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## Fish Hatchery Genetic Management Techniques: Integrating Theory with Implementation

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### **Abstract**

Artificial propagation of fish species in hatcheries has been conducted on a large scale for several decades. In recent years, however, there has been an increase in conservation hatcheries, which aim not only to produce fish for supplementing wild populations but also to preserve the genetic diversity and integrity of threatened or endangered species. Important considerations for the latter are maximizing genetic diversity and effective population size while minimizing inbreeding and adaptation to captivity. Several studies document the theoretical implementation of captive management strategies designed to achieve these goals. However, the practical application of many of these strategies to conservation hatcheries remains challenging, as the majority of the guidelines were developed for small zoo populations. The aims of this review are (1) to survey current fish conservation hatchery managers in order to assess current hatchery practices and goals; (2) to present available management strategies for conservation hatcheries that may minimize the genetic effects of artificial propagation; and (3) to present genetic management options and their trade-offs to managers developing fish conservation hatcheries. The results of the survey suggest that the majority of the responding conservation and nonconservation hatcheries use random broodstock selection and pairing techniques while valuing the importance of maintaining genetic diversity and effective population size and minimizing inbreeding. This article reviews the application of small-population management techniques to conservation hatcheries in an effort to increase their utility in recovery plans for endangered fish species.

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For over a century, the artificial propagation of fish species in hatcheries has been conducted on a large scale (Hard et al. 1992; Lichatowich 1999; Naish et al. 2008). Hatcheries have been noted as being attractive for highly fecund animals such as fish, as they may offer the best chance to recover populations through enhanced survival during protective propagation (Flagg et al. 1995; Flagg and Mahnken 2000; Pollard and Flagg 2004; O'Reilly and Doyle 2007). The majority of these efforts have been designed to enhance fisheries or to mitigate habitat alteration or harvest by replacing lost natural production (Mahnken et al. 1998; George et al. 2009). In recent years, there has been an increased interest in fish "conservation hatcheries," which share their goals and techniques with captive breeding programs designed for recovering endangered species (Pollard and Flagg 2004; Kozfkay et al. 2008; Conrad et al. 2013; Fisch et al. 2013; Osborne et al. 2013).

Conservation hatcheries aim to rebuild depleted stocks by minimizing the ecological and genetic impacts of releasing hatchery fish on wild populations (Flagg and Nash 1999; Utter and Epifanio 2002). Managing and operating conservation hatcheries requires that the unique characteristics of a species, its native habitat, and artificial propagation constraints be considered (Flagg and Nash 1999). The U.S. Endangered Species Act (ESA) recognizes artificial propagation as a potentially important conservation tool (Pollard and Flagg 2004), and hatchery fish can be protected under the ESA along with their natural counterparts. Recovery plans utilizing artificial propagation generally require collection of wild broodstock, culturing offspring from wild adults, and releasing offspring to supplement the wild population (Hard et al. 1992).

The purpose of this review is to summarize genetic management techniques, many of which originate from the zoo population management community, in the context of conservation hatcheries. The considerations that distinguish fish hatcheries from zoo populations include larger population sizes, higher fecundities, and high mortality. These differences make the relevance of intensive population management in fish hatcheries uncertain and create logistical challenges. This review is intended to present the various genetic management options to hatchery managers by providing a brief overview of broodstock selection methods, followed by an in-depth discussion of the broodstock spawning protocols that may be implemented in conservation hatcheries. In addition, it presents the results of a hatchery management survey and discusses the trade-offs of each spawning management strategy, which may vary with the goals of the hatchery. We sought to review the application of various management techniques to conservation hatcheries by addressing the following questions: (1) What are the main goals of conservation hatcheries? (2) What spawning management techniques are currently being used in conservation hatcheries? and (3) How do spawning management techniques differ depending on the goals of the hatchery?

## GOALS OF CONSERVATION HATCHERIES

The overarching goal for conservation hatcheries is to prevent the extinction of threatened or endangered stocks by enhancing natural production. Such hatcheries aim to minimize the genetic and ecological impacts of hatchery techniques during the collection, mating, and rearing of fish in an attempt to preserve the original genetic composition of the wild population in captivity (Pollard and Flagg 2004). To determine the current goals and techniques of conservation hatcheries throughout the United States, we conducted a survey of hatchery managers and fisheries professionals in January 2013. Survey respondents were identified by their affiliation with a fish hatchery in the United States, their e-mail addresses were collected, and a survey was distributed electronically. Question topics included the current hatchery management techniques used, the goals of hatchery management, the size of the programs, and the feasibility of implementing more rigorous hatchery management techniques (See Table A.1 in the appendix to this article).

A total of 251 individuals from different hatcheries or hatchery monitoring and evaluation programs were contacted; the 37 (14.7%) who responded represented 36 unique programs and a variety of species, such as Cutthroat Trout *Oncorhynchus clarkii*, steelhead *Oncorhynchus mykiss*, Chinook Salmon *Oncorhynchus tshawytscha*, Coho Salmon *Oncorhynchus kisutch*, Sockeye Salmon *Oncorhynchus nerka*, and Atlantic Salmon *Salmo salar*. A two-tailed Fisher's exact test was used to examine the significance of the association between categories for each of the questions. Twenty-eight of these programs were described by the respondents as conservation, integrated, or captive broodstock programs and some had additional production goals. Eight of the programs were defined as nonconservation programs with singular goals for mitigation and general production. The use of genetic management varied by the number of females spawned and the level of natural escapement of the programs surveyed (Figure 1). We hypothesized that hatcheries that spawned a larger number of females would more likely use random mating techniques due to the technical constraints imposed by the size of the hatchery. The results of the survey indicate that the largest class of hatchery (>1,000 females spawned) used random mating only, which is consistent with our expectations. We also hypothesized that the use of genetic management rather than random mating would depend on the level of natural escapement. The results of the survey indicate that some hatcheries with high levels of natural escapement use genetic management, which is contrary to the hypothesis that larger hatchery programs (defined by either the number of females spawned or the level of natural escapement) only use random mating.

Overall, the majority of the conservation hatcheries indicated that preserving genetic diversity, managing for effective population size, minimizing inbreeding, and providing fish for introduction were very important goals of their program, with 80, 55, 60, and 73% of the respondents rating these respective goals as

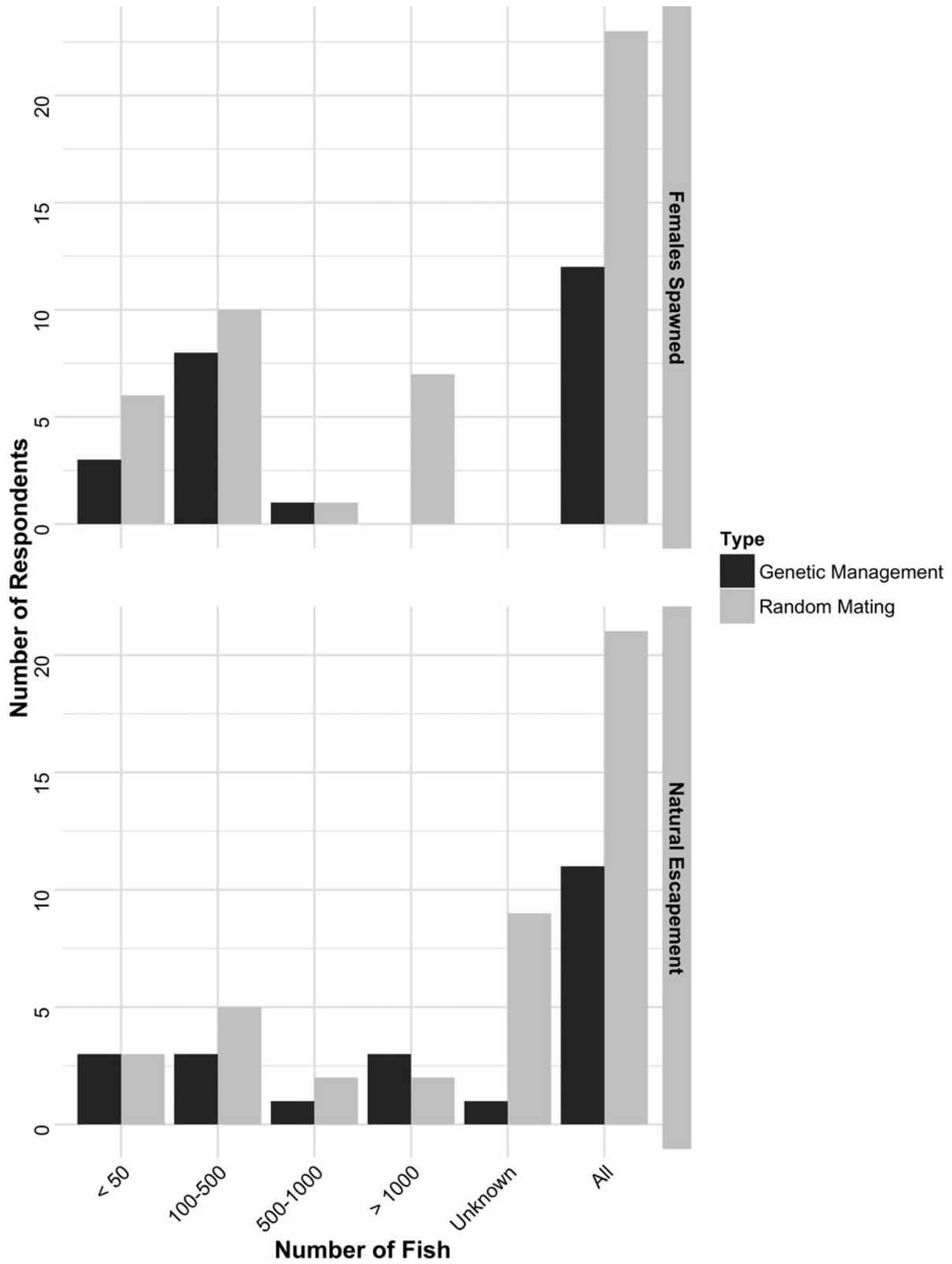


FIGURE 1. Survey results indicating the range of fish hatchery program size as defined by the number of females spawned and the level of natural escapement in relation to whether genetic methods or random mating was utilized.

TABLE 1. Survey respondents' rankings of the importance of four considerations—preserving genetic diversity, managing effective population size, minimizing inbreeding, and enabling reintroduction to the natural environment—to hatchery operations, by type of hatchery (conservation or nonconservation). The differences between hatchery types were all significant. Asterisks indicate that the question was unanswered by one or more respondents.

Consideration and ranking	Number of respondents	Type of hatchery		<i>P</i> -value
		Conservation	Nonconservation	
<b>Genetic diversity</b>				
Very important	28	27	1	<0.001
Somewhat important	5	1	4	
Not important	2	0	2	
Total	35*	28	7	
<b>Effective population size</b>				
Very important	19	18	1	0.006
Somewhat important	13	0	4	
Not important	2	0	2	
Total	34*	27	7	
<b>Inbreeding</b>				
Very important	21	20	1	0.014
Somewhat important	10	6	4	
Not important	4	2	2	
Total	35*	28	7	
<b>Reintroductions</b>				
Very important	25	23	2	0.008
Somewhat important	5	4	1	
Not important	4	1	3	
Total	34*	28	6	

very important (Table 1). In general, the conservation programs placed higher value on these goals than the nonconservation programs, a result that was significantly different when analyzed using Fisher's exact test. Broodstock selection and pairing methods varied by type of hatchery. The nonconservation hatcheries used random mating (and marks/tags) to both select and pair fish, while the conservation hatcheries used a variety of genetic methods (genetic broodstock selection, random mating, genetic relatedness, inbreeding avoidance matrices, and/or pedigrees). In most cases where genetic methods were being utilized, a combination of methods were used. However, the majority of hatcheries surveyed randomly selected and mated fish (62%).

The majority of the hatcheries surveyed consider genetic factors to be important to their program and, at a minimum, use genetic analyses for monitoring and evaluation. Additionally, the majority of respondents indicated that maintaining genetic diversity and effective population size while minimizing inbreeding were important goals of their program. However, there were no clear trends as to when genetic techniques were being utilized based on either the number of spawners or the level of natural escapement. Other considerations need to be taken into account for one to determine whether a hatchery will use genetic techniques, and this likely depends on the program's goals rather than its size. The results of the survey reveal a gap between the desired goals of a conservation hatchery and current hatchery ge-

netic management practices. As a result of the increasing desire to incorporate genetics into conservation hatchery management, this review outlines various hatchery management techniques that have been and may be employed in conservation hatcheries to achieve some of the above goals (Figure 2).

#### HATCHERY GENETIC MANAGEMENT TECHNIQUES

Multiple guidelines have been set forth for collecting and mating hatchery broodstock and rearing and releasing hatchery fish (Hard et al. 1992; Kapuscinski and Miller 1993; Miller and Kapuscinski 2003). Formal recommendations have been developed by NOAA–Fisheries for planning and evaluating artificial propagation programs under ESA Section 4(d) rules, Section 7 consultations, and Section 10 permits (termed hatchery and genetic management plans; NOAA 1999), and additional guidelines have been developed by the Pacific Salmon and California Hatchery Reform Scientific Review groups (HSRG 2009; California HSRG 2012). These general guidelines are prescriptive for best management practices in a hatchery. They include recommendations for assessing the effects of hatchery programs on listed populations, the relationship of a program to other management objectives for the species, hatchery facilities and water sources, broodstock origin, identity and collection, mating, incubation, rearing, release, and monitoring. Extensive research has been done on many of these topics (Flagg and Nash 1999;

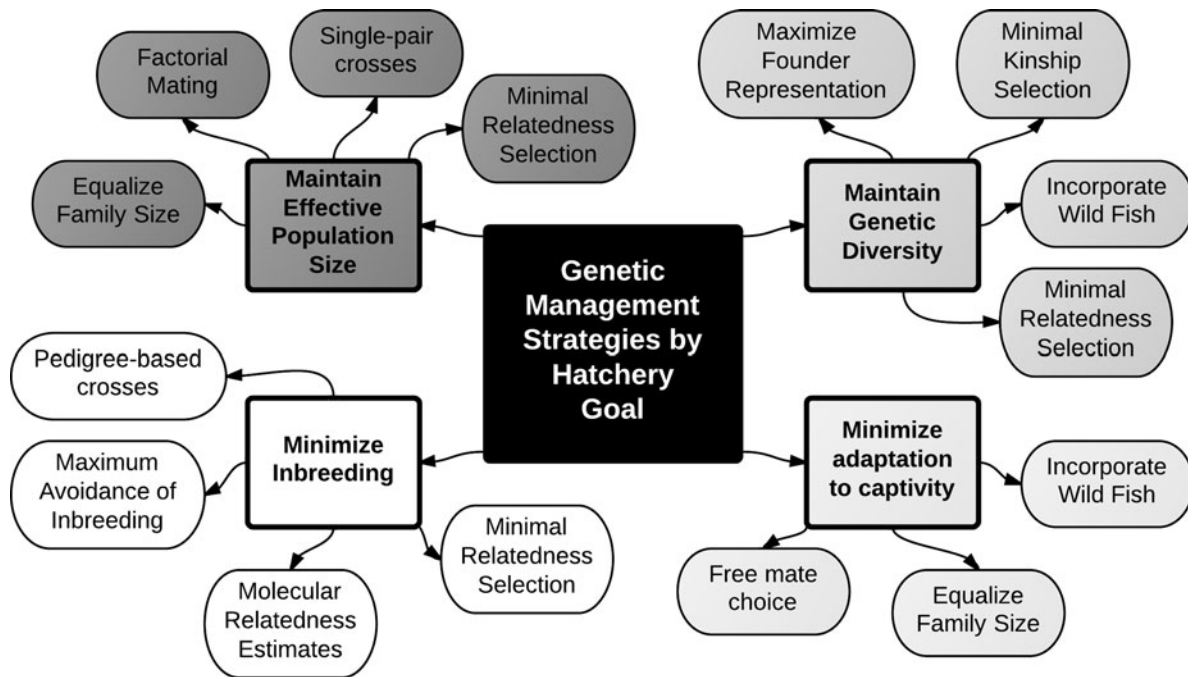


FIGURE 2. Summary of the goals of conservation hatcheries and the genetic management methods best suited to achieve each of these goals.

Pollard and Flagg 2004; Fraser 2008), although an in-depth analysis of possible mating strategies and their implementation in a hatchery has not yet been conducted. We provide an in-depth description of broodstock spawning protocols that may be employed in conservation hatcheries to preserve genetic diversity, maximize effective population size, and minimize inbreeding.

**Broodstock Spawning**

Broodstock spawning strategies range from randomly mating fish in the hatchery to the genetic selection of individuals to create desired pair crosses. Conservation hatcheries may use different combinations of broodstock spawning strategies depending on the goals of the program. There are two components to the goal of preserving genetic diversity that may warrant different broodstock spawning strategies: (1) maximizing effective population size and thus overall genetic diversity and (2) using nonrandom mating to increase the diversity of genotypes within a generation to a level above that expected from random mating. The spectrum of broodstock spawning strategies and their trade-offs depending on the goals of the conservation hatchery are discussed in the following sections and are summarized in Table 2.

*Random mating.*—The majority of fish hatcheries use a random mating scheme in which parents are mated at random with regard to phenotypic and genotypic characteristics; such schemes may include single-pair crosses or factorial mating designs (Becker 1984; Gharrett and Shirley 1985; Hard et al. 1992). In fact, 62% of the respondents from the conservation hatcheries in our survey used a random mating scheme. Single-

pair crosses involve mating a single male with a single female, and ideally full-sibling families should be kept separate until individuals can be uniquely marked physically (Gharrett and Shirley 1985; Hard et al. 1992) or genetic marking can be implemented (Saillant et al. 2009; Steele et al. 2012). A pedigree can then be maintained using the marked individuals, which is especially useful in small populations and allows for monitoring genetic contributions to the next generation (Hard et al. 1992). Pedigrees are very rarely tracked in fish hatcheries (5 of the 36 hatcheries that responded to our survey tracked pedigrees and/or calculated genetic relatedness), most likely due to the logistical difficulty this poses, but this may become more widespread with parentage-based tagging (Steele et al. 2012). In addition, single-pair mating schemes result in the reproductive potential of mates being linked to one another, causing individual contributions to be lost if either mate fails to produce viable gametes (Busack and Knudsen 2007). To reduce the risk of loss due to infertile males, a modified single-pair mating scheme or factorial breeding scheme is recommended for small numbers of captive spawners (Hard et al. 1992; Fiumera et al. 2004). Based on simulations, Bentsen and Olesen (2002) recommend that there be a minimum of 50 pairs of spawners in each generation and that an equal number of progeny from each cross be randomly reared to maturity to avoid high inbreeding rates in captivity.

*Factorial mating.*—Factorial or partial-factorial mating involves crosses between all possible parents or matings of single females to overlapping pairs of males and is recommended by numerous studies (Campton 2004; Fiumera et al. 2004; Pollard and Flagg 2004; Dont-Nivet et al. 2006; Busack and

TABLE 2. Summary of hatchery genetic management techniques recommended in the literature, together with their benefits and costs and examples of conservation hatcheries employing them.

Management technique	Benefits	Costs	Source(s)	Conservation hatchery examples
<b>Broodstock selection</b>				
15–100 founders	Maximize founder genetic diversity	Taking fish from depleted wild populations; facility size constraints (can split among several facilities)	Frankham (2010); Witzemberger and Hochkirch (2011); Bentsen and Olesen (2002)	
Minimize generations in captivity	Minimizes adaptation to captivity	Time in captivity bounded by the response time of habitat restoration necessary to boost wild production; not necessarily feasible	Williams and Hoffman (2009); Frankham (2010)	Dungeness River spring Chinook Salmon (WDFW 2002)
Use locally adapted stocks	Minimize outbreeding depression	Taking fish from depleted wild populations	Allendorf and Ryman (1987); SRT (1998); Flagg and Nash (1999); Frankham et al. (2002)	Feather River Hatchery spring-run Chinook Salmon program (Cavallo et al. 2009)
Minimize domestication selection	Minimize effect of domestication selection and outbreeding depression on wild populations; important for species with short generation times	Difficult to implement due to multiple causes of domestication selection	King (1965); Allendorf (1986, 1993); Frankham et al. (2000); Allendorf and Luikart (2007); Williams and Hoffman (2009)	Northwest Fisheries Science Center NATURES program (Maynard and Flagg 2001)
<b>Broodstock spawning</b>				
Random mating: single-pair crosses	Simple mating design allows for tracking of pedigree; no half-siblings in the population	Reproduction potential of mates linked	Gharrett and Shirley (1985)	Feather River Hatchery spring-run Chinook Salmon program (Cavallo et al. 2009)
Random mating: factorial mating designs	Maximizes offspring production; maintains effective population size; benefit when population sizes are large	Labor intensive to implement in large populations; can lead to inbreeding if large numbers of individuals are not used	Hard et al. (1992); Fiumera et al. (2004); Pollard and Flagg (2004); Dont-Nivet et al. (2006); Busack and Knudsen (2007); Neff et al. (2011)	Sacramento River winter-run Chinook Salmon (Hamelberg 2011); Kootenai White Sturgeon conservation aquaculture program (Kincaid 1993; KTOI 2007; P. J. Anders [paper presented at the Annual Northwest Fish Culture Conference, 2004])
Free mate choice	Allows for sexual selection; improved fitness	Logistical constraints; can lead to inbreeding in small populations, high variance in reproductive success and reduced effective population sizes	Berejikian and Ford (2004); O'Reilly and Doyle (2007); Pitcher and Neff (2007); Berejikian et al. (2011)	Rio Grande Silvery Minnow (USFWS 2009)

TABLE 2. Continued.

Management technique	Benefits	Costs	Source(s)	Conservation hatchery examples
Maximum avoidance of inbreeding and circular breeding	Retains high levels of genetic diversity if applied from the first generation in captivity in large populations	Requires large populations; requires maintaining different lines separately; logistical constraints	Kimura and Crow (1963); Kincaid (1977)	
Inbreeding avoidance matrix (using molecular relatedness estimates)	Minimizes inbreeding; benefits small populations (<10,000 individuals)	Requires molecular markers; high variance of molecular relatedness estimators makes this technique imprecise	Norris et al. (2000); Waples and Do (1994); Hansen and Jensen (2005); Dont-Nivet and Vandeputte (2011)	Southern Coho Salmon (Sturm et al. 2009; Conrad et al. 2013); Snake River Sockeye Salmon (Kozfkay et al. 2008)
Minimal relatedness selection	Results in lower mean coancestry and increased genetic diversity compared with random mating	High variance of molecular relatedness estimators makes this technique imprecise	Doyle et al. (2001); del Mar Ortega-Villaizan et al. (2011)	Mekong Giant Catfish (Sriphairoj et al. 2007); Red Seabream (Doyle et al. 2001); Japanese Flounder (Sekino et al. 2004)
Minimal kinship selection (using pedigree reconstruction)	Minimizes mean kinship, maintains higher effective population size than random mating	Requires individual identification; may increase inbreeding if not avoided	Ballou and Lacy (1995); Montgomery et al. (1997); Fernandez and Toro (1999); Toro et al. (1999)	Delta Smelt conservation hatchery (Fisch et al. 2013)
<b>Rearing and release</b>				
Equalization of family sizes	Reduces domestication selection; maximizes effective population size; minimizes inbreeding	Trade-offs between genetic and demographic benefits; impractical for species with long generation times and low fecundity	Hard et al. (1992); Allendorf (1993); Waples and Do (1994); Frankham et al. (2000); Frankham et al. (2002); Rodriguez-Ramilo et al. (2006); Frankham (2008)	Harvey Creek Hatchery for North Fork Stillaguamish River Chinook Salmon (Eldridge and Killebrew 2008); Sacramento River winter-run Chinook Salmon (Hedrick and Hedgecock 1994; Hedrick et al. 1995; Hedrick et al. 2000; Hamelberg 2011); Delta Smelt conservation hatchery (Fisch et al. 2013)

Knudsen 2007). In our survey, over half of the programs used a factorial random mating scheme. Fiumera et al. (2004) used simulations to demonstrate that in supplementation hatchery programs a factorial mating scheme maximized offspring production while maintaining effective population size. In an aquaculture program involving selection, partial and full factorial

mating were the most efficient mating schemes to preserve long-term genetic variability and single-pair schemes were the least efficient (Dont-Nivet et al. 2006). A more general study by Busack and Knudsen (2007) demonstrated that, in one case, full-factorial mating resulted in a 33% increase in the effective number of breeders over that with single-pair mating. However,



full-factorial mating is generally only feasible in small populations (Neff et al. 2011). When there is low variance in family size in single-pair matings, males are used an unequal number of times, or populations are small, the benefit of a factorial mating scheme over single-pair crosses rapidly decreases as the size of the broodstock increases (Busack and Knudsen 2007). When performing factorial mating, it has been recommended that managers avoid combining sperm from different individuals prior to fertilization, as sperm competition may decrease the contribution of some males, reducing the effective population size (Withler 1988; Campton 2004). In very small populations and where the technology exists, it is recommended to cryopreserve a fraction of the milt from each male to be able to use it in years when the number of available males is low; however, the trade-offs are that the viability of frozen sperm is low (Hard et al. 1992), the procedure is very labor intensive, and a smaller number of eggs can be fertilized.

An extension of random mating is allowing individuals to have free mate choice, which may provide an advantage by producing offspring that have benefitted from sexual selection (Berejikian and Ford 2004; O'Reilly and Doyle 2007). This can be accomplished by allowing captive-reared adults to spawn naturally in the wild, allowing communal spawning in a hatchery facility (Gruenthal and Drawbridge 2012), collecting fertilized eggs from the wild (USFWS 2009), or implementing a quantitative genetic breeding design (Berejikian and Ford 2004; O'Reilly and Doyle 2007; Pitcher and Neff 2007; Berejikian et al. 2011). The fitness of captive-reared offspring may also be increased by sexual selection and the benefits associated with it, such as improved immune responses (Landry et al. 2001; Berejikian and Ford 2004; Foresberg et al. 2007; Fraser 2008). Theory suggests that there should be some advantages to captive mating strategies that mimic those that occur in the wild; however, this may lead to large variance in reproductive success among individuals, reducing the effective population size (Hard et al. 1992; Maynard and Flagg 2001; Christie et al. 2012).

In practice, factorial mating schemes are applied to many fish conservation hatcheries, including those for Sacramento River winter-run Chinook Salmon and Rio Grande Silvery Minnow *Hybognathus amarus*. The Livingston Stone National Fish Hatchery uses a partial factorial mating scheme with Sacramento River winter-run Chinook Salmon (Hamelberg 2011). The eggs from each female are divided into two equal groups and each group is fertilized by milt from two separate males, producing two half-sibling family groups. Each female is only spawned once, and each male is spawned a maximum of four times (Hamelberg 2011). The artificial propagation program for Rio Grande Silvery Minnow uses random single-pair crosses or communal mass spawning allowing for mate choice, and the population is genetically monitored each year to assess the effective number of breeders (USFWS 2009; Osborne et al. 2013). Osborne et al. (2013) assessed the genetic outcomes for these mating designs within the Rio Grande Silvery Minnow arti-

cial propagation program and determined that genetic diversity and egg productivity did not significantly differ between the spawning designs. However, there was evidence for variance in reproductive success among individuals and a decline in allelic richness in all spawning designs due to unequal parental contributions (Osborne et al. 2013).

While the variants of a random mating scheme are easy to implement, result in large production, and can contribute to maintaining genetic diversity, random mating alone may result in decreased genetic diversity, increased inbreeding, a decrease in effective population size and adaptation to captivity depending on the scheme used and the size of the population (Reisenbichler and McIntyre 1977; Falconer 1981; Ralls and Ballou 1983; Ryman and Laikre 1991; Frankham 1995; Reisenbichler and Rubin 1999; Heath et al. 2003; Waples and Drake 2004; Araki et al. 2007a, 2007b, 2009; Frankham 2008; Table 2). In addition, some of these mating schemes (such as factorial mating) may be time-consuming to implement, as was demonstrated by Currens et al. (1998). It is important to assess the trade-offs of each of these random mating techniques in the context of the hatchery population size and the feasibility of implementing them.

*Maximum avoidance of inbreeding scheme and circular breeding.*—The maximum avoidance of inbreeding scheme (MAI) involves equalizing family sizes and employing a circular mating pattern (Kimura and Crow 1963; Princee 1995). This scheme mates females from one subpopulation to males of different subpopulations each year and is recommended when a large proportion of the pedigree is unknown, when species are maintained as groups, and when new subpopulations need to be incorporated into the scheme (Ballou and Lacy 1995; Princee 1995; Windig and Kaal 2008; Frankham 2010). This management strategy has been shown through simulations to retain high levels of genetic variation if it is applied from the first generation in captivity (Ballou and Lacy 1995; Frankham 2010). Another variant of circular mating is rotational line crossing, where sequential crosses are made among three or more separate lines to minimize matings between related individuals (Kincaid 1977). Rotational line crossing reduces the rate of inbreeding accumulation within each line, although it is dependent on high founder genetic diversity, preferably starting with a minimum of 50 fish per line from different strains (Kincaid 1977). The complexity of the mating schemes and the requirement for a large number of founders make these techniques very difficult to implement and follow precisely (Kincaid 1977; Ballou and Lacy 1995). The authors have no knowledge of strict MAI or circular/line breeding schemes being applied in fish conservation hatcheries, although these strategies may be useful in species with different subpopulations, year-classes, or replicate hatchery populations to avoid inbreeding and retain genetic diversity in captivity.

*Molecular relatedness estimates.*—When known pedigrees are not available (as is the case with the majority of fish hatcheries), molecular data have been used to estimate pairwise relatedness among the founders of captive populations and to reconstruct pedigrees (Brock and White 1992; Geyer et al.

1993; Haig et al. 1995; Jones et al. 2002; Gautschi et al. 2003; Russello and Amato 2004). Relatedness estimators estimate the probability that an allele sampled at random due to identity by descent from a common ancestor is shared by two individuals. The performance of relatedness estimators is based on the number of DNA markers, the number of alleles, allele frequency distributions, and the composition of the relationship categories present (Queller and Goodnight 1989; Ritland 1996; Lynch and Ritland 1999; Van de Casteele et al. 2001; Wang 2002; Milligan 2003). Small-population management generally assumes that wild-caught individuals brought into captivity are not inbred and are unrelated. Recent research suggests that the violation of these assumptions has little long-term impact on the performance of genetic management methods for most zoo-based captive breeding programs (Ballou 1983; Rudnick and Lacy 2008; Ivy et al. 2009).

With the use of molecular relatedness estimators, mating schemes can also be designed to avoid mating related individuals (Waples and Do 1994; Norris et al. 2000; Hansen and Jensen 2005; Dont-Nivet and Vandeputte 2011). These schemes are advantageous since they minimize the rate of inbreeding and decrease the risk of inbreeding depression (Hansen and Jensen 2005). This is especially important when large numbers of offspring that represent a small number of founders are released (Ryman and Laikre 1991). Dont-Nivet and Vandeputte (2011) performed stochastic simulations to compare genetic diversity and levels of inbreeding in populations in which full-sibling matings were avoided or allowed when selecting individuals for mating. They determined that when population sizes were very large (>10,000 individuals), avoiding full-sibling matings became less relevant (Dont-Nivet and Vandeputte 2011). In small populations, which by definition include almost all of those in conservation hatcheries, avoiding full-sibling matings resulted in higher genetic diversity in the hatchery only as long as the practice was continued. However, this effect disappeared after the fish were reintroduced to the wild, a result that is supported by numerous other studies (Waples and Do 1994; Caballero et al. 1996; Wang 1997; Norris et al. 2000; Hansen and Jensen 2005).

Another approach is to manage captive populations using molecular relatedness estimator values as proxies for pedigree-based kinships (hereafter termed minimal relatedness [mr] selection; Doyle et al. 2001; del Mar Ortega-Villaizan et al. 2011). Minimal relatedness selection has previously been referred to as minimal kinship (MK) selection, but we distinguish it from pedigree-based MK selection because it is based on molecular relatedness estimators and is not a strict application of MK selection as defined by Ballou and Lacy (1995). Doyle et al. (2001) incorporated molecular data into hatchery genetic management by using molecular estimates of relatedness as a proxy for pedigree-based kinships, and Toro et al. (1999) investigated the benefits of using molecular markers to minimize the homozygosity by descent in captive populations to minimize the average group coancestry. Doyle et al. (2001) empirically demonstrated that

higher levels of genetic diversity were retained using molecular relatedness estimates and minimum relatedness analyses in a captive population of Red Seabream *Pagrus major* (also known as Madai) than was the case with random mating. These authors used molecular relatedness estimates to preferentially mate subsets of breeders so as to equalize founder contributions instead of simply avoiding the mating of related individuals; this resulted in lower mean coancestry and greater heterozygosity and allelic diversity (Doyle et al. 2001). Using this method, it is possible to incorporate information about individual relatedness into the design of mating schemes with the intention of retaining founder genetic diversity (Doyle et al. 2001). A simulation study examined the performance of mr selection in a Barfin Flounder *Verasper moseri* captive broodstock program and determined that it retained both genetic and allelic diversities better than random mating (del Mar Ortega-Villaizan et al. 2011).

In practice, molecular relatedness estimators have been applied in a handful of fish hatcheries, either simply to avoid the mating of related individuals or through actual mr selection (Doyle et al. 2001; Sekino et al. 2004; Sriphairoj et al. 2007; Kozfkay et al. 2008; Sturm et al. 2009; Conrad et al. 2013). Conservation hatcheries using molecular relatedness to develop breeding matrices that avoid inbreeding include those for southern Coho Salmon and Snake River Sockeye Salmon (Kozfkay et al. 2008; Sturm et al. 2009; Conrad et al. 2013). The Snake River Sockeye Salmon Captive Broodstock Program implements a two-phase genetic management strategy. It first employs broodstock selection based on reconstructing family relationships (via relatedness or pedigree information) to rank and choose spawners and then uses an inbreeding avoidance matrix to pair spawners (Kozfkay et al. 2008). The implementation of inbreeding avoidance matrices in this program selects fish to spawn by means of a relatedness cutoff determined by known relationships less than a full-sibling relationship and aims to minimize the loss of genetic diversity, minimize inbreeding, and provide a means for incorporating returning anadromous adults into the captive broodstock (Kozfkay et al. 2008). Similarly, the southern Coho Salmon breeding program at the Monterey Bay Salmon and Trout Project's Kingfisher Flat Genetic Conservation Fish Hatchery develops a spawning matrix based on molecular relatedness estimators for each male and female pair (Sturm et al. 2009; Conrad et al. 2013). Simulations are not conducted with the suite of microsatellite markers used, and the relationship category cutoff values used are those under theoretical expectations. Potential males for each female are listed in the spawning matrix if the relatedness estimate is less than a half-sibling level of relatedness, with the goal of minimizing inbreeding to maintain genetic diversity in the captive population (Sturm et al. 2009; Conrad et al. 2013). Conrad et al. (2013) determined that there was a significant negative effect of the relatedness coefficient between mated pairs on progeny survival and that the use of genetic broodstock management increased offspring survival and reduced inbreeding.

Minimal relatedness selection has been employed for Red Seabream, Japanese Flounder *Paralichthys olivaceus*, and Mekong Giant Catfish *Pangasianodon gigas* (also known as Giant Catfish) (Doyle et al. 2001; Sekino et al. 2004; Sriphairoj et al. 2007). Sekino et al. (2004) used mr selection to give high spawning priority to individuals with rare genotypes in a nonpedigreed captive population of Japanese Flounder. Using this method, they demonstrated retention of allelic diversity and gene diversity in the selected breeders, although some related fish were mated using this method (Sekino et al. 2004). Another example of the application of molecular relatedness estimators to design a mating scheme is the broodstock management plan for the critically endangered Mekong Giant Catfish (Sriphairoj et al. 2007). Using the above-described mr selection, the authors predicted that the captive population would retain greater genetic diversity with a larger effective population size in addition to setting a relatedness cutoff above which individuals would be considered related and would not be mated (Sriphairoj et al. 2007).

Using molecular relatedness estimators greatly increases the degree of control that managers have over the genetic diversity in their hatcheries compared with variants of random mating, allowing managers to track individuals and only mate those with low relatedness estimates. Sekino et al. (2004) caution that using molecular relatedness estimators may not necessarily assign individuals to the true kinship category, as they are subject to high variance. This may introduce errors in breeder selection when these methods are used to make breeding recommendations, making their utility in genetic management questionable—although using a large number of diverse loci may increase confidence in the estimates (Brock and White 1992; Geyer et al. 1993; Haig et al. 1995; Jones et al. 2002; Gautschi et al. 2003; Russello and Amato 2004).

*Minimal kinship selection.*—Computer simulations and empirical studies have demonstrated that the best captive breeding strategy for maintaining genetic diversity is one that minimizes overall kinship (i.e., relatedness) in a population (Ballou and Lacy 1995; Montgomery et al. 1997; Fernandez and Toro 1999; Toro et al. 1999). This strategy is recommended for formally managed species within regional zoo associations to preserve genetic diversity and reduce inbreeding (Ballou and Lacy 1995; Cronin et al. 2006).

Minimal kinship selection aims to minimize a population's average kinship by breeding genetically underrepresented individuals with low mean kinships ( $mk$ ). Minimal kinship selection differs from mr selection because it involves calculating a pedigree-based kinship value rather than an estimate of molecular relatedness. Kinship ( $f$ ) between two individuals is the probability that two alleles at a given locus, one randomly drawn from each individual, are identical by descent from a common ancestor (Falconer 1981). Mean kinship measures an individual's genetic distinctiveness and is calculated as the average kinship between that individual and all living individuals

in the population, including itself, namely,

$$mk_x = \frac{\sum_{y=1}^N f_{xy}}{N}, \quad (1)$$

where  $f_{xy}$  is the kinship between  $x$  and all other breeding individuals in the population and  $N$  is the population size. If mating were random, the average mean kinship of the captive population would be the expected mean inbreeding coefficient of the subsequent generation's offspring, which is equal to the proportional loss of gene diversity in the next generation (Ballou and Lacy 1995). Traditionally, MK selection is based on pedigree calculations of  $mk$ , and recent advances in molecular techniques provide the opportunity to apply MK selection by reconstructing a population's pedigree (Fisch et al. 2013; O'Reilly and Kozfkay 2014).

A potential drawback of strict MK selection is the possibility of mating related individuals, resulting in increased inbreeding in the population (Ballou and Lacy 1995). Simulations performed by Fernandez and Caballero (2001) revealed that MK selection minimized the loss of allelic and gene diversity but failed to maintain population fitness as much as other mating schemes, such as random mating. This was due to the accumulation of inbreeding, as MK selection does not explicitly avoid mating relatives. This makes it especially important to employ other methods to explicitly avoid mating related individuals when using MK selection (Sekino et al. 2004). In addition, strict MK selection is difficult to apply in a hatchery, as the desired pair crosses may not be able to be made due to asynchronous maturation of spawners.

We actually have no knowledge of strict MK selection being used in a fish hatchery. However, one conservation hatchery is known to have implemented a modified version of it, the Delta Smelt *Hypomesus transpacificus* captive breeding program at the University of California–Davis's Fish Conservation and Culture Laboratory (Fisch et al. 2013). Each generation, individual fish are uniquely tagged, the pedigree is reconstructed using microsatellite loci, and pedigree-based values of mean kinship are estimated for each tagged individual in the captive population. Females are selected as breeders as they become mature throughout the season, and males that are unrelated to the females are selected (and thus that minimize the overall mean kinship in the population) to create single-pair crosses. Equalization of family size occurs in two stages: the number of fertilized eggs reared from each family is equal, and the number of offspring mated from each single-pair cross is selected to equalize founder contributions (Fisch et al. 2013). Fisch et al. (2013) evaluated the performance of this modified MK selection method and determined that it retained greater gene diversity over the long term (100 years) than theoretical expectations in an idealized population, in addition to maintaining a higher effective population size than that produced by random mating.

## Rearing and Release of Fish

*Equalization of family sizes.*—An important strategy that can be applied with any mating scheme is the equalization of family sizes (EFS), whereby each family contributes the same number of offspring to the next generation (Hard et al. 1992; Allendorf 1993; Waples and Do 1994; Frankham et al. 2000, 2002; Rodriguez-Ramilo et al. 2006; Frankham 2008). Methods for employing EFS include culling the number of offspring from each spawning pair, mating the same number of offspring from each spawning pair to produce the next generation, and releasing the same number of offspring from each spawning pair into the wild to reduce the reproductive variance between families (Allendorf 1993; O'Reilly and Doyle 2007; Frankham 2008). Culling individuals in a fish hatchery is most easily done at the juvenile stage. While it may not guarantee equal family size at the adult stage due to subsequent differential mortality, it will deemphasize the advantage that any one family has had within the hatchery.

Equalizing founder representation has numerous genetic and fitness benefits, including reduced inbreeding, genetic drift, and domestication selection (Allendorf 1993; Fraser 2008). This technique maximizes the effective population size and minimizes the direct effects of selection for hatchery-related traits by minimizing the variance in family size (Falconer 1981; Simon et al. 1986; Lande and Barrowclough 1987). However, it may reduce the degree of selection that would occur in the wild, which may lead to a decline in the fitness of the captive population (Bryant and Reed 1999).

In practice, conservation hatcheries are beginning to employ EFS to increase effective population size in captivity. The Harvey Creek Hatchery employs random factorial mating for North Fork Stillaguamish River Chinook Salmon (a population that is part of the Puget Sound evolutionarily significant unit) to reduce the variance in reproductive success of the parents (Eldridge and Killebrew 2008). The Sacramento River winter-run Chinook Salmon conservation hatchery also employed EFS in addition to using a partial factorial mating scheme, EFS has been shown to maintain the effective population sizes of the captive and wild populations (Hedrick and Hedgcock 1994; Hedrick et al. 1995; Hedrick et al. 2000; Hamelberg 2011). Finally, EFS can be used in combination with other mating schemes besides random mating, such as in the Delta Smelt conservation hatchery (Fisch et al. 2013).

The many genetic benefits of EFS make it an important strategy for fish conservation hatcheries aimed at preserving genetic diversity, although EFS is costly to implement and does not come without trade-offs. Getting legal permission to cull ESA-listed species may be difficult, and culling may reduce population sizes or releases to undesirable levels. Williams and Hoffman (2009) describes EFS as an impractical strategy for species with certain life histories, such as those with long generation times and low fecundity. In addition, in some empirical studies EFS did not significantly improve reproductive fitness

over variable family size treatment and random mating, although it did maintain lower levels of inbreeding and reduced the rate of genetic adaptation (Frankham and Loebel 1992; Loebel et al. 1992). Equalization of family sizes—in conjunction with other spawning techniques best suited for the species in question—is important for managers to consider when creating a captive broodstock for conservation.

## APPLICATION TO HATCHERY MANAGEMENT

Captive populations maintained for the sole purpose of preserving the wild gene pool of a species are generally relatively small, with the goals of being demographically stable and genetically diverse (Ralls and Ballou 1986). Theoretically, the application of small-population management techniques to conservation hatcheries may preserve genetic diversity, minimize inbreeding, and increase the effective population size of captive populations better than random mating (Allendorf 1993; Waples and Do 1994; Doyle et al. 2001; Hansen and Jensen 2005; del Mar Ortega-Villaizán et al. 2011). If a manager aims to maximize genetic diversity and effective population size and minimize adaptation to captivity, this could be accomplished by starting with a large number of founders, minimizing the number of generations in captivity, using locally adapted stocks, employing factorial mating designs, and implementing procedures to equalize family sizes (Hard et al. 1992; Allendorf 1993; Pollard and Flagg 2004; Williams and Hoffman 2009; Frankham 2010). If resources permit, larger conservation hatcheries can use molecular markers to estimate relatedness or reconstruct pedigrees to ensure that related individuals are not being spawned and to equalize family size at the time of spawning (Sturm et al. 2009; O'Reilly and Kozfkay 2014). In creating a conservation hatchery to rebuild stocks by supplementing the wild population, a manager should consider maximizing genetic diversity and minimizing adaptation to captivity through different mating strategies and the equalization of family sizes; such a strategy should minimize the negative effects of supplementing wild populations with captive fish, such as the Ryman-Laikre effect (Ryman and Laikre 1991).

The combination of techniques employed may depend on the logistical constraints of the hatchery. For instance, to maximize genetic diversity in captive broodstocks, the number of founders may be selected so that the captive population is diverse enough to adequately represent the wild source population while taking into account the capacity of the hatchery (Crow and Kimura 1970; Bentsen and Olesen 2002; Frankham 2010; Witzemberger and Hochkirch 2011). Managers can estimate the proportion of expected heterozygosity in a wild population that will be captured by a given number of founders using the following equation:

$$H_f = H_w \times \left[ 1 - \frac{1}{2N} \right], \quad (2)$$

where  $H_f$  is the mean expected heterozygosity in the  $N$  founders and  $H_w$  is the mean expected heterozygosity in the wild population (Crow and Kimura 1970). Maximizing the level of heterozygosity in captivity is important because even an  $H_f/H_w$  ratio of 0.9 represents a loss in heterozygosity relative to what would be expected if all progeny were half-siblings. In addition, wild fish may be brought into captivity every generation (when possible) to increase the number of founders and to incorporate wild genes into the captive population (Mobrand et al. 2005; HSRG 2009; California HSRG 2012).

## CONCLUSION

While conservation hatcheries may have different goals, various techniques from small-population management can be employed to meet a program's specific goals for a species of conservation concern (Figure 2). The size of a captive broodstock population and the management techniques employed may determine whether single-pair crosses or factorial mating designs are feasible. For large captive populations, for which managers can select fish for breeding so as to minimize inbreeding (e.g., programs implementing molecular techniques to identify relatives and/or reconstruct pedigrees), single-pair crosses may outperform factorial mating designs in minimizing inbreeding and maximizing genetic diversity (Gharrett and Shirley 1985; Hard et al. 1992; Fiumera et al. 2004; Pollard and Flagg 2004). However, for smaller populations or those for which mates are selected randomly for breeding, partial or full factorial designs may be more appropriate (Hard et al. 1992; Fiumera et al. 2004; Pollard and Flagg 2004).

The main goals of conservation hatcheries are to enhance natural production while minimizing the loss of genetic diversity. As conservation hatcheries begin to adopt mating schemes consistent with small-population management theory, careful genetic and demographic monitoring must occur. This will allow evaluation of the performance of these techniques for hatchery use, in order to adaptively manage conservation hatchery programs. Theoretical and empirical studies also need to be conducted to extend small-population management techniques to fish hatcheries that are specific to highly fecund species and large captive populations. Many of the programs with more labor-intensive mating schemes have only been implemented recently and have not yet been evaluated for their long-term effectiveness or to see whether the benefit to the population justifies the cost (Fraser 2008). More research is needed to quantify the potential benefit of these intensive genetic management options to conservation hatchery programs. Finally, as recommended by Fraser (2008), it is very important to document and publish the mating schemes and techniques used in conservation hatcheries, making these techniques more available to others. Through advancing technology, the application of small-population management techniques to maintain genetic diversity, and intensive monitoring, conservation hatcheries may become a more routine part of recovery plans for endangered fish species.

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### APPENDIX: SURVEY INSTRUMENT

TABLE A1. Survey questions asked of hatchery managers in January 2013.

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1. Job title, hatchery program name, facility, state, agency and species, year program was initiated
  2. How would you currently categorize the hatchery program (definitions provided for each): conservation, segregated, integrated, production for recreation, commercial or tribal harvest, captive broodstock, other?
  3. What methods are employed to select broodstock for spawning: random, genetic stock ID, genetic broodstock selection, marks and tags, other?
  4. Is the founding broodstock of local origin or out-of-basin origin?
  5. Which methods are employed to pair broodstock for spawning: random, inbreeding avoidance matrix, genetic relatedness/pedigree, other?
  6. Do you employ factorial mating during artificial spawning? If yes, how difficult is it to implement: easy, moderate, difficult?
  7. Do you equalize family size in the resulting brood generation? If yes, at what life stage do you equalize family size: egg, juvenile, adult, multiple stages? How difficult is this technique to implement: easy, moderate, difficult?
  8. Do you use cryo-preserved milt? If yes, how difficult is this technique to implement: easy, moderate, difficult?
  9. Do you use genetics for monitoring and evaluation? If yes, how do you employ genetic information: monitor changes in genetic diversity, monitor effective population size, monitor relative reproductive success of hatchery and wild fish, other?
  10. What is the approximate level of wild/natural escapement to the spawning grounds of the stock of interest in the past five years (<50 fish, 100–500 fish, 500–1,000 fish, >1,000 fish, unknown?)
  11. How important is preserving genetic diversity to your program: very important, somewhat important, not important?
  12. How important is managing for effective population size to your program: very important, somewhat important, not important?
  13. How important is minimizing inbreeding to your program: very important, somewhat important, not important?
  14. How important is producing fish for reintroduction to the natural environment to your program: very important, somewhat important, not important?
  15. How important is creating a captive broodstock/gene bank to your program: very important, somewhat important, not important?
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