully describe and measure what constitutes "quality" sturgeon habitat (which differs with life stage) could lead to costly long-term culture and stocking efforts that have little chance of success. Spawning of hatchery progeny with each other in the wild (as siblings and half-sibs) is more likely to occur in stocked rivers with extirpated natural populations. Either way stocking proceeds it is assumed that management strategies are now in place that will protect fish to maturity (*e.g.*, limited bycatch mortality), and this may not be true. Any restoration or enhancement proposal using cultured sturgeon must, among other things, justify selection of waters to be stocked, identify possible sources of broodfish, and describe elements of population and habitat surveys to be performed.

I. PLANNING, EVALUATION AND TAGGING REQUIREMENTS

Recommendation 1: Planning, Monitoring and Reporting

Management jurisdictions involved in stocking programs for Atlantic sturgeon should provide a detailed proposal to the ASMFC Sturgeon Technical Committee for review and recommendation to the Board. Such plan will include goals and objectives, population surveys, broodstock sources and selection criteria, numbers, sizes and locations to be stocked, and timelines. The plan should also include annual monitoring of the status of their population, the effects of stocking, and possible interactions with shortnose sturgeon if they co-exist. Stocking and monitoring results should be reported to ASMFC each year by October 1 through annual state compliance reports.

Recommendation 2: Habitat Quality and Population Surveys

Prior to initiation of a large-scale sturgeon stocking program, areas targeted for stocking will be evaluated for presence-absence of remnant populations, determination of the relative quality and quantity of available habitat (*e.g.*, water quality, substrate, flow characteristics, food availability) and possible human impacts on these environments (dams, dredging, water withdrawals, *etc*.). Small- scale releases of marked cultured sturgeons may be useful a component of these evaluations.

Recommendation 3: Tagging

All stocked cultured sturgeon including released broodfish should be marked. The Atlantic Sturgeon Technical Committee or a special subcommittee should examine the entire range of tagging options to include wild-caught and hatchery origin fish of all sizes and in all locations. A comprehensive tagging protocol should be developed for ASMFC states and become part of these stocking guidelines, when available.

II. BROODSTOCK SELECTION AND MINIMUM NUMBERS

Recommendation 4: Source of Broodstock

Wherever possible, broodstock should be taken from the same river in which stocking will occur. When such fish are not available, broodstock used to produce fish for stocking should be taken from a nearby source(s) which will allow maintenance of sturgeon abundance. Such sourcing of broodstock will consider the genetic as well as the logistical (fish availability) implications associated with the stocking plan.

Recommendation 5: Number of Spawners

> The stocking plan will incorporate broodstock collection and progeny production components that meet genetic criteria for maximizing effective population size of broodstock while achieving an inbreeding rate of less than 1%, preferably 0.5%. Plans may use various genetic and temporal approaches including cautious application of unbalanced sex ratios, egg or sperm splitting, and mixing of various genetic stocks. In other than small-scale research stocking efforts, proponents may have to commit to long-term stocking efforts.

III. FATE OF POST-SPAWN BROODSTOCK AND PROGENY

Recommendation 6: Fate of Post-Spawn Broodstock

> Broodstock should typically be spawned only once unless there is genetic justification to reuse them. After use, they should be marked and returned to their river of origin whenever feasible.

Recommendation 7: Fate of Progeny

> If progeny produced are excess to ASMFC approved plan needs, they may be used for research purposes, educational exhibits, or provided to private aquaculture interests. Any excess progeny released into the wild for research or study purpose must be specifically approved in advance by ASMFC. If there is no need for these fish they should be properly euthanized.

SUMMARY

Culture and stocking of Atlantic sturgeon may play an important role in re-introduction or restoration of populations which are currently at or near all time low levels. Technology for such culture is largely developed, but major difficulties exist in securing adequate numbers of broodfish. Desirable minimum year-class effective population sizes for artificial culture will be difficult to achieve. However, effective generational populations can be developed using relatively small numbers of brood fish each year for many years. This lengthy commitment is also desirable due to life history characteristics of Atlantic sturgeon (long-lived, slow growing, late maturing, multi-year spawning periodicity) which requires considerable time to evaluate success and because of large costs associated with establishing culture and holding facilities.

Highest emphasis should be placed on stocking of rivers having suitable spawning and nursery habitat but whose natural populations are presumed to be extirpated or at very low abundance. Hatchery product evaluation should be an essential component of these stocking programs.

Basic information on natural reproduction parameters (fertilization rate, hatch rate, larval and juvenile mortality rates, etc.) are lacking for this species although some of this information is available from culture studies (Mohler 2004). Artificial production from even a few large female sturgeon could amount to great numbers of juveniles being produced for potential stocking efforts. However, since natural reproduction appears to be weak in most rivers targeted for restoration or enhancement, these stockings may substantially increase ultimate population sizes quickly but may not be in the best interest of the species. Adverse impacts associated with potential inbreeding depression and alteration of reproductive fitness of the resultant mixed populations is minimized by relatively small scale multi-year stockings over the course of the generation interval.

Stocking of very young fry may reduce perceived adverse impacts associated with domestication but exposes them to higher in-stream mortality (predation and food competition). No field data are available on the relationship between numbers of spawners and recruits, natural rates of egg deposition, fertilization, hatching, or survival of larvae and early juveniles. Results of the 1996 yearling Atlantic sturgeon stocking of the Nanticoke River, however, indicate that stocked and wild fish grew at similar rates; that feeding incidence was high and forage similar between to those reported for wild juveniles (Secor *et al.* 2000b; Welsh *et al.* 2002). On the other hand, an ecophysiological model indicated that 1996 may have been an unusually favorable year for juvenile sturgeon growth and survival, with summer production several-fold the mean predicted for the period 1993-2002 (Niklitschek and Secor 2005). Further, the distribution of Chesapeake Bay recaptures clearly showed a pattern of avoidance of hypoxic waters (Niklitschek and Secor 2005; USEPA 2003).

Although males may not mature for 8-10 years and females for 15-20 years, it is believed that survival of marked-stocked sturgeons and their relative abundance in the mixed populations can be adequately evaluated within about 5-10 years. Although natal stream fidelity is implied by population genetic studies, juveniles are known to move into non-natal systems. At present, it is uncertain when or how fish become imprinted. Managers must determine preferred stocking locations (freshwater vs. estuarine), seasons, and size of stocked fish to coincide with habitat and food requirements and availability. Prior to embarking on full-scale restoration or enhancement stocking of Atlantic sturgeon, sponsor agencies are encouraged to consider smaller experimental releases to help identify optimal stocking strategies.

In order to determine effectiveness of sturgeon stocking, tagging or marking and follow-up evaluation must be essential components of population enhancement programs. Production lots of small sturgeon in hatcheries can be marked with chemicals (e.g., calcein or tetracycline) or coded wire tags (CWT). Larger fingerlings could accommodate streamer tags, PIT tags, and even acoustic tags (Peterson *et al.* 2000; Secor *et al.* 2000b) but substantially higher costs are involved with the latter. Because of wide ranging migrations of stocked sturgeon, ASMFC member states should examine tag alternatives and adopt uniform tagging guidelines. USFWS is encouraged to continue maintenance of a sturgeon tag and recapture database.

CONCLUSIONS

Many Atlantic sturgeon populations presently exist at low levels. Managers are faced with the conundrum of adopting a laissez faire stance and allowing populations an opportunity to recover on their own, or of launching a stocking program. The potential for a laissez fair approach to work was greatly increased by the enactment in the late 1990s of protections against direct fishing for the species. However, while such an approach avoids the financial costs, logistic and genetic complications of a stocking program, if it fails it may result in the loss of that population and its genetic legacy.

Alternatively, a stocking program may be initiated. However, stocking programs require substantial sustained funding, an ongoing source of often-difficult-to-obtain broodstock, and a commitment to work within stringent operational guidelines in order to avoid genetic injury to the population.

It may be that a laissez faire approach is best suited to populations that show ongoing natural reproduction and that stocking is justified where natural reproduction is believed not to be occurring or where extirpation is suspected. However, each severely reduced Atlantic sturgeon population exists in its own unique circumstances which should be evaluated in light of the principles outlined in this set of guidelines.

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APPENDIX I

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