American Fisheries Society Symposium 66:307–327, 2009
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Genetic Conservation and Paddlefish Propagation

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Abstract.—The conservation of genetic diversity of our natural resources is overwhelmingly one of the central foci of 21st century management practices. Three recommendations related to the conservation of paddlefish *Polyodon spathula* genetic diversity are to (1) identify genetic diversity at both nuclear and mitochondrial DNA loci using a suggested list of 20 sampling locations, (2) use genetic diversity estimates to develop genetic management units, and (3) identify broodstock sources to minimize effects of supplemental stocking on the genetic integrity of native paddlefish populations. We review previous genetic work on paddlefish and described key principles and concepts associated with maintaining genetic diversity within and among paddlefish populations and also present a genetic case study of current paddlefish propagation at the U.S. Fish and Wildlife Service Gavins Point National Fish Hatchery. This study confirmed that three potential sources of broodfish were genetically indistinguishable at the loci examined, allowing the management agencies cooperating on this program flexibility in sampling gametes. This study also showed significant bias in the hatchery occurred in terms of male reproductive contribution, which resulted in a shift in the genetic diversity of progeny compared to the broodfish. This shift was shown to result from differential male contributions, partially attributed to the mode of egg fertilization. Genetic insights enable implementation of a paddlefish propagation program within an adaptive management strategy that conserves inherent genetic diversity while achieving demographic goals.

Genetic Principles and Hatchery Management

The conservation of genetic diversity of our natural resources is overwhelmingly one of the central foci of 21st century management practices. Concurrently, most fisheries management actions throughout the world aim to maintain optimal sustainability and variable user interests such as recreational and commercial harvest. The paddlefish *Polyodon spathula* is no exception

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to these general rules (see Kerns et al. 2009; Mestl and Sorensen 2009; Paukert 2009; Quist et al. 2009; Scholten 2009; all this volume). With the creation of the Mississippi Interstate Cooperative Resource Association (MICRA) in 1991, concerns for interjurisdictional management of paddlefish were brought in focus, including much-needed attention for the species' genetic resources. The formulation of the MICRA Paddlefish Genetics Plan (MICRA Paddlefish and Sturgeon Committee 1998) represented a compilation of guidelines related to the conservation of paddlefish genetic resources in light of increasing use of stocking as part of management. Three primary recommendations were made: (1) identify genetic diversity at both nuclear and mitochondrial DNA loci using a suggested list of 20 sampling locations, (2) use genetic diversity estimates to develop genetic management units, and (3) identify broodstock sources to minimize genetic effects of supplemental stocking on the genetic integrity of native paddlefish populations.

The use and implementation of propagation in the management of fish species has a long and sometime contentious history (Schramm and Piper 1995; Incerpi 1996; Waples 2002). The widespread use of propagation of paddlefish (O'Bara 2009, this volume; Paukert 2009; Scholten 2009) coupled with the identification of genetic structure throughout the native range of paddlefish (Carlson et al. 1982; Epifanio et al. 1996; Szalanski et al. 2000; Heist and Mustapha 2008) supports the consideration of genetic concerns in propagation and management of paddlefish. Conservation practices often focus on the genetic impacts of small population size and translocation or propagation of artificially reared individuals. In fisheries management, this has led to the development and implementation of conservation hatchery programs (Flagg and Nash 1999). Flagg and Nash (1999) define a conservation hatchery as "a rearing facility to breed and propagate a stock of fish with equivalent genetic resources as the native stock, and with the full ability to return to reproduce naturally in its native habitat." The needs and concerns associated with paddlefish conservation are consistent with the implementation of conservation-oriented management and propagation programs. In essence, a conservation hatchery program is a prerequisite to sufficient and efficient management and conservation of paddlefish. A number of considerations are implicit in the development and implementation of a conservation hatchery strategy. Key factors are what and how many sources of broodfish are necessary, the selection of fish to breed in the program, and rearing conditions.

In this chapter, we review previous genetic work conducted on paddlefish. We describe key principles and concepts associated with maintaining genetic diversity within and among paddlefish populations, including the stock concept and its impact on genetic management practices. We also present recent findings from a genetic case study of paddlefish propagation at the U.S. Fish and Wildlife Service (USFWS) Gavins Point National Fish Hatchery. This study is an in-depth genetic examination of the Lake Francis Case (middle Missouri River) multi-agency paddlefish propagation program, including the impacts of crossing strategy and the appropriateness of potential brood sources.

Fish Genetic Concepts and Principles

Responsible stocking practices preserve genetic variation within and among populations (Busack and Currens 1995). Reduction in genetic variation within populations can result in a decrease in fitness (performance) of fish due to inbreeding depression. Loss of genetic variation among populations can reduce fitness through outbreeding depres-

sion and can have long-term effects on biodiversity by eroding or retarding local adaptation. Inbreeding depression is the loss of fitness that accompanies crosses between related individuals and is caused by both a loss of overall heterozygosity (within-individual variation) and an increase in the likelihood that an individual will receive two copies of a recessive deleterious allele, one from each related parent via their common ancestor. Inbreeding depression has been demonstrated to produce negative impacts on many traits important to fish managers, including survival, growth rate, and the fraction of malformed fry (Kincaid 1983; Gall 1987; Edmands 2007).

While a fish culturist would probably not intentionally cross related individuals, hatchery practices can unwittingly increase the risk of inbreeding depression if the average relatedness of broodstock increases due to low effective population size (N_e). Effective population size is related to the number of breeders that actually contribute to future generations but is typically less than the number of breeders due to unequal sex ratios and/or a high variance in reproductive success among breeders (For an excellent review see Chapter 9 in Hallerman 2003). However, if care is taken to balance reproductive output among individuals, N_e can be almost twice the number of breeders (Allendorf and Luikart 2007). The rate at which inbreeding increases in a population is proportional to one-half N_e (Gall 1987). The oft-cited 50/500 rule states that the N_e of a captive population should be at least 50 at its founding and that a N_e of 500 is recommended for the long-term survival of a stock (reviewed in Allendorf and Ryman 1987). Hatchery managers can mitigate against reduction in N_e by not pooling gametes of the same sex, perhaps resulting in overrepresentation by a few individuals, and by keeping family lots separate to ensure that many different family lots are represented. If a large number of offspring from few parents is stocked into a wild population, the N_e of the wild population can be reduced such that inbreeding may threaten the future genetic health of the population. This phenomenon was described by Ryman and Laikre (1991) and has come to be known as the Ryman-Laikre effect. If the stock supplemented by hatchery fish has a large N_e, the Ryman-Laikre effect is unlikely to have any significant impact. The Ryman-Laikre effect is most likely to impact wild populations with low N_e, which ironically are those that are also most likely to benefit from supplementation.

Another potential genetic effect of hatchery practices is outbreeding depression, which has two causes: maladaptive genes and breakdown of intrinsic coadaptations (Templeton 1986). Fish stocks become adapted to their environment through Darwinian natural selection, and introduction of stocks from other regions may introduce maladaptive genes into the population. For example, Philipp and Whitt (1991) found that while native northern largemouth bass *Micropterus salmoides salmoides* readily hybridized with introduced Florida largemouth bass *M. s. floridanus*, in central Illinois lakes, pure northern largemouth bass had higher winter survival and faster young-of-the-year growth rates than Florida largemouth bass or either reciprocal hybrid. Philipp and Claussen (1995) found that growth and survival of *M. s. salmoides* stocks originating from northern and southern Illinois and stocked into northern, southern, and central Illinois were highest for fish stocked into their native region and were intermediate in central Illinois. Even stocks that originate in identical habitat but are reproductively isolated can experience outbreeding depression due to the intrinsic coadaptation, which can be thought of as adaptation to an isolated population's genetic environment rather than its external environment (Templeton 1986). This sort of outbreeding

depression is not expected to occur until the generation following the initial hybridization event and thus may escape scrutiny in the hatchery only to occur later in the wild. In a study in which hybrids were produced between even-year and odd-year runs of pink salmon *Oncorhynchus gorbuscha*, a species with a strict 2-year life cycle and thus reproductive isolation among stocks in the same river, Gharrett and colleagues (Gharrett and Smoker 1991; Gharrett et al. 1999) demonstrated that second-generation hybrids had lower return rates than either pure odd- or even-year fish or their F_1 hybrids. Outbreeding depression is still a controversial topic owing to the difficulty of measuring and generalizing its effects; nevertheless, the prudent approach is to avoid mixing genetically differentiated stocks except in cases of ameliorating inbreeding (Edmands 2007).

Genetic Stock Structure in Paddlefish

One way to assess the likelihood that fish from different regions could produce outbreeding depression is through studies of genetic stock structure. The stock concept has a storied history as a seminal feature in fisheries resource management (see Berst and Simon 1981; Booke 1999). A stock is a conglomeration of fish from a single or multiple populations that have sufficient migration (i.e., connectivity) to result in similar biological attributes such as population dynamic measures and genetic characteristics resulting in shared adaptations (Larkin 1972; Bailey and Smith 1981; Shaklee and Currens 2003). Proper delineation of stock structure in a resource is an integral part of modern fisheries management as it helps the resource manager minimize the risk of overexploitation and negative impacts associated with propagation (including supplemental stocking) by focusing the attention of management activities on a biologically relevant unit. Typically, these studies employ molecular markers to estimate whether there is significant differentiation among populations indicative of reproductive isolation. Adaptive and co-adaptive differences among stocks are far more likely to develop in the absence of gene flow among populations (however, see McKay and Latta 2002). The degree of genetic heterogeneity among populations is expressed as some estimate of Wright's (1969) F_{ST}, which is analogous to the *F*-statistic in analysis of variance and can be thought of as the standardized variance in allele (gene) frequencies among populations.

Numerous types of molecular markers have been used or could be used to investigate genetic population structure in paddlefish. To date, paddlefish have been studied using allozymes (Carlson et al. 1982; Fries and Hutson 1993; Epifanio et al. 1996), mitochondrial (mt) DNA (Szalanski et al. 2000), and microsatellites (Heist and Mustapha 2008). Future studies may involve single nucleotide polymorphisms (Morin et al. 2004). Each of these techniques is presumed to measure selectively neutral variation, that is, the genes measured have little or no effect on phenotype, although there are proven exceptions (Van Oosterhout et al. 2004; Grant et al. 2006). Techniques that screen many polymorphic markers simultaneously may permit the identification of genes that confer adaptive differences among stocks (e.g., Rogers and Bernatchez 2005). Each marker type has different characteristics, and thus, depending on the research goal, one available marker type might be preferable (Ferguson and Danzmann 1998).

Allozymes

Allozymes are proteins (typically enzymes involved in either glycolysis or the Kreb's cycle) that exhibit varying rates of migration during electrophoresis (reviewed in

May 2003). The term allozymes refers to multiple allelic forms at individual loci and are a subset of isozymes, which includes both allelic polymorphisms and products of different loci that resolve under the same conditions. To score allozymes, a researcher extracts intact enzymes from fresh or frozen tissue, typically by homogenizing tissue in a buffer solution. For allozymes to resolve, they must be intact (nondenatured), and thus, the technique relies on either very fresh tissue or tissue frozen (preferably below –20°C) very soon after death of the fish. The homogenate is applied to a separatory medium, typically a starch gel (Murphy et al. 1996) or cellulose acetate plate (Hebert and Beaton 1989). An electrical field is applied across the medium causing ionic proteins to migrate towards one or the other poles. By varying the chemical nature (especially pH) of the electrophoretic buffer, the rate and even direction of migration can be altered. After electrophoresis for minutes to hours, the gel or plate is stained with a solution typically containing an enzyme substrate and indicator dye. The location of enzyme activity is resolved as a colored or fluorescent band. Converting the gel image into genetic data can require considerable interpretation and cannot be detailed here, although several excellent guides are available (Utter et al. 1987; Murphy et al. 1996; May 2003).

Paddlefish allozymes have been scored by Carlson et al. (1982), Epifanio et al. (1996), and Fries and Hutson (1993). Carlson et al. (1982) observed genetic variation in only 2 of the 35 allozyme loci scored (6%) in 6 to 18 paddlefish from five locations in the Mississippi River drainage (Yellowstone, Missouri, Mississippi, Osage, and Cumberland rivers) and one individual from the Alabama River. The overall observed heterozygosity was 1.3%. One polymorphic locus, CK-B*, exhibited similar allele frequencies in all samples. The second, PGM-1*, was monomorphic in the Mississippi drainage; however, the one paddlefish surveyed from the Alabama River was homozygous for a different allele. Carlson et al. (1982) concluded that there was no significant heterogeneity in allele frequencies in CK-B* but that finding a homozygote for an alternate allele in the Alabama River indicated significant genetic heterogeneity between isolated drainages. Fries and Hutson (1993) examined allozymes in 10 hatchery-reared and 2 wild paddlefish and found heterozygosity at only 2 of 35 loci. The study by Epifanio et al. (1996) included larger sample sizes, more geographic samples, and a larger suite of allozyme loci, including most of those surveyed by Carlson et al. (1982) and Fries and Hutson (1993). Epifanio et al. (1996) found variation in 20 of 62 loci (32%), and using the conservative criterion of considering a locus polymorphic if the frequency of the most common allele was 0.95 or less in one or more geographic sample, 12 of 62 loci (19%) remained polymorphic. The overall observed heterozygosity was 3.2%. Epifanio et al. (1996) found two PGM-1* alleles in all geographic samples. Epifanio et al. (1996) concluded that there was significant heterogeneity in allozyme allele frequencies, even when the Alabama River drainage samples were removed from the data. The several-fold increase in variation detected by Epifanio et al. (1996) relative to Carlson et al. (1982) highlights the fact that allozyme studies can differ greatly in resolution. Undoubtedly, Epifanio et al. (1996) were able to resolve allelic differences missed by Carlson et al. (1982).

Mitochondrial DNA

Mitochondrial DNA consists of a double stranded loop of extrachromosomal DNA that in paddlefish is approximately 16,500 base pairs in length (Inoue et al. 2003). Mitochondrial DNA has some useful characteristics for studying population genetics,

including its compact size and supercoiled structure, which allows it to be physically isolated from nuclear DNA (Lansman et al. 1981). In vertebrates, mtDNA is maternally inherited and haploid, typically occurring as identical copies of one sequence in all cells. The combination of haploidy and maternal inheritance gives it an approximately fourfold reduction in Ne, resulting in increased genetic drift (compared to nuclear DNA loci) and hence a more rapid pace of genetic divergence among isolated lineages (Birky et al. 1983). Because it does not undergo recombination and accumulates mutations in a clock-like fashion (i.e., linear with time; Brown 1983), mtDNA can reveal evidence about the past lineage divergence and secondary contact. For example, Bernatchez and Wilson (1998) compared distributions of mtDNA haplotypes across 42 species of North American fishes and concluded that there was a latitudinal shift in mtDNA divergence associated with the southernmost advance of the Wisconsin glaciations. Populations of fishes occurring in formerly glaciated regions had lower levels of among-population divergence, indicating more recent shared ancestry, while fish from the southeastern United States (reviewed in Avise 1994) exhibited higher levels of divergence among Atlantic and Gulf of Mexico drainages indicative of more ancient lineage splits. By creating and destroying vast freshwater lakes, glaciers provided a mechanism for facilitating gene flow among freshwater fishes that was lacking in unglaciated regions.

Mitochondrial DNA can be analyzed by restriction fragment length polymorphism (RFLP) analysis of whole molecule mtDNA, RFLP of polymerase chain reaction (PCR) amplified fragments, and direct sequencing (reviewed in Billington 2003). Whole molecule methods, which require relatively large amounts of high quality tissue, were popular before there were sufficient data regarding mtDNA sequences for many species. PCR-RFLP can be performed on small amounts of dried or ethanol-preserved tissues and the design of universal PCR primers (Kocher et al. 1989) opened up PCR to a large number of species. Both whole-molecule and PCR-based RFLP techniques indirectly sample a subset of the variation in the surveyed region while direct sequencing scores all of the variation. RFLP was once a more efficient means of surveying variation, but with the development of inexpensive automated DNA sequencing, more studies are obtaining data directly from sequences.

To date, two studies have examined mtDNA in paddlefish. Epifanio et al. (1996) was able to isolate and analyze whole molecule mtDNA RFLPs in 73 of the 179 tissue samples they examined. They detected four composite haplotypes (mtDNA genotypes), with the most common haplotype occurring in 58 of the 73 specimens (79.5%). There was no significant heterogeneity in haplotype frequencies among sampled locations, and the eight Alabama River specimens examined all shared the common haplotype. Szalanski et al. (2000) used PCR primers developed for brown trout *Salmo trutta* to amplify the paddlefish mtDNA d-loop region (which is typically the most polymorphic segment of vertebrate mtDNA). They examined 93 individual paddlefish, 83 of which were from a single location (Gavins Point Dam tailwaters, Missouri River, Nebraska). They first sequenced 800 base pairs of D-loop in 10 individuals and found nine unique haplotypes. Based on the distribution of polymorphic restriction enzyme sites in the sequenced individuals, they chose three restriction enzymes and performed PCR-RFLP on all 93 individuals. RFLP detected six unique haplotypes. There was no heterogeneity in haplotype frequencies among Nebraska paddlefish from three different years (1995, 1998, and 1999), nor were there significant differences in hap-

lotype frequencies between Nebraska and the other sampled locations (Montana [Yellowstone River], South Dakota [Missouri River], and Louisiana [Mermentau River]), although sample sizes in these locations (seven, one, and two, respectively) were too low to powerfully test for genetic heterogeneity. We do not believe that these two studies have adequately surveyed mtDNA diversity in paddlefish. Both studies surveyed very few polymorphic mtDNA nucleotide sites, and between the two studies, only one location (Nebraska) had a sample size larger than 16 paddlefish. While there are already considerable allozyme and microsatellite (see below) data for paddlefish, these markers indicate recent levels of gene flow while the unique modes of inheritance and evolution of mtDNA make it a potentially useful marker for understanding whether there are any remaining remnants of historically isolated paddlefish populations, perhaps harboring unique genetic adaptations.

DNA Microsatellites

DNA microsatellites (O'Connell and Wright 1997) are repetitive DNA sequences that have a very high mutation rate due to a process known as slipped-strand mispairing (Levinson and Gutman 1987). While most allozyme loci possess one or two alleles, microsatellites often have 10 or more alleles and some may have more than 30. Microsatellites are the ideal markers for estimating relatedness (e.g., parentage) among individuals. The very high diversity of microsatellites means that it is extremely unlikely that any two individuals will share identical genotypes unless they are monozygotic twin. One application of parentage analysis for fisheries is genetic tagging. Provided that the broodstock used to produce fish for stocking can be genotyped, it is possible to distinguish unmarked hatchery fish from wild fish (DeHaan et al. 2008). Genetic tags can also be used to identify previously marked and captured individuals that have shed their physical tags (Feldheim et al. 2002). Despite having much higher levels of variation, microsatellite data are analyzed much like allozyme data. Although scoring of band sizes vary among laboratories making it necessary to standardize allele identities before independent data sets can be combined (Moran et al. 2006), even without such standardization, levels of variation should be consistent for the same loci and individuals. Thus, microsatellites are more objective than allozymes.

Heist et al. (2002) described eight polymorphic microsatellite loci for paddlefish, and an additional locus was described by Heist and Mustapha (2008). Heist and Mustapha (2008) used these loci to survey variation in 12 geographic samples of paddlefish collected throughout the range of the species, including samples from several reservoirs and a sample from the Tombigbee River, part of the Alabama River drainage, and also Bayou Nezpique, Mermentau River, which drains directly into the Gulf of Mexico west of the Mississippi River. Considerable heterogeneity in microsatellite allele frequencies was detected, and most pairwise tests of the null hypothesis that $F_{ST} = 0$ (i.e., no genetic divergence between population samples) were significant, as was the overall test of $F_{ST} = 0$. Paddlefish therefore show genetic structuring among sample sites and are not panmictic throughout the sampled area. The most distinct sample was the Tombigbee River followed by Grand Lake and Bayou Nezpique. Grand Lake and Tombigbee River also exhibited the lowest heterozygosities. A test for isolation by distance, which tested whether distance in river miles between samples was correlated with genetic differences among sites, was nonsignificant. Based on the degree of genetic heterogeneity among sampled regions and the lack of an isolation by distance effect, Heist and

Mustapha (2008) concluded that paddlefish populations were previously more connected, but that significant allele frequency differences developed recently in the presence of anthropogenic barriers to gene flow (dams). The low level of genetic variation in Grand Lake might be explained by a Ryman-Laikre effect perhaps enhanced by historical stocking of Grand Lake using Grand Lake broodstock. Heist and Mustapha (2008) recommended that future stockings might be more beneficial if they facilitated movement around dams, for example by stocking reservoirs with offspring of broodstock below the dam. However, Heist and Mustapha (2008) cautioned that movement of fish across great geographic distances and particularly latitudinal gradients should be avoided as should movement of fish across drainages.

Case Study: Genetic Evaluation of a Paddlefish Propagation Program at the Gavins Point National Fish Hatchery

Propagation Program Development

Paddlefish propagation programs have been put in place in various sections of the species' home range to recover declining or extirpated populations (Graham 1997). The Missouri River was fragmented in South Dakota by closure of four main-stem dams: Fort Randall in 1952, Gavins Point in 1955, Oahe Dam in 1962, and Big Bend Dam in 1964, which blocked paddlefish migrations and inundated spawning habitats (Friberg 1972). Paddlefish congregated in the tail waters were highly vulnerable to snagging by anglers, and within 3 years of legalizing that fishing method in 1957, 12,850 paddlefish were harvested in the tailrace of Fort Randall Dam with similar boom-bust fisheries observed downstream of Oahe and Big Bend dams (Friberg 1972). High exploitation and no evidence of natural recruitment in Lake Francis Case led to initiation of experiments by South Dakota Department of Game Fish and Parks (SDGFP) and the USFWS Gavins Point National Fish Hatchery (NFH), Yankton, South Dakota to develop artificial propagation methods for paddlefish in 1972, with the initial goal to maintain an exploitable population (Friberg 1973). The first fry stocking in Lake Francis Case occurred in 1974 (Unkenholz 1979), and generally 10,000–30,000 fingerlings produced at Gavins Point NFH have been stocked annually from 1991 to 2007 (U.S. Fish and Wildlife Service, unpublished data). Fingerlings have also been stocked (20,000–48,000 per year) in Lewis and Clark Lake from 1988 to 1992 (except 1990), with no stocking since 1992 (U.S. Fish and Wildlife Service, unpublished data). The Missouri River forms a geopolitical boundary, resulting in a cooperative propagation and management program between SDGFP, the Nebraska Game and Parks Commission (NGPC), and the USFWS. All paddlefish harvest has been prohibited above Gavins Point Dam since 1987; however, a co-managed snagging and archery sport fishery is still present downstream of Gavins Point Dam (Mestl and Sorensen 2009, this volume).

The broodfish for the propagation program are generally collected from Lake Francis Case but, in some years, were also collected in the Missouri River downstream of the confluence with the Niobrara River (Figure 1). Often, the number of males and females captured and used as broodfish is less than 25, with sex ratios skewed towards an excess of males. Skewed sex ratios and small numbers of broodfish can have a tremendous influence on the genetic diversity of progeny (Waples 1989; Frankham et al. 2001) and, thus, the propagated paddlefish. As genetic diversity levels are associated with long- and short-term viability

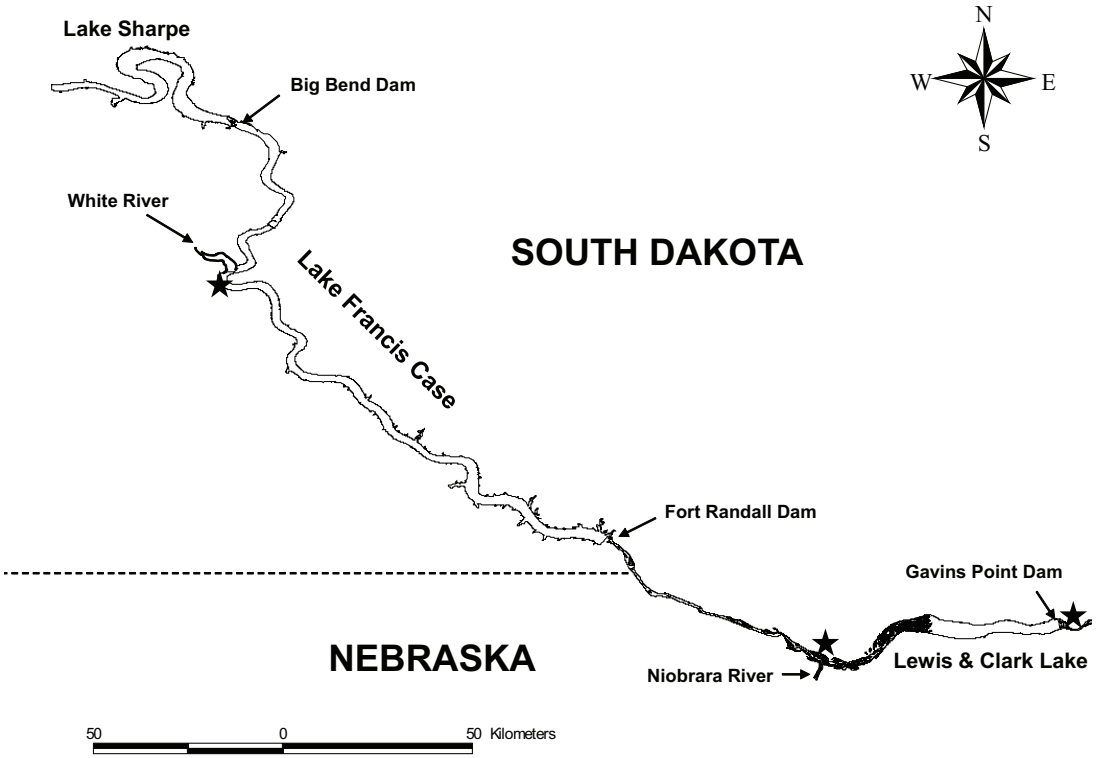

Figure 1. Map of the Missouri River in South Dakota and Nebraska, including the location of Gavins Point National Fish Hatchery and the three potential brood source collection sites used in this study (indicated by stars).

and adaptability of populations and species (Lande and Shannon 1996; Reed and Frankham 2003), a genetically sound propagation approach should be a high priority for any propagation program including paddlefish.

In 2005, a genetic review and monitoring program for paddlefish propagation at the Gavins Point NFH was initiated. The goal of this effort was to apply molecular genetic techniques to evaluate key methods and assumptions of the program to ensure that the genetic integrity of putative stocks were being preserved and that the genetic characteristics of the brood source populations were being sufficiently represented. The key objectives we addressed were (1) to determine if genetic differences exist among three sample locations for paddlefish used or potentially used for paddlefish brood sources, (2) to determine if significant biases in the rearing process result in genetic effects (i.e., were propagated fish genetically representative of the broodfish from which they were derived), and (3) to determine if significant deviations in male-reproductive success resulted from using a mixed-milt crossing strategy (i.e., unequal amounts of milt from multiple males used per female). The first objective ensures the use of a genetically representative brood source in propagation programs. The second and third objectives aimed to assess the level of reproductive variance (and hence N_e). One way to maximize N_e for a given number of broodstock when more males than females are available for breeding is to divide eggs from each female, fertilize each lot with sperm from a single male, and then rear families separately to equalize family

sizes. We wanted to directly assess the realized impact on N_e of not doing this and whether these laborious and resource-intensive procedures were justified. We present the results of this study here to exhibit how genetic marker data can be used to evaluate a well-established propagation/management program resulting in insights to allow for adaptive management.

Methods

Sampling Design

Our research focused on paddlefish production at the Gavins Point National Fish Hatchery in Yankton, South Dakota. This hatchery rears about 25,000 paddlefish fingerlings annually for subsequent stocking into the Lake Francis Case reservoir of the Missouri River (www.fws.gov/gavinspoint/paddlefish.htm).

At Gavins Point NFH, broodfish are induced to spawn with intramuscular injections of Luteinizing Hormone-Releasing Hormone. Males usually ripen earliest, and milt is precollected in plastic bags and kept refrigerated until needed. Females, once ripe after a second injection, are hand-stripped of eggs, often multiple times over the course of 24 h, with each batch of eggs fertilized with two or more males (milt volumes visually approximated). After fertilization, fuller's earth is added to the eggs and stirred for 20 min with a feather to prevent adhesion and clumping. Eggs are then allowed to water harden for about 1 h before transfer to McDonald incubation jars.

For our first objective, we attempted to sample more than 50 fish from each of three sources for paddlefish broodfish: (1) immediately downstream of Gavins Point Dam, (2) the confluence of the Niobrara River with the Missouri River in Lewis and Clark Lake, and (3) the confluence of the White River and the Missouri River in Lake Francis Case (Figure 1). Genetic characteristics of these three putative populations were compared to determine if significant differentiation existed. If differences were observed, it would suggest genetic discontinuity of the paddlefish in this region due to dam construction or natural population structuring from unique spawning runs. Either situation can result in one or more brood sources not being suitable to supplementary stock certain regions.

For our second objective, we sampled $N \geq 50$ fingerlings stocked out from the 2006 production year. We compared the genetic diversity of these fish with the genetic diversity of the adults used to produce that year-class. If significant deviations existed between the broodfish used for that year's production and the resulting stocked fish, we would infer that some form of male-mediated selection or bias was occurring.

For our third objective, we used genetic paternity analysis of larval fish produced by a variety of crossing strategies in 2007. A common strategy in the Gavins Point NFH is to cross a female with between 3 and 10 males (mixed-milt design) with little attention paid to quantity of male milt used (i.e., equal volumes approximated by eye). An alternative approach is to use an equalized milt approach wherein the eggs of a single female are fertilized with an approximately equal volume of milt (10 mL/male) from a set number of males (equal-milt design) simultaneously added to the eggs. To test the impact of male contribution, we tested three replicates of the mixed-milt design and two replicates of the equal-milt design from four to five males crossed with each female. Female families were reared to hatching separately, and at least 75 larval fish were sampled for determination of paternity. We expected some random deviation from evenly distributed paternity, but significant deviation from equal contribution (as tested by chi-square analysis) would show a realized bias associated with either sperm competition or male–female compatibility.

Samples ($N > 25$) of actual and potential brood sources were collected in 2004, 2005, and 2006 from three primary sites in the upper Missouri River: (1) immediately downstream of Gavins Point Dam, (2) the confluence of the Niobrara and Missouri rivers (i.e., Lewis and Clark Lake), and (3) the confluence of the White and Missouri rivers (i.e., Lake Francis Case). Paddlefish were collected with drifted large meshed gill nets by USFWS and SDGFP from the White River, Lake Francis Case, in Lewis and Clark Lake near the Niobrara River, and near the James River downstream of Gavins Point Dam. Additional samples downstream of Gavins Point Dam were collected with static large-meshed gill nets set in the tailrace by NGPC as part of annual paddlefish tagging efforts (Walburg 1971; Stastny 1990; Mestl and Sorensen 2009) or from anglers as part of the fall snagging fishery. Tissue samples in 2004, 2005, and 2006 were obtained by the USFWS from broodfish used in annual spawning operations. Approximately 100 larval fish from individual female families were sampled in these same years from the Gavins Point NFH for assignment of male paternity and reproductive contribution. Tissue samples consisted of either an anal fin, caudal fin (for fingerlings), or opercular flap clip or a larval fish (immediately posthatch). All tissues were preserved in 95–100% ethanol before transport to the Molecular Conservation Genetics Laboratory at the University of Wisconsin-Stevens Point.

DNA Analysis

All sampled tissues had DNA extracted using a Qiagen DNeasy tissue kit[1] (Qiagen, Inc., Valencia, California, USA) according to the manufacturer's recommended protocol. For fin and opercular tissue, we used approximately 25 mg of tissue, and for the newly hatched larvae, the entire larval fish was used for extractions. Extracted DNA was checked for quality by electrophoresing all samples on a 1% agarose gel to check for high molecular weight DNA and quantified using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). All sampled DNA was normalized to 20 ng/μg prior to genotyping.

Multi-locus, microsatellite genotyping was conducted using five microsatellites (Psp-20, -21, -26, -28, and -32) described by Heist et al. (2002). We fluorescently labeled the forward primer for each primer set, amplified individual loci using the conditions prescribed in Heist et al. (2002), and genotyped the samples on an ABI 377XL DNA Prism DNA sequencer (Applied Biosystems, Inc., Foster City, California, USA). All samples included a ROX-labeled GeneFlo-625 (Chimerx, Inc., Milwaukee, Wisconsin, USA) in-lane standard to facilitate accurate sizing using Genescan (ABI) software. All broodstock fish were genotyped a minimum of two times to ensure accuracy of genotypes for parental assignment. Where errors in genotype scoring occurred, a third genotype was collected.

Genetic diversity within the potential broodstock populations and the stocked fish (Objective 2) was estimated as the mean number of alleles per locus (A), number of private alleles (alleles occurring only in a single population), and observed (H_o) and expected (H_e) heterozygosity. Conformance to Hardy-Weinberg (HW) expectations was tested at each locus using the exact HW test of Guo and Thompson (1992) as implemented by Genepop v4.0 (Rousset 2008). Tests of genotypic disequilibrium, the independence of genotypes at each locus, were likewise performed using Genepop v4.0 (Rousset 2008). Interpopulation genetic differences were assessed between potential brood sources and the

[1] Use of trade names throughout manuscript does not constitute endorsement by the U.S. Federal Government.

stocked paddlefish in a pairwise fashion by conducting either a Fisher's exact test of genic differentiation (test of independence of allele frequencies) or a test of genotypic differentiation (test of independence of genotype frequencies) as implemented in Genepop v4.0 (Rousset 2008). In all cases, a sequential Bonferroni correction was used to correct for multiple pairwise tests (Rice 1989) with initial nominal $\alpha = 0.05$.

Genetic paternity of the paddlefish crosses was conducted by comparing the genotypes of a sample of larval fish to the parental genotypes. In all cases, tests were confined to a single female with known potential male contributors. We used the software package Cervus 3.0 (Kalinowski et al. 2007), which uses a likelihood approach to determine the most likely pair of parents for a given individual. In our case, we used the known parental genotypes and a strict (95%) confidence criteria.

Following the results of paternity testing, we calculated the predicted effective number of breeders (N_b) in our test crosses with the observed N_b based on the observed variance of male reproductive success according to Hedrick (2005). The N_b of a species approximates the genetic number of fish contributing to a given cohort. This number is not solely contingent on census numbers but is impacted by unequal sex ratios and variance in reproductive success (Waples 1989; Frankham et al. 2001). In cases of unequal sex ratio, the expected N_b in a given year is predicted to be

$$N_b = \frac{4N_{\mathrm{bf}}N_{\mathrm{bm}}}{N_{\mathrm{bf}} + N_{\mathrm{bm}}},$$

where N_{bf} is the effective number of breeding females (in our case, just the number of females used) and N_{bm} is the effective number of breeding males calculated, assuming random differences in production as the total number of males used or, if differences exceed random expectations (i.e., biased survival/reproduction), calculated according to the following formula:

$$N_{\mathrm{bm}} = \frac{N_m \bar{k}_m - 1}{\bar{k}_m - 1 + \left(V_{\mathrm{km}} / \bar{k}_m \right)},$$

where N_m is the number of males in the cross, $\bar{k}_m$ is the mean number of offspring produced per male, and V_{km} is the variance in the number of progeny produced by all males in the cross (Hedrick 2005). Methods for assessing the significance of the observed N_b versus expected N_b based strictly on the number of broodfish used are available when the studies look at adult returns of hatchery fish (Waples 2002; Moyer et al. 2007). However, in our study, only family variance at the larval stage was estimated. Therefore, only absolute values and percent reductions of observed N_b versus expected N_b were estimated.

Results

Sample sizes for the three potential brood sources ranged from 31 to 83, and the intrapopulational genetic diversity measures were consistent with the rangewide study of Heist and Mustapha (2008) (Table 1). Heterozygosity values were fairly high (mean $H_e = 0.6075$), and the mean number of observed alleles were also quite high (mean $A = 7.50$). No significant deviations were observed for either HW equilibrium or gametic phase disequilibrium among the three brood sources following sequential Bonferroni correction. Significant deviations from HW expectations were observed at two loci for the 2006 fingerling fish (Psp-26 and -28). Tests of interpopulation differentiation among the three brood sources were nonsignificant (minimum P = 0.319). Tests of genotypic differentiation among the fish stocked in 2006 and the Niobrara River confluence broodfish used to produce them were highly significant ($P = 0.00023$), suggesting that a significant

Table 1. Intrapopulation genetic diversity measures from three potential paddlefish brood stock sources from the Missouri River in South Dakota and Nebraska where *N* is the sample size, H_e is expected heterozygosity, H_o is observed heterozygosity, *A* is the mean number of alleles/locus, and A_p is the number of private alleles observed. The 2006 hatchery progeny were produced at US Fish and Wildlife Service Gavins Point National Fish Hatchery from brood fish collected in Lewis and Clark Lake.

Population	N	H_e	H_o	A	A_p
Below Gavins Point Dam - Tailrace	83	0.6307	0.6392	9.40	4
Lake Francis Case/White River Confluence	41	0.6164	0.6146	7.60	1
Lewis and Clark Lake/Niobrara River Confluence	31	0.6340	0.6411	8.00	1
2006 Stocked Fish	56	0.5489	0.6143	5.00	0

bias in reproduction and/or survival was occurring.

Parentage analysis showed consistent bias in paternal contribution regardless of mixed-milt versus equal-milt types of crosses performed (Table 2). Male contribution to hatched larvae from an individual female cross ranged from 1% to 86%. All crosses showed significant deviations from a random expectation using a chi-square analysis ($P < 0.00001$ in all tests). The distribution of success was not consistent with a strict fertility explanation, as males were used multiple times in the mixed-milt crosses and showed disparate results among crosses. For example, in the mixed-milt cross of female B, male #11 contributed 59% of sampled larvae, whereas in female C, this same male contributed only 3%. The end result is that significant bias occurred in terms of male reproductive success under both a mixed-milt and an equal-milt design. The impact of this bias can be seen in predictions of N_b (Table 2). The proportional reduction in N_b due to nonrandom bias in male reproductive success ranged from 4.8% (cross A) to 21.2% (cross C). For example, under a random bias in male reproductive success, the N_b of crosses D and E would have been expected to be 3.33. However, when the variance of male reproductive success is taken into account, cross D exhibits an $N_b = 3.10$, approximately 7% lower than expected if male contribution deviated only according to random expectations. Cross E exhibited an even larger bias resulting in a 10.1% reduction in observed N_b.

Discussion

No apparent differences existed among the potential brood sources considered for supplying gametes for the Gavins Point NFH's paddlefish propagation program, suggesting that all three putative sources could be used for gametes in this area. Two of the three locations used for brood sources in this study, Lake Francis Case and Lewis and Clark Lake, were within the middle Missouri River drainage, highlighted for genetic assessment in the MICRA paddlefish genetics plan (MICRA Paddlefish and Sturgeon Committee 1998). However, the Missouri River from Gavins Point Dam downstream to the Osage River was not included as a locality in need of assessment in the MICRA genetics plan. Our third site, from Gavins Point Dam to the James River, was genetically similar to the middle Missouri River and can be included within the greater middle Missouri River management unit. Although the primary sources of fish propagated by this program are thought to be devoid of natural recruitment, the probability exists that stocked fish will be able to interact with wild, naturally recruiting paddlefish populations as a result of the species' propensity to migrate (Rosen et al. 1982). Currently, emigration rates of pad-

Table 2. Paddlefish paternity assignment for two types of crosses, mixed-milt and equal-milt, made during 2006 propagation efforts at the Gavins Point National Fish Hatchery where Female is the single female family designation, Male represents the males used to cross an individual female, Prop. is the relative proportional contribution of a given male to a female's production, df is the chi-square test degrees of freedom, $\chi 2$ is the chi-square test statistic, P is the chi-square estimated p-value versus equal contribution, V_k is the variance of male reproductive success in a given cross, $\bar{k}$ is the mean number of offspring per male, N_b is the estimate of the effective number of breeders given V_k, N_b exp. represents the expected effective number of breeders if male reproductive success differs according to random (Poisson) expectations, and % is the percent reduction in N_b given the bias in male reproductive success.

Female	Male	N	Prop.	df	χ^2	P	V_k	$\bar{k}$	N_b	N_b exp.	%
					Mixed-milt crosses						
A	M1	18	0.247	7	34.507	<0.0001	44.982	9.125	3.386	3.556	4.8
	M2	3	0.041	–	–	–	–	–	–	–	–
	M3	3	0.041	–	–	–	–	–	–	–	–
	M4	15	0.205	–	–	–	–	–	–	–	–
	M5	17	0.233	–	–	–	–	–	–	–	–
	M6	3	0.041	–	–	–	–	–	–	–	–
	M7	4	0.055	–	–	–	–	–	–	–	–
	M8	10	0.137	–	–	–	–	–	–	–	–
B	M1	1	0.014	8	172.23	<0.0001	167.444	7.778	2.836	3.600	21.2
	M2	3	0.043	–	–	–	–	–	–	–	–
	M3	1	0.014	–	–	–	–	–	–	–	–
	M5	1	0.014	–	–	–	–	–	–	–	–
	M7	3	0.043	–	–	–	–	–	–	–	–
	M8	9	0.129	–	–	–	–	–	–	–	–
	M9	1	0.014	–	–	–	–	–	–	–	–
	M10	10	0.143	–	–	–	–	–	–	–	–
	M11	41	0.586	–	–	–	–	–	–	–	–
C	M2	61	0.859	3	140.78	<0.0001	832.917	17.750	2.855	3.200	10.8
	M3	3	0.042	–	–	–	–	–	–	–	–
	M10	5	0.070	–	–	–	–	–	–	–	–
	M11	2	0.028	–	–	–	–	–	–	–	–
					Equal-milt crosses						
D	M2	19	0.216	4	34.39	<0.0001	151.300	17.600	3.102	3.333	6.9
	M7	24	0.273	–	–	–	–	–	–	–	–
	M11	6	0.068	–	–	–	–	–	–	–	–
	M12	34	0.386	–	–	–	–	–	–	–	–
	M13	5	0.057	–	–	–	–	–	–	–	–
E	M2	30	0.370	4	46.47	<0.0001	188.200	16.200	2.996	3.333	10.1
	M5	26	0.321	–	–	–	–	–	–	–	–
	M8	2	0.025	–	–	–	–	–	–	–	–
	M10	1	0.012	–	–	–	–	–	–	–	–
	M11	22	0.272	–	–	–	–	–	–	–	–

dlefish through Fort Randall and Gavins Point dams are unknown, but natural reproduction, documented in Lewis and Clark Lake (Unkenholz 1979), is hypothesized to provide a source of recruitment to the only open sport fishery downstream of Gavins Point Dam (Walburg 1971; Stastny 1990; Mestl and Sorensen 2009). This emigration coupled with the documented genetic structure throughout the species'

range (Heist and Mustapha 2008) make it imperative to employ a genetically sound strategy to minimize risks to the genetic integrity of native paddlefish populations and encompass consistent management approaches within biologically relevant reaches of the Missouri River (Rosen et al. 1982).

The significant genetic differences observed between the stocked out fingerlings and the known broodfish used to spawn were consistent with differential production and/or survival in the hatchery, which is problematic for several reasons. First, differential survival of offspring is consistent with domestication selection wherein the traits of individual genetic lineages that make fish more fit in the hatchery environment may prove detrimental after release in the wild (Hindar et al. 1991; Johnsson and Abrahams 1991; Weber and Fausch 2003; Huntingford 2004). Under these circumstances, the best result one could hope for is a selection differential so severe that stocked paddlefish fail to survive; however, stocked paddlefish in Lake Francis Case are surviving (Walburg 1971; Stastny 1990; Mestl and Sorensen 2009). Alternatively, the selection differential is often not sufficient to result in death, but instead introduces the risk of introgressive reproduction with native fish; in this region of the Missouri River, the only known naturally spawning fish are in the remnant riverine section of Lewis and Clark Lake (Unkenholz 1980). The domesticated characters can then be introduced into native gene pools, potentially harming the very species the propagation was meant to protect. A study of Atlantic salmon *Salmo salar* showed a significant inverse relation between the proportion of genetic introgression of domestic genes into wild fish and overall survival attributed to outbreeding depression (McGinnity et al. 2003). Under this scenario, excessive hybridization of domestic fish (stocked at a large excess to wild fish) could result in the lowering of mean fitness of the native species.

The high variance observed in male reproductive success is consistent with several other studies that have found sperm competition, differential hatching, and order of fertilization to be critical factors in explaining high male variance (Gharrett and Shirley 1985; Liley et al. 2002; McLean et al. 2008). The impact of this phenomenon is that genetic guidelines used to establish a minimum effective number of breeders per year underestimate the numbers of broodfish actually needed to maintain the genetic characteristics of the population(s). Subsequently, this increases the risk of genetic erosion of founding paddlefish populations and/or increased risk of a Ryman-Laikre effect in the receiving paddlefish populations (reviewed previously; Ryman and Laikre 1991). For the paddlefish program, the MICRA guidelines suggested a minimum of 25 pairs of breeding fish for restoration work and as little as two to five pairs for supplemental stocking (MICRA Paddlefish and Sturgeon Committee 1998). This recommendation equates to an annual N_b of 50 for restoration efforts and between 4 and 10 for supplementation. Over a typical generation time for paddlefish (10–15 years), this equates to an effective population size (N_e) of 500–600 for restoration and 40–150 for supplementation. Our findings suggest that an approximately 10% reduction in N_e will be realized if multi-male spawning occurs, due to the interaction of an unequal sex ratio and high variance of male reproductive success.

In an ideal circumstance, hatchery propagation of paddlefish would adhere to a 1:1 male:female sex ratio, with a single male spawning a single female with no multiple use of males (Miller and Kapuscinski 2003). Under this scenario, the 1:1 families would be hatched separately before combining the families. Even under this scenario, differential survival could occur, but this would

at least get the families through the initial male-biased restriction of offspring. We realize this scenario is generally untenable for the current hatchery system given the large hatching space required. Further, the biology and/or gear bias of paddlefish often results in a larger number of males versus females being captured, further making idealized 1:1 crosses difficult.

Intermediate steps can pragmatically remediate some of the negative genetic aspects of the high variance observed in male reproductive success or differential male contributions. Adherence to a 1:1 crossing strategy is likely improbable given that male paddlefish are captured in greater numbers than females and females numbers are often limited. One approach could be to bring the excess males to the hatchery and fertilize a strict number of males/female, with males not being used for more than a single female (Miller and Kapuscinski 2003). Each female provides multiple egg takes after injection with luteinizing hormone, and each batch of eggs could be fertilized with a unique male(s). Additionally, if excess males are captured, cryopreservation of milt could be pursued to enable greater flexibility in family crosses but entails the costs of long term storage.

This study was conducted to better understand the impacts of current hatchery strategies on the genetic diversity of paddlefish produced at the Gavins Point NFH. During this study, we were able to confirm that three potential sources of broodfish are genetically indistinguishable, allowing the management agencies cooperating on this program flexibility in sampling gametes. We also showed significant bias occurred in terms of reproductive contribution, resulting in a shift in the genetic diversity of fish produced in the hatchery versus the genetic characteristics of the broodfish used to produce them. This shift was further shown to likely be the result of differential male contributions, partially attributed to the mode of fertilizing eggs. Minor modifications outlined above can lessen the negative impacts associated with biased sex ratios and high variance in reproductive success while still able to meet the dual management goals of protecting genetic diversity and achieving annual stocking targets for population supplementation. We included this study in this chapter to illustrate the value of genetic data to assess and monitor current propagation approaches and integrating genetic data into an adaptive management strategy for future paddlefish propagation.

Summary

Genetic diversity in paddlefish across its native range is beginning to yield key findings associated with managing this highly vagile, ancient species. Despite the work reviewed in this chapter, we stress that more work associated with better understanding of (1) the fine-scale genetic structuring of paddlefish within and among major watersheds, (2) the realized impacts (both positive and negative) of paddlefish propagation, and (3) potential unique or rare genetic variants that may be vital for long-term sustainability of paddlefish is necessary to properly address MICRA's stated goals for paddlefish genetic resource management. We believe the paddlefish represents a crucial element in the big river ecosystems of North America, as well as a model organism for better understanding the ecology and genetic resources of long-lived freshwater fish species. We hope the review and research we have presented in this chapter aids the reader in better understanding what we currently know and future uses of genetic techniques in managing this species.

Acknowledgments

This study would not have been possible without the openness and cooperation of the staff at the U.S. Fish and Wildlife Ser-

vice (USFWS), Gavins Point National Fish Hatchery in Yankton, South Dakota: Herb Bollig–Project Leader (now retired), Keith McGilvray–Assistant Project Leader, Craig Bockholt, and Marvin Ehlers. Field collections of paddlefish broodstock were assisted by Kristen Grohs, Wayne Stancill, and Greg Wanner at USFWS, Great Plains Fish and Wildlife Conservation Office, Pierre, South Dakota and Jason Sorensen and staff from South Dakota Department of Game, Fish, and Parks office in Chamberlain. Additional paddlefish tissue samples below Gavins Point Dam were collected by Gerald Mestl and Kirk Steffensen and their staff from the Nebraska Game and Parks Commission in Lincoln. Assistance processing genetics samples at the University of Wisconsin-Stevens Point Molecular Conservation Genetics Laboratory was provided by Ed Murphy, Justin VanDeHey, Mike Hughes, and Todd Kittel. A special thanks is given to Ryan Franckowiak for genetics laboratory management and assistance and parentage analysis. Partial funding for this work was provided by a U.S. Department of the Interior Fish and Wildlife Service Intra-Agency Agreement No. 64310 with the U.S. Geological Survey.

References

Allendorf, F. W., and G. Luikart. 2007. Conservation and the genetics of populations. Blackwell Scientific Publications Publishing, Oxford, UK.

Allendorf, F. W., and N. Ryman. 1987. Genetic management of hatchery stocks. Pages 141–159 *in* N. Ryman, and F. Utter, editors. Population genetics and fishery management. University of Washington Press, Seattle.

Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.

Bailey, R. M., and G. R. Smith. 1981. Origin and geography of the fish fauna of the Laurentian Great Lakes basin. Canadian Journal of Fisheries and Aquatic Sciences 38:1530–1561.

Bernatchez, L., and C. C. Wilson. 1998. Comparative phylogeography of nearctic and palearctic fishes. Molecular Ecology 7:431–452.

Berst, A. H., and R. C. Simon, editors. 1981. Proceedings of the stock concept international Symposium (STOCS). Canadian Journal of Fisheries and Aquatic Sciences 38:1457–1923.

Billington, N. 2003. Mitochondrial DNA. Pages 59–100 *in* E. M. Hallerman, editor. Population genetics: principles and applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland.

Birky, C. W. J., T. Marayama, and P. Fuerst. 1983. An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. Genetics 103:513–527.

Brown, W. M. 1983. Evolution of animal mitochondrial DNA. Pages 281–323 *in* M. Nei, and R. K. Koehn, editors. Evolution of genes and proteins. Sinauer Associates, Sunderland, Massachusetts.

Booke, H. E. 1999. The stock concept revisited: perspectives on its history in fisheries. Fisheries Research 43:9–11.

Busack, C. A., and K. P. Currens. 1995. Genetic risks and hazards in hatchery operations: fundamental concepts and issues. Pages 71–80 *in* S. H. L. Jr., and P. R. G., editors. Uses and effects of cultured fishes in aquatic ecosystems. American Fisheries Society, Symposium 15, Bethesda, Maryland.

Carlson, D. M., M. K. Kettler, S. E. Fisher, and G. S. Whitt. 1982. Low genetic variation in paddlefish populations. Copeia 1982:721–725.

DeHaan, P. W., G. R. Jordan, and W. R. Ardren. 2008. Use of genetic tags to identify captive-bred pallid sturgeon (*Scaphirhynchus albus*) in the wild: improving abundance estimates for an endangered species. Conservation Genetics 9:691–697.

Edmands, S. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. Molecular Ecology 16:463–475.

Epifanio, J. M., J. B. Koppelman, M. A. Nedbal, and D. P. Philipp. 1996. Geographic variation of paddlefish allozymes and mito-

chondrial DNA. Transactions of the American Fisheries Society 125:546–561.

Feldheim, K. A., S. H. Gruber, J. R. C. de Marignac, and M. V. Ashley. 2002. Genetic tagging to determine passive integrated transponder tag loss in lemon sharks. Journal of Fish Biology 61:1309–1313.

Ferguson, M. M., and R. G. Danzmann. 1998. Role of genetic markers in fisheries and aquaculture: useful tools or stamp collecting? Canadian Journal of Fisheries and Aquatic Sciences 55:1553–1563.

Flagg, T. A., and C. E. Nash, editors. 1999. A conceptual framework for conservation hatchery strategies for Pacific salmonids. Department of Commerce, NOAA Technical Memorandum, NMFS-NWFSC-38, Silver Spring, Maryland.

Frankham, R., J. D. Ballou, and D. A. Briscoe. 2001. Introduction to conservation genetics. Cambridge University Press, New York.

Friberg, D. V. 1972. Paddlefish abundance and harvest within a population lacking recruitment, Big Bend tailwaters, 1969–71 South Dakota. South Dakota Department of Game Fish and Parks, National Marine Fisheries Service Completion Report 4–61-R Segments 1, 2, 3, Pierre.

Friberg, D. V. 1973. Investigation of paddlefish populations in South Dakota and development of management plans, 1972. South Dakota Department of Game Fish and Parks, Dingell-Johnson Project F-15-R-7, Study IX, Jobs 1–5, Pierre.

Fries, L. T., and P. F. Hutson. 1993. Genetic analysis of paddlefish: a comparison of individuals from Missouri, South Dakota, and Texas. Texas Parks and Wildlife Department, Data Management Series 87, Austin.

Gall, G. A. E. 1987. Inbreeding. Pages 47–87 *in* N. Ryman, and F. Utter, editors. Population genetics and fishery management. University of Washington Press, Seattle.

Gharrett, A. J. and S. M. Shirley. 1985. A genetic examination of spawning methodology in a salmon hatchery. Aquaculture 47: 245–256.

Gharrett, A. J., and W. W. Smoker. 1991. Two generations of hybrids between even- and odd-year pink salmon (*Oncorhynchus gorbuscha*): a test for outbreeding depression. Canadian Journal of Fisheries & Aquatic Sciences 48:1744–1749.

Gharrett, A. J., W. W. Smoker, R. R. Reisenbichler, and S. G. Taylor. 1999. Outbreeding depression in hybrids between odd- and even-broodyear pink salmon. Aquaculture 173:117–129.

Graham, K. 1997. Contemporary status of the North American paddlefish, *Polyodon spathula*. Environmental Biology of Fishes 48:279–289.

Grant, W. S., I. B. Spies, and M. F. Canino. 2006. Biogeographic evidence for selection on mitochondrial DNA in North Pacific walleye pollock *Theragra chalcogramma*. Journal of Heredity 97:571–580.

Guo, S. W. and E.A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48: 361–372.

Hallerman, E. M. 2003. Population genetics: principles and applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland.

Hebert, P. D. N., and M. J. Beaton. 1989. Methodologies for allozyme analysis using cellulose acetate electrophoresis. Helena Laboratories, Beaumont, Texas.

Hedrick, P. W. 2005. Genetics of populations, 3rd edition. Jones and Bartlett Publishers, Boston.

Heist, E. J., and A. Mustapha. 2008. Rangewide genetic structure in paddlefish inferred from DNA microsatellite loci. Transactions of the American Fisheries Society 137:909–915.

Heist, E. J., E. H. Nicholson, J. T. Sipiorski, and D. B. Keeney. 2002. Microsatellite markers for the paddlefish (*Polyodon spathula*). Conservation Genetics 3:205–207.

Hindar, K., N. Ryman, and F. Utter. 1991. Genetic effects of cultured fish on natural fish populations. Canadian Journal of Fisheries and Aquatic Sciences 48: 945–957.

Huntingford, F. A. 2004. Implications of domestication and rearing conditions for the behavior of cultivated fish. Journal of Fish Biology 65(Supplement A):122–142.

Incerpi, A. 1996. Hatchery-bashing: a useless pastime. Fisheries 21:28.

Inoue, J. G., M. Miya, K. Tsukamoto, and M. Nishida. 2003. Basal actinopterygian relationships: a mitogenomic perspective on the phylogeny of the "ancient fish." Molecular Phylogenetics and Evolution 26:110–120.

Johnsson J. I., and M. V. Abrahams. 1991. Interbreeding with domestic strain increases foraging under threat of predation in juvenile steelhead trout (*Oncorhynchus mykiss*): an experimental study. Canadian Journal of Fisheries and Aquatic Sciences 48:243–247.

Kalinowski, S. T., M. L. Taper, and T.C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099–1006.

Kincaid, H. L. 1983. Inbreeding in fish populations used in aquaculture. Aquaculture 33:215–227.

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Pääbo, F. X. Villablanca, and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences of the United States of America 86:6196–6200.

Lande, R. and S. Shannon. 1996. The role of genetic variation in adaption and population persistence in a changing environment. Evolution 50:434–437.

Lansman, R. A., R. O. Shade, J. F. Shapira, and J. C. Avise. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. Journal of Molecular Evolution 17:214–226.

Larkin, P. A. 1972. The stock concept and management of Pacific salmon. Pages 11–15 *in* R. C. Simon and P. S. Larkin, editors. The stock concept in Pacific salmon. University of British Columbia, H. R. MacMillan Lectures in Fisheries, Vancouver.

Levinson, G., and G. A. Gutman. 1987. Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Molecular Biology & Evolution 4:203–221.

Liley, N. R., P. Tamkee, R. Tsai, and D. J. Hoysak. 2002. Fertilization dynamics in rainbow trout (*Oncorhynchus mykiss*): effect of male age, social experience, and sperm concentration and motility on in vitro fertilization. Canadian Journal of Fisheries and Aquatic Sciences 59: 144–152.

May, B. 2003. Allozyme variation. Pages 23–36 *in* E. M. Hallerman, editor. Population genetics: principles and applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland.

McGinnity, P., P. Prodohl, K. Ferguson, R. Hynes, N. O'Maoileidigh, N. Baker, D. Cotter, B. O'Hea, D. Cooke, G. Rogan, J. Taggart, and T. Cross. 2003. Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. Proceedings of the Royal Society of London Series B 270:2443–2450.

McKay, J. K., and R. G. Latta. 2002. Adaptive population divergence: markers, QTL and traits. Trends in Ecology and Evolution 17:285–291.

McLean, J. E., T. R. Seamons, M. B. Dauer, P. Bentzen, and T. P. Quinn. 2008. Variation in reproductive success and effective number of breeders in a hatchery population of steelhead trout (*Oncorhynchus mykiss*): examination by microsatellite-based parentage analysis. Conservation Genetics 9: 295–304.

Mestl, G., and J. Sorensen. 2009. Joint management of an interjurisdictional paddlefish snag fishery in the Missouri River below Gavins Point Dam, South Dakota and Nebraska. Pages 235–259 *in* C. Paukert and G. Scholten, editors. Paddlefish management, propagation, and conservation in the 21st century: building from 20 years of research and management. American Fisheries Society, Symposium 66, Bethesda, Maryland.

Miller, L. M., and A. R. Kapuscinski. 2003. Genetic guidelines for hatchery supplementation programs. Pages 329–355 *in* E. M. Hallerman, editor. Population genetics: principles and applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland.

MICRA (Mississippi Interstate Cooperative Resource Association) Paddlefish and Sturgeon Committee. 1998. MICRA paddlefish genetics plan. Mississippi Interstate Cooperative Resource Association, Bettendorf, Iowa. Available: www aux.cerc.cr.usgs.gov/MICRA/pfgenetics.pdf (January 2009).

Moran, P., D. J. Teel, E. S. LaHood, J. Drake, and S. Kalinowski. 2006. Standardizing multilab oratory microsatellite data in Pacific salmon: an historical view of the future. Ecology of Freshwater Fish 15:597–605.

Morin, P. A., G. Luikart, and R. K. Wayne. 2004. SNPs in ecology, evolution and conservation. Trends in Ecology & Evolution 19:208–216.

Moyer, G. R., M. S., Blouin, and M. A. Banks. 2007. The influence of family-correlated survival on N_b/N for progeny from integrated multi- and single-generation hatchery stocks of coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Sciences 64:1258–1265.

Murphy, R. W., J. M. J. Sites, D. G. Buth, and C. H. Haufler. 1996. Proteins: isozyme electrophoresis. Pages 51–120 *in* D. M. Hillis, C. Moritz, and B. K. Mable, editors. Molecular systematics. Sinauer Associates, Inc., Sunderland, Massachusetts.

Grady, J. M., and B. S. Elkington. 2009. Establishing and maintaining paddlefish populations by stocking. Pages 385–396 *in* C. Paukert and G. Scholten, editors. Paddlefish management, propagation, and conservation in the 21st century: building from 20 years of research and management. American Fisheries Society, Symposium 66, Bethesda, Maryland.

O'Connell, M., and J. M. Wright. 1997. Microsatellite DNA in fishes. Reviews in Fish Biology & Fisheries 7:331–363.

Hansen, K. A., and C. P. Paukert. 2009. Current management of paddlefish sport fisheries. Pages 277–290 *in* C. Paukert and G. Scholten, editors. Paddlefish management, propagation, and conservation in the 21st century: building from 20 years of research and management. American Fisheries Society, Symposium 66, Bethesda, Maryland.

Philipp, D. P., and J. E. Claussen. 1995. Fitness and performance differences between two stocks of largemouth bass from different river drainages within Illinois. Pages 236–243 *in* H. L. Schramm, Jr. and R. G. Piper. Uses and effects of cultured fishes in aquatic ecosystems. American Fisheries Society, Symposium 15, Bethesda, Maryland.

Philipp, D. P., and G. S. Whitt. 1991. Survival and growth of northern, Florida, and reciprocal F_1 hybrid largemouth bass in central Illinois. Transactions of the American Fisheries Society 120:58–64.

Quist, M. C., M. J. Steuck, and M. M. Marron. 2009. Commercial harvest of paddlefish in the upper Mississippi River. Pages 345–355 *in* C. Paukert and G. Scholten, editors. Paddlefish management, propagation, and conservation in the 21st century: building from 20 years of research and management. American Fisheries Society, Symposium 66, Bethesda, Maryland.

Reed, D. H., and R. Frankham. 2003. The correlation between population fitness and genetic diversity. Conservation Biology 17:230–237.

Rice, W. R. 1989. Analyzing tables of statistical significance. Evolution 43:223–225.

Rogers, S. M., and L. Bernatchez. 2005. Integrating QTL mapping and genome scans towards the characterization of candidate loci under parallel selection in the lake whitefish (*Coregonus clupeaformis*). Molecular Ecology 14:351–361.

Rosen, R. A., D. C. Hales, and D. G. Unkenholz. 1982. Biology and exploitation of paddlefish in the Missouri River below Gavins Point Dam. Transactions of the American Fisheries Society 111:216–222.

Rousset, F. 2008. Genepop'007: a complete reimplementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8: 103–106.

Ryman, N., and L. Laikre. 1991. Effects of supportative breeding on the genetically effective population size. Conservation Biology 5:325–329.

Scholten, G. D. 2009. Management of commercial paddlefish fisheries in the United States. Pages 291–306 *in* C. Paukert and G. Scholten, editors. Paddlefish management, propagation, and conservation in the 21st century: building from 20 years of research and management. American Fisheries Society, Symposium 66, Bethesda, Maryland.

Kerns, J. A., P. W. Bettoli, and G. D. Scholten. 2009. Mortality and movements of paddlefish released as bycatch in a commercial fishery in Kentucky Lake, Tennessee. Pages 329–343 *in* C. Paukert and G. Scholten, editors. Paddlefish management, propagation, and conservation in the 21st century: building from 20 years of research and management. American Fisheries Society, Symposium 66, Bethesda, Maryland.

Schramm, H. L., Jr., and R. G. Piper, editors. 1995. Uses and effects of cultured fishes in

aquatic ecosystems. American Fisheries Society, Symposium 15, Bethesda, Maryland.

Shaklee J. B., and K. P. Currens. 2003. Genetic stock identification and risk assessment. Pages 281–328 *in* E. M. Hallerman, editor. Population genetics: principles and applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland.

Stastny, W. M. 1990. Bionomics of paddlefish (*Polyodon spathula*) in the Missouri river between Fort Randall and Gavins Point Dams. Master's thesis, University of South Dakota, Vermillion.

Szalanski, A. L., R. Bischof, and G. Mestl. 2000. Population genetic structure of Nebraska paddlefish based on mitochondrial DNA variation. Transactions of the American Fisheries Society 129:1060–1065.

Templeton, A. 1986. Coadaptation and outbreeding depression. Pages 105–116 *in* M. E. Soule, editor. Conservation biology: the science of scarcity and diversity. Sinauer Associates Inc., Sunderland, Massachusetts.

Unkenholz, D. G. 1979. Investigation of paddlefish populations in South Dakota and development of management plans. South Dakota Department of Game Fish and Parks, Dingell-Johnson Project F-15-R-14, Jobs 3 and 7 completion reports, Pierre.

Unkenholz, D. G. 1980. Paddlefish spawning movements and reproductive success in the Missouri River below Fort Randall Dam, 1979. South Dakota Department of Game Fish and Parks, Progress report number 80–3, Pierre.

Utter, F., P. Aebersold, and G. Winans. 1987. Interpreting genetic variation detected by electrophoresis. Pages 21–45 *in* N. Ryman, and F. Utter, editors. Population genetics and fishery management. University of Washington Press, Seattle.

Van Oosterhout, C., M. K. van Heuven, and P. M. Brakefield. 2004. On the neutrality of molecular genetic markers: pedigree analysis of genetic variation in fragmented populations. Molecular Ecology 13:1025–1034.

Walburg, C. H. 1971. Loss of young fish in reservoir discharge and year class survival, Lewis and Clark Lake, Missouri River. Pages 441–448 *in* G. E. Hall, editor. Reservoir fisheries and limnology. American Fisheries Society, Special Publication 8, Bethesda, Maryland.

Waples, R. S. 1989. A generalized approach for estimating effective population size from temporal change in gene frequency. Genetics 121:379–391.

Waples, R. S. 2002. Evaluating the effect of stage-specific survivorship on the N_e/N ratio. Molecular Ecology 11:1029–1037.

Weber, E. D. and K. D. Fausch. 2003. Interactions between hatchery and wild salmonids in streams: differences in biology and evidence for competition. Canadian Journal of Fisheries and Aquatic Sciences 60: 1018–1036.

Wright, S. 1969. Evolution and the genetics of populations. Volume 2, the theory of gene frequencies. University of Chicago Press, Chicago.