BREEDING PLAN FOR NECHAKO WHITE STURGEON

NECHAKO WHITE STURGEON RECOVERY INITIATIVE

October 2005

Disclaimer

This breeding plan is intended for the recovery of Nechako River white sturgeon. It was prepared in consultation with a number of recovery team members from this and other provincial white sturgeon recovery initiatives. Its purpose is to provide breeding guidelines for conservation culture to help protect the genetic diversity in the remaining population, as well as to address issues associated with small population demographics. It does not necessarily represent the official positions of the agencies or views of individuals involved in the recovery process. The guidelines provided here are part of an adaptive management process and are subject to change as new information becomes available, as well as to constraints associated with program budgets and biological realities.

This document should be cited as follows:

Nechako White Sturgeon Recovery Initiative. 2005. Breeding Plan for Nechako White Sturgeon. Prepared by Susan M. Pollard, BC Ministry of Environment. 35 pp.

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INTRODUCTION

In 1994, the BC Conservation Data Centre provincially 'red-listed' white sturgeon and identified the Nechako River population as 'critically imperilled'. This listing triggered a comprehensive 5-year study on the Fraser River populations of white sturgeon, including the Nechako River, to establish status and population sizes. Results of this study, and subsequent modelling exercise, concluded the following for the Nechako River population of white sturgeon:

1. The population experienced a precipitous decline in juvenile recruitment starting in the early 1960's with no measurable juvenile recruitment for the past 40 years based on population age-structure assessments and modelling exercises (RL&L 2000, Korman and Walters 2001);

2. The Nechako population is a genetically distinct population, isolated from Fraser mainstem populations based on genetic and movement studies (Smith et al. 2002, Nelson et al. 1999, RL&L 2000);

3. The estimated total population size in 2000 was 571 fish (95% CI of 403 and 851) (RL&L 2000) ;

4. A high probability of extinction is expected within 2 to 3 decades (Korman and Walters 2001) without immediate intervention to prevent further declines.

Further to these conclusions, and in combination with Fraser River and Columbia Basin results, COSEWIC (Committee on the Status of Endangered Wildlife in Canada) listed all white sturgeon in BC as *endangered* in November 2003, and a decision on listing under SARA (Canadian Species At Risk Act) is due in 2006. Regardless of the outcome under SARA, the Province recognizes the urgency of the situation, particularly for the Nechako River population, and the need to take immediate measures to prevent further declines in population size and loss in genetic diversity. In January 2001, the provincial Nechako White Sturgeon Recovery Initiative was established to address the critical state of the population. The purpose of the recovery initiative is to stabilize the remaining population by preventing further declines, and ultimately to rebuild a self-sustaining population.

Even if the recruitment problem is immediately rectified, the mature population would continue to lose numbers of mature fish for the next 25 years to natural mortality given the lag time between juvenile recruitment and maturation for this population. Without immediate intervention, genetic diversity will also continue to be eroded. The specific reason(s) for recruitment failure remain unknown, and while analysis of this problem is progressing, recruitment restoration cannot be guaranteed in the near future. Given that the population size is predicted to drop by 50% every 10 years or less, conservation fish culture seems paramount to preventing extinction of this population, at least for the immediate future until wild recruitment can be re-established.

GOALS

The Nechako conservation fish culture program has two main goals:

Conservation Goal - To prevent extinction and preserve remaining genetic diversity in the short-term, and to restore age structure and maintain genetic diversity over the long-term by maintaining an adequately-sized founder population;

Research Goal - To provide cultured juvenile white sturgeon to assist in identifying life stage(s) where recruitment failure is occurring and specific mechanisms responsible for this failure, and to optimize hatchery release strategies to meet the conservation goal.

Both goals are high priorities and can generally be simultaneously implemented. Unless river conditions are altered to begin supporting wild recruitment, the only source of significant recruitment for the Nechako white sturgeon population in the near future will be through the culture facility releases. The size of the culture program must be adequate to address the genetic and demographic issues related to a very limited founder population. Similar to the Upper Columbia program, the purpose of the Nechako culture program is to provide a stop-gap measure to re-establish age structure and to preserve genetic variation until a self-sustainable population has been re-established. However, the longevity of this species and delayed onset of sexual maturation necessitate a long-term commitment for the conservation fish culture program of at least one generation <u>in the continued absence</u> of natural recruitment. In the short term, the post-release evaluation following experimental releases of individuals is essential to develop release protocols and to assist in determining the cause(s) for recruitment failure, although this may depend on the minimum size that fish can be marked. Conservation fish culture is no substitute for natural production, and many uncertainties and risks exist. Under existing conditions (i.e., no recruitment in wild) the potential benefits outweigh the risks. Should conditions in the river system improve so that wild recruitment is possible, the recovery initiative must re-evaluate the conservation fish culture component. Under such conditions, a hatchery program could pose a risk in delaying recovery of the wild population removing wild broodstock that could otherwise spawn naturally or by swamping natural production.

The purpose of this document is to provide a breeding plan for the Nechako River White Sturgeon Recovery Initiative. Many of the concepts outlined here have been developed for the Upper Columbia White Sturgeon Breeding Plan (Pollard 2002). However, this plan has been adapted to address the population demographics and issues specific to the Nechako population of white sturgeon. In addition, new research and empirical data associated with conservation culture programs for white sturgeon (i.e., Kootenay River and Upper Columbia breeding programs) have provided some insights into appropriate culture practices for white sturgeon. Thus, the recommendations in this document will also consider these findings. The recommendations in this plan also attempt to address the uncertainty relating to the recruitment failure and the likelihood of decreased broodstock availability over time. The plan does not attempt to account for facility constraints such as capacity. In summary, the purpose of this breeding plan is to provide guidelines that support a conservation-based approach to the culture of Nechako River population of white sturgeon for the purposes of recovery. These guidelines are limited to preserving the remaining genetic diversity and preventing the demographic extinction of the Nechako population. Efforts to minimize risks to other Fraser River populations as outlined in the genetic risk assessment (Williamson et al. 2003) are mentioned only briefly here and should be addressed more thoroughly elsewhere in the Recovery Initiative.

UPDATING THE BREEDING PLAN

Since the Nechako White Sturgeon Recovery Initiative was first created, a number of factors have changed and should be considered, including population status, progress on other recovery initiatives involving sturgeon culture and new research results. Specifically these are outlined in the following sections.

1. Population status

Since the recovery plan was originally developed, additional information on the status of the Nechako River white sturgeon population has been collected or updated as follows.

In 2000, RL&L (2000) estimated a population size of 571 fish including all age classes for the Nechako population. We are now five years past these estimates – given an expected 8% natural mortality annually, the population may now be less than 400 fish.

Based on the 2000 population size estimate and observed sex ratios in mature fish, Korman and Walters (2001) estimated approximately 150 mature females were available in 2000. Further, they estimated only 25 mature females would remain in 2025. Given that possibly only 20-25% of the population is believed to be reproductively mature in any given year (based on an assumed periodicity of 4-5 years for females), these estimates result in only a maximum of 30 mature females available for spawning in 2000, and 5 in 2025. More recently, Golder (2003) found a sex ratio of approximately 1:3 for females:males based on captures and sex determination of 15 large fish in the Nechako River, but in reality actual sex ratios remain unknown (S. McAdam, pers. comm.). In particular, sex-determination of recaptured fish in the Kootenay River was found to be incorrect up to 27% of the time (Paragamian et al., 2002, see 2. below). Based on this, it should be assumed that sex ratios in the Nechako are 1:1 until more information is available. This would result in slightly higher estimates for predicted females available.

Despite considerable egg/larval sampling effort in 2003, including downstream of a female believed to have spawned, no eggs or larvae were retrieved (Golder 2003). In 2004, 4 eggs and one larva were retrieved (Triton 2004), from 399 hours of mat sampling. In 2005 no spawning was detected despite 31,847 hours of mat sampling (Triton 2005, draft). These findings suggest spawning intensity may be highly variable, and that very limited numbers of spawners may be available in some years. However, while wild egg/larvae collections for captive rearing will be challenging it should be pursued.

In 2003, a single fish was observed to move from the Nechako River to the Fraser River and similarly a single fish was observed to move from the Fraser River to the Nechako River (Golder 2003). While this represents the first documentation of such movement, it highlights the potential for fish to leave the Nechako system and the need for a conservative approach that minimizes possible interactions with Fraser River mainstem populations through adequate monitoring efforts.

2. Kootenay breeding program

The updated Kootenay breeding plan (KTOI 2004) emphasizes the need for more rigorous population enhancement than originally outlined in Kincaid's (1993) plan, in response to updated information on population status, as well as initial hatchery findings. In particular, this plan takes into account the updated demographics of the wild population, the reproductive strategy of this species compared to salmonids, and post-release survival information.

Demographics: The Kootenay River population is currently estimated to be less than 500 adults (for 2005). With a decline rate of 50% every 7.4 years, fewer than 50 adults are expected by 2030, and fewer than 30 females are estimated to be spawning in any given year after 2015. While these values are dismal, the Nechako population estimates may be as bad or possibly worse.

Hatchery survival rates and uncertainty: Average annual survival rates for hatchery-reared juveniles is approximately 60% for the first year following release and 90% during all subsequent years. This is based on approximately 10 years of post-release survival studies in the Kootenay River. Preliminary results for the Columbia hatchery suggest similar or better post-release survival rates for the first few years (i.e., 70% in first post-year followed by 92% after first year, S. McAdam, pers. comm.). While these results are encouraging they do not consider long-term annual survival rates. Unfortunately, estimates of equilibrium population size for adults in the next generation based on continued hatchery production are highly sensitive to even minor changes in annual survival rates. Thus, it is not realistic at this point to back-calculate with certainty how many annual releases are necessary to meet the recovery goal for population size.

Population parameters: Multiple biopsies conducted on individual wild fish in the Kootenai River estimated spawning intervals for females (based on 10 fish) ranged from 2-10 years but were generally 4-5 years. Furthermore, these biopsies concluded that sex determination of immature or resting mature fish is very difficult, and in the case of the Kootenai population, of 49 fish determined to be females initially, 27% were later determined to be males based on gonadal development. Similarly, of 110 fish determined to be males, 11% were later determined to be females. These results make any conclusions on sex ratios in the adult population questionable. Finally, there is some evidence that fin-ray ageing significantly under-estimated the age of fish. This being the case, many uncertainties increase and recovery time may take longer than originally expected.

Life history: The specific life history and reproductive strategies of white sturgeon have been factored into the most recent recommendations. These strategies are very different from those of salmonids, and breeding plan designs should consider these differences (Table 1).

Table 1. Life history and reproductive attributes of white sturgeon (from Table 2 in KTOI 2004)*.

Life history or reproductive attribute	Benefits
Iteroparity, overlapping generations	Multiple opportunities to pass gametes on to subsequent generations within a single lifespan; enlarges trans-generation gene flow mosaic Enlarges intergeneration gene flow mosaic; reduces probability of inbreeding
Differential, sex-specific age at first maturity	Reduces reproductive synchrony of male and female siblings and half-sib family members
Differential, sex-specific spawning periodicity	Reduces reproductive synchrony of male and female siblings and half-sib family members
Spawner aggregations, possibly communal courtship, potential for multiple males to spawn with a single female*	Enlarges trans-generation gene flow mosaic; reduces probability of inbred progeny

* Note: that this table was copied from KTOI 2004 with the exception of this attribute (originally stated as 'communal, broadcast spawning'). There is some disagreement with regards to the communal, broadcast spawning attribute. While pre-spawning behaviour and courtship may be communal (i.e., congregations of sturgeon have been observed), this does not imply complete random mixing of gametes among individuals. Eggs are broadcast by the female, but there is some visual and genetic evidence of pairing – observations suggest that there is often a single male closest to the female when eggs and milt are released. At this point, there may be limited potential for other males to fertilize eggs, or the female may release more eggs later. Fertilized eggs are then 'broadcast' in the water current. There is no reason at this point to believe communal fertilization is a generalization for white sturgeon (S. McAdam, pers. comm.).

3. Research

A recent study modelling the genetic and demographic implications of conservation aquaculture for white sturgeon found that attempts to equalize families (i.e., culling large families) did not reduce genetic risks significantly (i.e., those associated with genetic swamping), and in some cases actually reduced population size (Jager 2005). This finding supports earlier recommendations by Lande (1988) to focus on demography in recovery efforts rather than genetic risks in situations where demographic factors may be of greater immediate importance to a wild population than genetic factors. These results have some significant ramifications for release goals and family rearing. However, it should be noted that this modelling exercise makes some assumptions about two very sensitive parameters. Specifically, the model assumed that variation in survival among families is bimodal; there is a strong possibility that survival differences are actually much greater than this, resulting in a genetic swamping effect by some families. Similarly, the model assumed that the level of density-dependent mortality is fairly low. This may be the case in the initial years of the hatchery program but given that large numbers of releases and good post-release survival, the population could rapidly approach carrying capacity resulting in much greater density-dependent mortality. Some very preliminary observations in the Columbia program suggest that density-dependent effects may be occurring but confirmation is necessary (S. McAdam, pers. comm.).

BREEDING PLAN OPERATIONAL GUIDELINES

The Nechako population of white sturgeon presently appears to be at greater immediate risk of extinction than any other listed population of white sturgeon in British Columbia. The total estimated population size and numbers of observed spawners and spawning events are the lowest, and the onset of maturation is the latest. This revised breeding plan attempts to incorporate an approach that puts more emphasis on demographic factors (i.e., affecting short-term population viability), while ensuring genetic factors (i.e., more important in long-term population viability) are not ignored. Specifically, the guidelines provided below are focussed on optimizing two components of the conservation goal of the culture program: short-term demography requirements (i.e., to prevent extinction within the next generation) and long-term genetic requirements (i.e., to prevent further loss in genetic diversity). Where both cannot be simultaneously optimized, demography must take precedence over genetic equalization requirements unless recruitment conditions change. Given the genetic risks inherent in this approach, it is essential that an adequate monitoring program be developed that not only tracks the status of the Nechako population but also considers the straying potential into the Fraser River mainstem.

Incorporating Uncertainty

Uncertainty exists for all components of the recovery plan including conservation fish culture, and therefore requires an approach which will incorporate new information as it becomes available. Thus, this breeding plan should be considered a living document which can be updated as needed.

Duration

Under continuing recruitment failure, the conservation culture component of the recovery plan is expected to be a long-term commitment. However, it is still to be considered as an interim measure, and only one of several components of the recovery plan that focuses considerable emphasis on habitat restoration. Should recruitment improve, the continuation of the conservation culture component would have to be re-evaluated, revised accordingly and potentially reduced or even eliminated. It will be terminated if and when natural reproduction is successful enough to rebuild and maintain the desired population size.

Specifically, fisheries managers should be prepared to have the program running annually for a minimum of one generation, in the continued absence of any significant natural recruitment, to re-establish age structure. Generation length as defined by the IUCN (IUCN 2001) as the average age of spawning within a cohort. For the late-maturing Nechako River white sturgeon population, this period is estimated to be approximately 45 years (S. McAdam, pers. comm.). If after one generation, natural recruitment has not been restored, the conservation culture program must be re-evaluated in terms of its original goals, as well as the overall goals of the recovery plan.

Recruitment Goal

In the original breeding plan for the Kootenay River white sturgeon, Kincaid (1993) concluded that a recruitment goal of "4-10 adults per family" would be sufficient to ensure a slowly expanding population size that would not overwhelm the mature natural population. Since 1993, a more aggressive approach to prevent demographic extinction has been deemed necessary for both the Kootenay and Upper Columbia breeding programs. Paragamian et al. (2002) estimated a total population size of 630 in 2002 with a projected 50 adults remaining by 2030 in the Kootenay River. They emphasized the need to weigh demographic risks against genetic risks. Similarly, the Upper Columbia breeding plan (Pollard 2002) stated that the dire situation faced by the Upper Columbia population required a more aggressive approach at least initially to 'jump-start' the rebuilding process and minimize small population demographics problems in the future.

An aggressive approach is also recommended for the Nechako white sturgeon population which may be at an even greater immediate risk of extinction than the other two populations. It is expected that the next generation will be produced entirely or primarily via the conservation culture program. The remaining wild population is the founder population upon which all future recruitment will depend, assuming conditions do not change. Therefore, the conservation culture program must be designed to: (1) protect the genetic diversity remaining in this population; and, (2) produce enough individuals to ensure that the next generation of spawners does not suffer from small population demographics (e.g., inbreeding, inability to find mates).

The recruitment goal for the breeding plan can be equated to the recovery target for the Nechako white sturgeon population. The recovery target has been set at 2,500 breeding adults within a generation, which is the minimum number of individuals required to downgrade a population from endangered to vulnerable when the rate of population decline is 20% or more over two generations (IUCN 2001). This is approximately half of what Korman and Walters (2001) estimated as the population size in 1980. Ideally, this equates to 50-60 breeding adults produced in the recovery facility annually over one generation length (i.e., 45 years). However, this value does not take into account habitat carrying capacity considerations or uncertainty. In reality the uncertainties associated with survival and maturation makes this type of back-calculation somewhat useless at present. For example, a 3% increase or decrease in mortality rate produces a 3-fold difference in expected adult numbers in the next generation (KTOI 2004) (Table 2).

Table 2. Comparison of numbers of spawner recruits at 30 years of age using a 3% range in survival rates, starting with 12,000 fertilized eggs. These scenarios all assume 70% survival for first year post release, and assume release occurs at 0 yrs of age.

Age in years	Numbers of Fish Remaining in Population		
	93% annual survival	90% annual survival	87% annual survival
1	8400	8400	8400
2	7812	7560	7308
3	7265	6804	6357
4	6756	6123	5531
5	6283	5511	4812
10	4371	3254	2398
20	2115	1134	595
30	**1023**	**395**	**148**

Effective Population Size

A minimum of 50 adults per generation is recommended to prevent short-term genetic losses associated with inbreeding and random genetic drift. However, in order to maintain the original level of genetic variation (including rare alleles) and long-term adaptive potential, a minimum effective population size (N_e) of 500 or more is recommended (Franklin 1980) which equates to approximately 10 adults per year to contribute each generation. This number is approximately equivalent to the estimated size of the Nechako white sturgeon population in 2000 (RL&L 2000). Ideally, all 500 individuals would be spawned in the conservation fish culture program over its duration to address long-term genetic requirements since no significant natural recruitment is expected. This is an impossible goal but underscores the importance of maximizing the number of adults captured for broodstock each year. Annual natural mortality and senility will reduce N_e of the existing population and will make the availability and capture of broodstock more difficult. This problem will be compounded by the issue of spawning periodicity.

Front-loading Concept

Even if production at a recovery facility were to be initiated immediately, it is estimated that fewer than 50 mature females may remain in the wild population when the first cohort begins to mature and be recruited into the spawning population. Of this number of females, potentially only 20-25% may spawn in a particular year given an estimated spawning periodicity of 4-5 years (based on Kootenai estimates). Lack of synchronicity alone in a population this small could mean no wild fish successfully spawn in a given year. These estimates highlight the urgency of the situation and the importance of incorporating a 'front-loading' approach to the program to maximize the number of wild fish used as spawners while they are still accessible for broodstock collection, rather than equally distributing the use of wild adults through the lifespan of the program.

Broodstock Collection

Annual broodstock numbers

The minimum number of fish to protect the remaining genetic diversity in the population is five pairs of fish per year for the next 45 years, based on a minimum genetic N_e requirement of 500 and assuming a generation length of 50 years. Ideally, this number would be collected consistently every year from the existing founder population, and all matings would be successful in producing individuals to be recruited into the next generation of spawners. This guideline is not appropriate for the Nechako for two main reasons. Population projections estimating the population to be declining by 50% every 10 years (Korman and Walters 2001) suggest that the collection of wild broodstock will become increasingly difficult over time. In fact, there will be a period of several years where very few remaining wild spawners are available and the first cultured cohorts have not reached maturity (Korman and Walters 2001). Secondly, the goal of five pairs per year for 50 years does not incorporate the fact the program will rely increasingly on facility-produced adults in the second half of the program's expected lifespan as cohorts mature i.e., the next generation. Finally, seven years of intensive sampling in the Nechako (1995-2001) only captured eighteen pre-spawner males and six pre-spawner females indicating that the desired number of spawners may already be difficult to obtain, although more recent studies have observed congregations of a few dozen spawners (Triton 2004), captive holding of interspawning adults to be brought to maturation is also a possibility.

The next 5 to 10 years are critical in obtaining wild broodstock to establish a founding adult population of adequate size for the next generation. Efforts to maximize broodstock numbers annually must be made. As an initial broodstock goal, collections should attempt to collect 10 or more each of males and females. Currently, it is unknown whether or not this is realistic and achievable in any given year. The proposed rearing facility in Vanderhoof currently has the capacity to rear 12 families separately (R. Billings, pers. comm.). However, some level of flexibility has been built into the plans (i.e., ability to support a number of additional temporary early rearing tanks for more families).

Broodstock spawning success

The number of captured individuals that will actually contribute to spawning activities each year of the program may be variable. A significant proportion (30-40%) of non-ripe females brought into captivity may not progress to the ripe stage because of physiological changes associated with the stress of capture (Conte et al. 1988). Initially, the spawning success rate in retaining mature Upper Columbia females for spawning was inconsistent. In 2003, the eggs of one of the six females collected for spawning had inexplicably undergone attrition (been re-absorbed) prior to induction efforts (L. Siemens, pers. comm.). Since this time, the percent to successfully spawn has improved significantly, likely due to the fact that collections are delayed until spawning season actually begins as opposed to collecting maturing fish earlier in the season (i.e., reach the point of 'no turning back' (L. Siemens, pers. comm.)). In fact, success was 100% in 2004 and 2005 (R. Ek, pers. comm.). Some failure associated with females has also occurred with the Kootenay program, possibly due to the inability to control water temperatures at the Bonners Ferry facility. Since the temperature can be strictly controlled in the proposed recovery facility on the Nechako River, this may not be a problem. Finally, fertilization may not be 100%. For example, fertilization to hatch survival rates at Hill Creek in 2001 ranged from 0% to 92% in 2004-200 for the Upper Columbia population (R. Ek, pers. comm.). While fertilization has been successful, some failure in families post-fertilization has been observed and appears to be related to males (R. Ek, pers. comm.).

Additional broodstock options

Given the limited size of the Nechako population, the ability to capture wild broodstock in pre-spawning condition may become increasingly limited. Three other options should be developed to provide additional options.

1. Long-term captivity of wild-caught broodstock until maturation is reached

Bringing fish to maturation in captivity may become increasingly necessary to meet annual wild broodstock capture and release goals. Long-term (up to two years) maintenance of wild-caught adult sturgeon in captivity is conducted regularly for Snake River white sturgeon at the College of Southern Idaho for conservation and aquaculture purposes (T. Patterson, pers. comm.). However, it has only been carried out on an experimental basis in B.C. at Malaspina College. This option has significant associated costs and uncertainties but given the situation for the Nechako River, it deserves serious consideration in breeding facility planning.

2. Cryopreservation

The cryopreservation of milt is a viable option to preserve male gametes and should be researched further. Cryopreservation trials for the Upper Columbia white sturgeon population were initiated in 2002 to test the effectiveness of this tool and to develop appropriate protocols. Preliminary results indicated only very low success in short-term preservation, but results from 2005 indicate that 15-20% of sperm were viable in short-term cryopreservation (T. Yesaki, pers. comm.). Efforts to refine the freezing/thawing protocol will continue to optimize this methodology as an option for the recovery program. Cryopreserved gametes could be extremely valuable in the future as broodstock become increasingly scarce. As cultured cohorts mature, cultured females' eggs could be crossed with wild cryopreserved sperm to maintain a wild component to the program. Cryopreservation should be conducted in all years when an excess of milt is available, and in years when no females are available to be spawned.

3. Wild-spawned eggs/larvae

The benefit of wild eggs and larvae is that they are the product of natural mate selection and mating, and this genetic source can only benefit the program by increasing the amount of variation preserved. Significant numbers of wild-caught viable eggs have been collected previously at the Waneta spawning site for the Upper Columbia program. In an experimental study for the Upper Columbia program in 2001, eggs were collected on three separate days as follows: 21 eggs on July 3; 25 eggs on July 4; and, 258 eggs on July 6. Of these eggs, only 23 eggs from the July 6 collection survived to a size at which they could be released. Interestingly, of the 22 fish released, one individual has already been recaptured (R. Ek, pers. comm.).

To date, only 4 eggs and a single yolk sac larva have been sampled from the Nechako River (all in 2004, Triton 2004), but this resulted from only 399 hours of mat sampling in effective locations. In other years over 30,000 hours of sampling yielded no eggs. Efforts to improve the ability to capture viable eggs and larvae should be made.

Broodstock maintenance

A secure, short-term holding facility for spawners will be required to induce spawning. Induction involves a combination of temperature/ photoperiod/hormone treatments. Given that wild pre-spawning adults are already in short supply, long-term holding facilities including maturation tanks (see Conte et al. 1988) to hold fish for one or more years may also be necessary. Long-term captivity of the broodstock will require the development of specific feeding protocols. Such a program will require a fairly sophisticated physical plant/recovery facility and a high degree of technical expertise with the support of professional fish culture biologists, technicians and managers to succeed.

Broodstock re-use

It is recommended that all broodstock be marked or permanently tagged, sampled for tissue (for DNA identification) and released back into the wild once they have been spawned (although reconditioning, including return to fish-based diet, should be considered if spawners were taken off a natural fish diet). In addition, all excess milt should be cryopreserved. It was recommended in the original Kootenay breeding plan that no individual fish should be spawned more than twice throughout the duration of the program to ensure genetic contributions to the next generation are equalized as much as possible (Kincaid 1993). However, sturgeon likely contribute to the next generation multiple times throughout their life span in a natural population. Furthermore, re-use may be necessary for the Nechako population where it will be more important to ensure that any spawners are available for mating. Therefore, any adults in pre-spawning condition including those previously used as broodstock should be included in future broodstock collections.

Asynchrony in spawners

Early results from the Upper Columbia program (2001-2002) indicated that not all broodstock were ready to spawn at the same time during the spawning season. However, more recently, synchrony in spawners has been greatly improved by delaying collections until June when fish are closer to spawning time (R. Ek, pers. comm.). Females spawn more closely together and it is easier to bring on a number of males once the females are ready. In fact, some males can be brought on 2 to 3 times (R. Ek, pers. comm.). To simplify the spawning design, efforts should be made to synchronize spawning. However, if this is not possible, spawning can be modified following the recommended mating schemes below depending on the number of males and females available at a given time during the spawning period.

Sperm can be kept viable for 4-5 days using refrigeration and oxygen (Ron Ek, pers. comm.). In addition, it is relatively straightforward to induce mature males to spawn throughout the spawning season (L. Siemens, pers. comm.). Mature females take 20-40 hours to spawn after induction (Conte et al. 1988). In theory, mature captive female sturgeon should not be induced to spawn until preferably two (although one may be the extreme case) ripe males can be confirmed. Realistically, it may be difficult or impossible to get successful ovulation at the desired time. For example, the two females used in the Upper Columbia program in 2001 were spawned almost a month apart (July 30 and August 23). This component of the plan will need some further experimentation, and consultation with experts in the field is recommended. Techniques to store milt over the spawning period should be developed to provide 'back-up' and ensure no opportunities to spawn females are missed.

Mating Schemes

Mating schemes are designed to maximize the genetic effective population size (N_e), thus reducing the likelihood of inbreeding. This is particularly important where broodstock numbers are low. Ideally, the genetic contributions of all spawners are equal within years. The simplest plan is to conduct 1:1 spawning where each male and each female is used only once. However, where gamete viability is variable or unknown, sex ratios are unequal, or numbers are critically low (i.e., each individual represents >10% of the total broodstock at the time of spawning), variations on the 1:1 plan are strongly recommended to maximize the genetic diversity captured.

Use of half-sib families

There has been some failure associated with egg viability in the Kootenay[1] and Upper Columbia[2] programs in the past, although this has been reduced significantly for the Upper Columbia in the past two years (R. Ek, pers. comm.). Furthermore, there has been some suggestion that the fin deformities seen in cultured progeny in the Upper Columbia program and other areas is associated with specific males but has yet to be confirmed (S. McAdam, pers. comm.). Finally, it may be very difficult to obtain recommended numbers of broodstock, or successfully induce spawning. Given these conditions, a factorial-type (full or partial) mating design is highly recommended. In a full factorial design, the gametes of each sex are split so that each male is mated with

[1] Note that 6 of 8 families created in 1999 (using 4 females and 8 males) had a high rate of early survival during the onset of self-feeding, and hatch success averaged nearly 80% for these families. The remaining 2 families had extremely low rates of success (less than 200 individuals).

[2] Of the 6 families created in 2001 (using 2 females and 5 males), 4 families (using female 1) had a high egg to hatch survival rate of over 95% while 2 families (using female 2) had less than 30% to 40% survival.

each female, whereas a partial factorial design involves crosses of subsets of broodstock. Both designs can create a large number of half-sib families in offspring that could potentially increase inbreeding levels in the next generation if the half-sibs were to mate. However, the lack of synchronicity between sexes for age at first maturity and for spawning periodicity will act to reduce the likelihood of half or full siblings mating naturally. Family tags will ensure that future matings between siblings in the hatchery will not occur. The production of half-sib families, especially when limited numbers of fish are available, will maximize the number of individuals contributing that year and the total amount of genetic variability captured, even if some families fail. Given the above, the genetic risks associated with half-sib families are considered acceptable. The creation of large numbers of such families may exceed facility limitations but some flexibility in facilities will allow the temporary creation of families during the very early stages of egg incubation until family viability is confirmed.

Spawning matrix design

Some debate about the most appropriate spawning design to capture the genetic variation remaining in the adult population is ongoing. One position is to maximize the number of genetic combinations by conducting full-factorial matrix spawning to produce a large number of half-sib families (i.e., N_{female} x N_{male} families). This has the benefit of ensuring that a single non-viable individual will not render all of its mate's gametes non-viable also. This design is not completely incompatible with the mating strategy of sturgeon species (i.e., potential for more than one male per female and vice versa), or the observation that no obvious pairing was observed during gamete release in large congregations of other species of sturgeon (i.e., for lake sturgeon, S. McAdam, pers. comm.). As already mentioned, the potential for inbreeding in the future among this large number of half-sib families is reduced due to sex-based asynchrony in age at first maturation and periodicity.

However, full matrix spawning does have some drawbacks. Obviously, this design does not reflect what happens in nature; while some multiple partnering is likely, the full matrix (i.e., every male spawning with every female) is not. This design has the potential to produce large numbers of families which should be reared separately until they can be marked creating logistical problems. Furthermore, it is probably not necessary when larger numbers of both sexes (i.e., >5 of each) are captured. Finally, it may not actually reflect what occurs naturally in the wild and still does have some risk of inbreeding in the future. For example, recent surveys in the Nechako River that focussed on finding spawning fish and habitat observed small congregations of adult fish displaying spawning behaviour (i.e., in 2004, 22-36 fish were observed). Of particular interest is the obvious pairing by several sturgeon, although groups of 3-4 sturgeon were occasionally observed. The release of gametes by a single pair was also observed (Triton 2004). These observations differ somewhat from those in the Columbia and Kootenay rivers where no pairing has been observed visually (but note that neither has full communal spawning). However, preliminary genetic results from a parentage analysis on offspring collected from Waneta spawning area strongly suggest that pairing (i.e., one to one spawning or close to this) is the predominant mating scheme for spawners in the upper Columbia system. Pairing behaviour may be an artefact of reduced breeding numbers or imbalances in numbers of males or females present, or may reflect the natural spawning behaviour of specific populations.

In conclusion, full matrix spawning is probably beneficial and logistically straightforward when low numbers of one or both sex is available. This will ensure as many individuals as possible contribute to the next generation and increases genetic combinations among individuals even where only very low founding numbers are available. However, when numbers of both sexes are larger, partial factorial design, ensuring each individual contributes at least once should be adequate (unless gamete viability is variable or questionable).

The matrix in Table 3 provides guidelines that attempt to maximize the amount (and combinations) of genetic material captured in crosses by protecting against possible spawning failure of some individuals (by creating half-sib families) while considering logistics involved with large numbers of crosses. Regardless of design, sperm from different males should never be mixed for fertilization process.

Table 3. Spawning options under various options for broodstock available

Number of females available (N_f)		Number of Males available (N_m)				
		0	1	2-5	6-9	10 or >
	0	----------	Cryopreserve sperm for future	→	→	→
	1	Attempt to use cryo-sperm, preferably with more than one male per female using all females	1 x 1 mating	Use all males, create N_m half-sib familes,	→	→
	2-5	↓	Create N_f half-sib families, also split eggs for 2 or more females and attempt to use cryo-sperm (i.e., use each cryo-male with more than one female to avoid family effects)	Ensure all males and all females are each spawned to produce at least 2 families initially	Ensure all females are used at least twice in N_m or > half-sib families using all males at least once	→
	6-9	↓	↓	Ensure all females are each used to create 2 half-sib families – has the potential to exceed facility capacity but excess families can be culled once early survival is confirmed	↓	Ensure all females are spawned with at least one male, create additional half-sib families, especially with lower numbers of females or if gamete quality questionable
	10 or >	↓	↓	↓	↓	↓

Rearing and Release

Family separation

It is essential to rear families separately until they can be differentially marked. The current breeding facility design incorporates a capacity to rear 12 families to up to 2 years of age (R. Billings, pers. comm.). However, these plans have included a level of flexibility to permit additional temporary rearing containers for larvae. In addition, should the program determine that a younger age of release is more appropriate, larger rearing tanks can be converted to provide rearing capacity for more families or larger numbers of fish (R. Billings, pers. comm.).

Family marking

Marking of all individuals should be conducted so that, at a minimum, family and cohort year can be determined. In this way, a number of key parameters including post-release survival and movement, as well as growth rates and condition, can be measured and tracked. Furthermore, family tagging will enable future removal of individuals prior to maturation should it be determined that release numbers were excessive (in terms of carrying capacity or genetic swamping). The importance of the ability to distinguish families in cohorts has been highlighted by the as yet unresolved fin deformities identified in cultured Upper Columbia white sturgeon.

Marking fish with individual-level markers (i.e., PIT tags) is essential in order to obtain estimates of certain parameters like growth rates, movement, individual survival and population estimates. They will also enable the assessments at the family and cohort level. Fish can be PIT-tagged once they achieve a size of approximately 20-30 g. This may be possible by October of the spawn year (i.e., approximately 4 months old) for Nechako fish if plans to rear the fish on the relatively warm river water (17-20^{o}C) are carried out. It is strongly recommended that the first 2-3 years of the program incorporate a PIT-tagging component that marks every fish released.

Family-level marking using scute removal will differentiate families and cohorts. This type of marking can be conducted on younger fish year at 10-15 g and will permit earlier mixing of families and reduce facility requirements per family. However, preliminary evaluations of this method in the Columbia system suggest that scute markings can only be correctly identified 70-80% of the time due to inconsistencies in how scutes are counted (S. McAdam, pers. comm.). Additional types of family-level markers should be investigated.

Cohort marking (and possibly even family level) using coloured elastomere implants should be investigated as an alternative or possibly an additional way to mark fish. This is a highly visual mark which would assist field observations particularly in monitoring escapes. Unfortunately, the technique is only temporary (i.e., a couple of years), so that any marking would have to be repeated or be used for short term assessments only.

Once important parameters such as post-release survival have been determined with confidence and the Initiative is confident that family contributions in the river are not excessive or require culling (i.e., re-capture and removal), it may no longer be necessary to mark every fish to the family level but marking of every hatchery fish must continue to be done annually.

Family equalization

Ideally, family sizes would be equalized prior to the release of juveniles into the wild environment. This ensures equal contributions from each parent to the next generation and maximizes the genetic effective population size N_e of the population. This is particularly important when very limited genetic material is available initially, and reduced numbers can increase the potential for loss of genetic variation through genetic drift and for inbreeding (i.e., when population size <50). This recommendation is emphasized repeatedly throughout the literature for salmonid hatchery programs, and was recommended in the original Kootenai breeding plan for white sturgeon (Kincaid 1993).

In addition, equalization ensures that all families have an equal opportunity to survive and contribute in theory. This is important because large families that appear to excel in a captive environment may not necessarily have the appropriate fitness traits in the wild (i.e., selective pressures differ between captive and wild environments). For example, captive fitness traits such as size and growth rate do not appear to correlate with post-release survival in the wild for the Kootenai breeding program (R. Beamesderfer, pers. comm.). For this reason, it is strongly recommended that the breeding program avoid selective culling procedures (i.e., removing small, slow-growing fish) and encourage the survival of all individuals while in the recovery facility environment (i.e., rear separately according to size).

In practice, achieving family equalization in the breeding program may create serious limitations (i.e., when one family is very small) . First, the late maturation of the white sturgeon means that there is plenty of time for natural selection to 'level the playing field' for hatchery families of varying sizes once released in the wild and prior to spawning in an estimated 15 to 30 years. Since natural recruitment is currently non-existent, 'swamping' of wild progeny by hatchery families is also not an issue. Furthermore, the results of a recent modelling exercise evaluating the importance of family equalization in sturgeon culture suggest that equalization may not have significant benefits on N_e and could reduce total population size when density-dependent mortality was low (Jager 2005). Finally, variation in release numbers among families has not been large for the Kootenay population (i.e., range of 3,000-4,500, KTOI 2004). It should be noted that in 2004 and 2005, the Upper Columbia program was able to meet both equalization and release goals at 2,000 fish/family for six families (R. Ek, pers. comm.).

It has been deemed more important to maximize release numbers for the Kootenay breeding program than to try to equalize release numbers from each family due to the acute demographic risk this population faces (KTOI 2004). This will ensure that enough numbers are present in the next generation to actually provide a spawning population (particularly given that long term survival of hatchery fish is unknown) and enable high confidence in post-release survival and other estimates of life parameters (P. Anders, pers. comm.). Finally, equalization would be more of a concern if the range in family size varied by more than a factor of 10.

Similarly, the Nechako population faces severe risk of demographic extinction within the current generation. More emphasis should be placed on meeting demographic and experimental requirements in the short-term (i.e., next 2-3 years). However, this plan recognizes the potentially significant risks in ignoring equalization completely. Thus, equalization should be attempted when possible. If not possible, efforts to balance contributions better without compromising total release goals should be made where the potential for significant imbalances in contributions from families exists (i.e., in the order of magnitudes). This more conservative approach compared to the Kootenay plan also recognizes the risks associated with potential strays.

Release goals

Numbers

Under current conditions, it appears that the next generation of Nechako white sturgeon will be based entirely on hatchery fish unless natural recruitment improves. This being the case, there is no likelihood of swamping wild cohorts with hatchery fish. The two key goals of the release strategy are:

1. To ensure that adequate numbers of each family survive to adulthood to provide a viable population of spawners in the long-term (includes demographic and genetic goals);

2. To provide adequate numbers for experimental purposes in the short-term.

Past targets for the Kootenay breeding program have been 1,500 fish/family and six families per year for a total annual release of 9,000 2-year old fish. Most recently, this recommendation has increased to up to 10,000 age 0 fish/family for up to 12 families. This could potentially result in a total of up to 120,000 fish/year. This significant increase is intended to better address the acute demographic status of the population, as well as address the increased mortality associated with releasing smaller fish (see Size section below). This goal also considers the fact that long-term survival rates are unknown and just a 3% difference in annual mortality results in a 3-fold difference in expected equilibrium numbers in the future spawner population (KTOI 2004).

Similarly, post-release survival rates for the Nechako population are unknown but small differences in annual mortality will also produce differences in the size of the future spawning population that are much larger in magnitude given the delayed maturity of this population (i.e.., see Table 2). However, unlike the Kootenay population which is ‘contained’ within the system, the Nechako population is not physically isolated by a barrier to the other Fraser populations. The behaviour of hatchery raised juveniles in terms of migratory behaviour is unknown, and released fish have the potential to leave the system and interact with other populations. Efforts to imprint hatchery fish to the Nechako River will be taken, including the use of river water to rear fish and experimental releases to determine the most appropriate locations for release sites. However, it is unclear if, when or how sturgeon imprint to their natal systems and what the most effective means are to prevent ‘straying’. Furthermore, large numbers of releases that exceed the habitat's capacity have the potential to increase straying rates (i.e., fish are forced to look for other habitat).

Thus, the three major risks of releasing large numbers of fish per family each year for the Nechako population include: (1) exceeding capacity of the system resulting in reduced condition and survival; (2) increasing the potential to stray to the Fraser River mainstem; and, (3) increasing the potential to genetically 'swamp' the population if large differences in family size exist.

Ideally, annual release goals would be determined by back-calculating how many progeny are required to meet future spawner number goals in the population within the habitat's carrying capacity. Unfortunately, the necessary information to do this calculation is not currently available. The sensitivity of slight changes in annual survival rates on spawner recruitment associated with delayed maturation emphasizes the importance of experimental releases to confirm post-release survival rates. Thus, some level of risk will be necessary in the short-term to calculate key parameters. Regular monitoring and adjustment of annual goals will reduce risks significantly, and enable the recovery team to determine appropriate population goals over the longer term. In the longer term, removal of tagged fish would be an option should the recovery team determine that too many fish were originally released.

As such, it is recommended that the annual release numbers be large enough to determine survival rates with confidence. To date, the number provided by the Recovery Team to achieve this adequately is 12,000 fish/year with the flexibility to increase numbers to some degree. This goal does not take into account the assessment of different release ages (discussed below) and larger goals may be necessary in the first 2-3 years to establish appropriate size, location and season for release. Release goals per family will have to be adjusted annually to meet this total release goal depending on the number of families available. In theory, this could range from an extreme case of 12,000 fish/family if only one family is created to 1,000 fish/family if 12 families are available. Swamping under such cases (i.e., where only one family is available) is a concern. This risk may be acceptable in the short term (i.e., 2-3 years) in order to provide adequate numbers for experimental purposes to estimate survival, growth and movement. However, this risk should be weighed against the amount of post-release monitoring and re-capture effort that will be included. If it makes little or no difference to confidence in survival estimates whether 6,000 or 12,000 are released in the case of only a single family being available, then releases should lean towards conservative numbers (i.e., minimum number needed to estimate survival).

Over the longer term, it could be argued that demographic needs outweigh genetic concerns associated with over-representation. For instance, if production of only 1-2 families becomes a regular occurrence due to severe difficulties in obtaining broodstock, it would be more beneficial to meet annual release goals than equalization goals (i.e., there may not be enough fish contributing to the next generation to worry about swamping effects in a given year). However, it is expected that efforts to bring wild broodstock to maturity in captivity would be increased to avoid this situation if such conditions persist. Secondly, the impacts of this swamping effect will likely be dampened over the long term given the number of years contributing to a single generation. Finally, family and total release sizes can be refined over time to focus more on equalization and swamping concerns as more information becomes available. A few initial years of over-representation will not likely have a huge impact over the longer term.

In conclusion, family equalization should not be dismissed entirely, but compromises within reason may be necessary to ensure enough fish are available each year, particularly in the 'start-up' years, to provide reliable estimates on early post-release survival and other important parameters. One key component to the success of this program is regular monitoring. As the possible genetic risks associated with release numbers increase, so too should the monitoring and re-capture efforts (Williamson et al. 2003). Given the risk of straying, regular monitoring must be expanded to the Fraser River system as well, and any tagged fish in the Fraser River should be removed.

Size

The specific bottleneck to recruitment is currently unknown but assumed to occur within the first 2 months of post-hatch life (S. McAdam, pers. comm.). Thus, release strategies will focus on rearing fish to sizes beyond this stage of development. However, the optimal size to maximize long-term post-release survival is unknown. Currently, survival rates for Kootenai fish released at 2 years of age are 60% for the first year post-release and 90% per year in subsequent years (R. Beamesderfer, pers. comm.). Preliminary results for the Upper Columbia breeding program suggest similar or higher rates (S. McAdam, pers. comm.).

To date, both Kootenay and Upper Columbia recovery programs have released 1+ and 2 year old fish at 30-60 g. However, the recommendation in the updated breeding plan for the Kootenay population is to conduct fall and spring larger-scale releases of younger (0-1 yr), smaller (10-15 g) fish. This has three main benefits: (1) it reduces time in the hatchery under artificial selection; (2) it enables the production of larger numbers of families; and (3) it reduces the likelihood that cohorts experience rearing catastrophes while in the hatchery environment (e.g., temperature failures, disease, etc.) (KTOI 2004).

The current planned capacity for the Nechako facility is 10,000 1 year old 40 g fish per year (R. Billings, pers. comm.). However, this could be increased to up to 30,000 0+ year old fish for fall release at 20 g, plus an additional 10,000 40 g 1 year old fish in the spring. This size assumes good growth rates associated with rearing on warm river water (mean 17^{o}C to fall release). Because fall-released fish will go well-fed into a relatively low-activity season from a growth and feeding perspective, it is suspected that initial survival may be as high or better than releasing larger, older fish in the following spring. Furthermore, predation while hatchery fish are adapting to the wild environment is expected to be lower during the winter (S. McAdam, pers. comm.). Because of the importance of knowing survival rates and other parameters, all fish must be large enough to be individually marked. Experimental releases at different sizes, potentially exceeding the suggested 12,000 fish/year goal, as proposed above are highly recommended to compare survival. Finally, experimental releases to identify the specific developmental period where the recruitment bottleneck occurs may require the release of even younger age groups. These releases should not be included in the 12,000 fish/year goal.

Timing and location

Timing (and hence size) and location of release will also affect survival rates and the likelihood of downstream movement into the Fraser River. Thus, efforts to optimize these two release variables must be made. For example, higher flows shortly after the release may have contributed to the downstream movement of naïve juveniles in the Upper Columbia River (S. McAdam, pers. comm.). For this reason, releases later in the season when flow rates have dropped should be considered. Optimal timing of release will also be affected by temperature. As mentioned above, fall releases may benefit from reduced activity by predators. However, if temperatures are too low, juveniles may not actually demonstrate any activity, including efforts to hide or protect themselves. Thus, timing will require some fine-tuning. Optimal location may be affected by temperature and flow rates to some extent.

Monitoring

Regular monitoring is the absolute key to the success of both the experimental and recovery goals of this breeding plan. Detailed records of all components of the broodstock collection, mating, rearing, recaptures and releases must be kept.

Broodstock collections and matings

All wild-caught broodstock should be individually tagged. In addition, biological information including lengths, ages, fecundities and condition should be recorded, and tissue samples for DNA analysis should be collected. This information will enable pedigree analysis in the future, as well as determining contributions over time for repeat spawners.

Family rearing

All families should be reared separately until they can be marked. Initial egg numbers, survival to various stages and variation in family size at time of mixing should be documented. In this way, early survival rates and family effects can be determined. All individuals should be marked initially using PIT-tags (or some other method of individual marking), but eventually marking could be reduced to family and year group. Furthermore, consideration of easily visible markers such as elastomere implants, particularly for consideration of stray fish into the Fraser River mainstem.

Release

Documentation of release methods including age, size, date and location should be recorded in detail. This will ensure that season, location and size at release are optimized to provide the best survival and reduced likelihood of straying.

Re-capture

Re-capture studies to determine survival rates should also document length, weight, location, and date for each fish. This will provide growth rates, movement information and condition assessments. Furthermore, all re-captures should be individually tagged (if they were not initially) so that they can be identified should they be re-captured in the future

SUMMARY

In summary, the primary goal of the breeding program is to restore and maintain the genetic and demographic integrity of the Nechako River white sturgeon population. In the absence of any significant natural recruitment, recruitment will be almost entirely dependent on the breeding program. Therefore, the size and design of the program must be adequate to address demographic and genetic issues associated with a small founder population.

The recovery recruitment goal is 2,500 adults per generation. This equates to approximately 50-60 adults per year for 45-50 years. The ideal number of adults required to meet this goal from a genetic perspective is at least 500 (or 5 pairs of breeders per year for 50 years). However, meeting demographic and genetic goals simultaneously may not be possible under existing or future population conditions. With every year that passes, another 8% of the total population is lost to natural mortality, and the already limited pool of potential spawners continues to dwindle.

This breeding plan emphasizes the need to incorporate the 'front-loading' concept to maximize the number of wild fish that contribute to the next generation of spawners. Furthermore, it considers demographic and experimental objectives to be a more immediate concern than meeting all genetic objectives in its short term release goals. Genetic equalization may be compromised initially to ensure that as many broodstock as possible are collected and mated each year, and that release numbers are adequate to avoid demographic extinction and the problems associated with critically low numbers of breeders available in the next generation (e.g., inbreeding, Allee effect). As information on survival rates, growth, and movement of hatchery released juveniles becomes available, it can be incorporated into a more refined release strategy that addresses carrying capacity and genetic concerns to a greater extent. In addition, measures can be taken in the future to offset the large numbers of fish recommended for initial releases should these numbers prove to be a problem. Given the potential risks involved, regular and continued monitoring is an essential component that must be included in all budgeting exercises.

It is important to recognize that the proposed conservation fish culture program for the Nechako white sturgeon population is an interim measure to prevent extinction until the cause for the recruitment failure is identified and addressed. This program must be considered experimental as similar conservation programs involving white sturgeon have not been conducted over long enough timeframes to evaluate success. However, if successful, this program will maximize the potential for natural recruitment in the future by preserving the remaining population's demographic and genetic integrity and restoring the age structure.

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