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SCIENTIFIC EVIDENCE TO INFORM SPAWNING PRACTICES AT FISHERIES AND OCEANS CANADA SALMON HATCHERIES IN THE PACIFIC REGION

Context

Release of Pacific salmon from hatcheries to support or enhance natural populations creates potential risks to the genetic integrity, genetic diversity, and fitness of wild salmon. Under *Canada's Policy for Conservation of Wild Pacific Salmon*, wild salmon are defined as salmon that have spent their entire lives in the wild and are the offspring of naturally-spawned parents (DFO 2005). The genetic risks to wild salmon associated with hatcheries are mitigated and minimized through genetic management of broodstocks. The Fisheries and Oceans Canada (DFO) Salmonid Enhancement Program (SEP) currently uses guidelines developed in the 1980s to direct broodstock collection and spawning within their hatchery lines (DFO 2016; see Appendix A). In recent years there have been substantial advances in scientific knowledge regarding the effects of hatcheries on wild salmon. A review of the genetic management of broodstock guidelines is timely to ensure operations are consistent with current science advice.

A recent Canadian Science Advisory Secretariat (CSAS) research document, *Genetically Based Targets for Enhanced Contributions to Canadian Pacific Chinook Salmon Populations* (Withler et al. 2018), established proportionate natural influence (PNI) designations for Canadian salmon populations to facilitate management of genetic risks associated with hatchery selection and domestication. The application of these designations is currently being developed for SEP's hatchery lines, which have objectives spanning harvest, assessment, rebuilding, conservation, and education/stewardship. Therefore, it is also essential to evaluate if the general broodstock collection and spawning guidelines require modifications depending on the particular management objective or PNI designation of a line enhanced by SEP.

DFO SEP has requested science advice on updates to the broodstock collection and spawning practices at SEP operated and supported hatcheries in the Pacific Region. This assessment and the advice arising from this Canadian Science Advisory Secretariat (CSAS) Science Response (SR) process will be used to update both the SEP genetic management guidelines (DFO 2016) and the SEP risk management framework for enhancing Pacific salmon (DFO 2013).This Science Response Report results from the regional peer review of March 5, 2024 on Scientific Evidence to Inform Spawning Practices at Fisheries and Oceans Canada Salmon Hatcheries in the Pacific Region.

The specific objectives of this report are to:

1. Assess potential genetic impacts of existing hatchery broodstock collection guidelines regarding the proportion of the escapement removed for broodstock, and the relative use of natural- versus hatchery-origin spawners compared to their representation in the escapement.
2. Examine published scientific knowledge to provide advice on best practices for maintaining genetic diversity in hatchery broodstock depending on broodstock size and enhancement

objectives, particularly with reference to the use of males, cryopreserved milt, sex ratios, and matrix spawning.

3. Evaluate the potential consequences of random versus intentional selection of hatchery spawners based on fitness-related traits (e.g., size, age, or run timing) with dedicated consideration of the use of jack males in hatchery broodstocks.

The information reported here is presented specifically in the context of Pacific salmon populations enhanced by hatcheries operated and supported by SEP. However, the underlying basic principles also have relevance to other hatchery operations involving Pacific salmon, other salmonids, and other species more generally.

Background

Essentially all hatchery enhancement for Pacific salmon in Canada involves collecting adults or gametes from the natural habitat, spawning the adults or gametes artificially in the hatchery, raising the offspring until a targeted life stage, and releasing the offspring into the natural habitat at the targeted life stage. These activities can support a range of objectives including conservation, rebuilding, assessment, stewardship, education, and harvest (DFO 2018), and populations of Chinook (*Oncorhynchus tshawytscha*), Coho (*O. kisutch*), Chum (*O. keta*), Pink (*O. gorbuscha*) and Sockeye (*O. nerka*) Salmon are enhanced by SEP. In the majority of these cases an underlying goal of enhancement is to maintain or increase the number of salmon in a population. At least in the short-term, hatcheries are able to generate these increases through three main mechanisms:

1. providing artificial habitat to support a greater number of spawners (i.e., spawning capacity);
2. improving spawning success; and
3. drastically reducing the mortality rate experienced by hatchery offspring throughout early life stages (Mobrand et al. 2005).

These mechanisms increase not only the number of spawners in the population, but also the recruits per spawner relationship for spawners within the hatchery compared to spawners in the natural habitat. The potential demographic benefit of these approaches is clear, and thus it is not surprising that use of hatcheries to produce Pacific salmonids is common throughout the Northern Pacific (e.g., Ruggerone and Irvine 2018).

Despite the potential benefits of hatcheries, the basic concept of enhancement produces inherent genetic risks for a population, which may lead to negative long-term effects despite short-term demographic gains (Busack and Currens 1995; Mobrand et al. 2005; Naish et al. 2007; McMillan et al. 2023). To restate the description above, hatchery practices expose a relatively small number of spawners to an artificial habitat, reduce or remove sexual selection during spawning, raise juveniles in an artificial habitat, and reduce natural selection throughout early life stages. These practices are key to increasing the expected recruits per hatchery spawner and the number of recruits overall, but they also result in disproportionate contributions of individual spawners to subsequent generations, relaxation of selection pressures associated with the natural habitat, and exposure to novel artificial selection pressures (i.e., domestication selection) (Hatchery Scientific Review Group [HSRG] 2004; Califiornia Hatchery Scientific Review Group [CHSRG] 2012; McMillan et al. 2023). All three of these unintended consequences of enhancement pose a threat to the conservation of genetic diversity in wild Pacific salmon, which in turn creates risks for the long-term fitness and productivity of wild salmon populations. Thus, it is critical to manage hatcheries in a scientifically defensible manner that minimizes these long-term risks.

Genetic diversity is defined by *Canada's Policy for Conservation of Wild Pacific Salmon* (i.e., the Wild Salmon Policy) as, "genetic variation and adaptations within and between populations of wild Pacific salmon" (DFO 2005). The breadth of this definition means that the term "genetic diversity" can be used to describe several aspects of genetic variation within a species of Pacific salmon, and related terms such as "genetic integrity" or "natural influence" are also used in the context of salmon conversation and management (e.g., Withler et al. 2018), creating the potential for ambiguity. Furthermore, risks to genetic diversity may pose threats to different aspects of genetic variation within a species. Therefore, prior to assessing the scientific evidence for best broodstock management practices in the context of SEP's genetic management guidelines, specific aspects of genetic diversity are briefly discussed below.

A more general scientific definition of genetic diversity is the total number and frequencies of genetic variants present in a group of organisms (i.e., the total genetic variation; Frankham et al. 2002). As highlighted by the Wild Salmon Policy, an important distinction is whether genetic variation is considered within a population or among different populations: population-level or species-level genetic diversity, respectively. Both levels of genetic diversity have key implications for conservation of healthy Pacific salmon populations. High genetic diversity within a population minimizes the likelihood of interbreeding between genetically related individuals, and provides the raw material upon which natural selection can act to drive adaptive processes (Kahilainen et al. 2014). Similarly, diversity among populations improves species resilience through the "portfolio effect" (Schindler et al. 2010, 2015), which is a concept borrowed from economic theory, suggesting that species with diverse populations are more resilient in the face of environmental change. Genetically diverse populations make it more likely that some populations will be resilient to a novel stressor, whereas others will not, rather than an all-or-nothing susceptibility across populations. Conservation of species-level genetic diversity is approximately synonymous with protection of genetic integrity, which refers to the reproductive isolation of a species or population and the maintenance of distinct genetic variation through the prevention of interbreeding between species or populations (e.g., Marie et al. 2010; Bourret et al. 2011; note that, in an unrelated cellular context, genetic integrity in scientific literature can also refer to the preservation of DNA sequence from damage or mutation). The concept of a population's genetic integrity illustrates the importance of clarity between population- and species-level genetic diversity. For example, a transplant of spawners from a river of high abundance to a river of low abundance has the potential to increase both the spawner abundance and population-level diversity in the recipient river; however, this same action would decrease any species-level diversity that was present between the two original populations. The balance of these risks versus rewards would likely be context dependent, particularly with respect to the differentiation between the two populations, and the conservation status of the recipient population.

A second important distinction within genetic diversity is between neutral and adaptive variation (e.g., Holderegger et al. 2006). Allele frequencies can change within or between populations as a result of random chance (i.e., genetic drift), or as a result of adaptive processes such as natural selection (Lande 1976). To varying degrees, Pacific salmon species display homing behaviour to return to their natal habitats to spawn as adults (Quinn 2005b), which produces high numbers of relatively small spawning populations. These conditions are conducive for neutral processes to play a large role in genetic differentiation among populations, and signatures of isolation by distance are often observed in population genetics studies of Pacific salmon (Taylor et al. 1994; Beacham et al. 2006a, 2006b, 2009, 2011, 2020). However, salmon also display local adaptation to their freshwater habitats (Taylor 1991; Eliason et al. 2011; Fraser et al. 2011), suggesting that both neutral and adaptive processes play a role in establishing and maintaining extant genetic diversity in these species. There is ongoing scientific debate on the conservation relevance of neutral and adaptive genetic variation (e.g., DeWoody et al. 2021; García-Dorado and Caballero 2021; Teixeira and Huber 2021). Essentially, one perspective suggests that adaptive variation underlies fitness, and thus adaptive variation should be the focus of conservation efforts. In contrast, the other perspective suggests that neutral variation provides the raw material for novel selection to act upon, adaptive variants under all scenarios are unknown, and putative neutral variation should not be discounted in conservation efforts. In the overall context of maintaining genetic diversity in Pacific salmon both perspectives have merit, and a balance between conservation of neutral and adaptive variation is likely most appropriate.

Within an enhanced population, hatchery production can pose risks to both neutral and adaptive genetic variation. The risks associated with adaptation to the hatchery environment and management of those risks have been considered in-depth elsewhere (e.g., HSRG 2004, 2009, 2014; Naish et al. 2007; CHSRG 2012; Anderson et al. 2020; McMillan et al. 2023), and reviewed previously through the Canadian Science Advice Secretariat (Withler et al. 2018). In brief, as discussed above, the artificial habitat of the hatchery alters the selection pressures experienced by hatchery spawners and hatchery-origin individuals, which changes the suite of traits that produce optimal fitness. In other words, the fitness optimum of hatchery-origin fish is different than that of natural-origin fish, and this effect can be amplified if broodstock collection does not proportionally represent the full trait distributions present in the population (e.g., McLean et al. 2005). Thus, hatchery-origin fish have lower reproductive success in the natural habitat than their natural-origin counterparts, and gene flow from the hatchery into the natural environment is expected to lower the average fitness of the population in the natural habitat (Araki et al. 2007a, 2008, 2009; Thériault et al. 2011; Christie et al. 2012a, 2014; Ford et al. 2016; Withler et al. 2018; Janowitz-Koch et al. 2019; Shedd et al. 2022). These risks to the adaptive state of the population can be mitigated by managing the relative rates of gene flow from the hatchery to the natural spawning grounds and from the spawning grounds to the hatchery, such that the natural adaptive influence in the integrated population is maintained (Ford 2002; HSRG 2009; Baskett and Waples 2013; Withler et al. 2018).

To understand the risks hatcheries pose to neutral genetic variation within an enhanced population, it is necessary to introduce the notion of an effective population size. An effective population size is a concept from population genetics that describes the size of an idealized population that would lose genetic variants through genetic drift (i.e., random chance) at the same rate as the real-world population (Wright 1931). To establish an idealized population, several simplifying assumptions are made that are optimal for the maintenance of genetic variation. The key assumptions are constant population size, non-overlapping generations, random mating, equal sex ratios and no selection (i.e., equal opportunities among parents for representation in the subsequent generation) (Charlesworth 2009). Real-world populations essentially never conform to these assumptions, and consequently effective populations sizes are generally smaller than census estimates of population size (Waples 1990; Frankham 1995). The benefit of these simplifying assumptions for conservation is that the rate of loss of genetic variants and the rate of inbreeding can be modeled in an idealized population, which means estimates of effective population size can inform the risk of loss of genetic variation at random from a population (Charlesworth 2009). These risks associated with genetic drift become more pronounced at small effective population sizes which also make selection and adaptation relatively ineffective (Adkison 1995). Both neutral and adaptive variation can be lost at low effective population sizes, as genetic drift is a random process posing a threat to all within-population genetic diversity.

In the absence of appropriate genetic management, hatchery production can cause a substantial reduction in effective population size of an integrated population even if the census population size increases as a result of the combined abundance of hatchery- and natural-origin fish (Christie et al. 2012b; Naish et al. 2013). In general, relatively few spawners are collected as hatchery broodstock, and the nature of hatchery spawning results in over-representation of these spawners' offspring in the subsequent generation when compared to the offspring of natural spawners (Anderson et al. 2020). Moreover, re-use of male spawners in the hatchery has the potential to exacerbate these effects. These decreases in effective population size associated with enhancement can lead to a Ryman-Laikre effect, in which repeated over-representation of relatively few spawners increases inter-individual relatedness leading to inbreeding depression and loss of population-level fitness (Ryman and Laikre 1991).

Taken together, there are undoubtedly benefits to hatchery production in a variety of contexts, and hatcheries have been successfully used as tools in the support or rescue of populations of Pacific salmon in several locations (Ackerman et al. 2014; Kline and Flagg 2014; Withler et al. 2014). However, it is also clear that the introduction of hatchery-origin fish into a natural system poses many genetic risks to the natural component of the population. It is crucial that hatchery practices are guided by the best science available to mitigate these risks and minimize the chance that long-term outcomes of enhancement will have negative consequences for wild Pacific salmon.

Analysis and Response

Enhancement strategy utilized by SEP hatcheries

Throughout the northern Pacific two main hatchery strategies are employed to manage the genetic interactions between hatchery- and natural-origin salmon. Segregated programs prevent hatchery-origin fish from spawning in the natural habitat and natural-origin fish are not used in broodstock such that there is no gene flow between the hatchery and natural environments (HSRG 2004, 2009; Mobrand et al. 2005; CHSRG 2012). In contrast, integrated programs allow hatchery-origin fish to spawn in the natural habitat and natural-origin fish to spawn as part of the broodstock such that there is deliberate gene flow between the two environments; integrated programs can manage the genetic interactions between hatchery- and natural-origin fish by controlling the relative directions of gene flow between the two habitats (HSRG 2004, 2009; Mobrand et al. 2005; CHSRG 2012).

These two hatchery strategies have different strengths and weaknesses. In a segregated program, natural selection pressures are absent in the broodstock, which increases the potential for relaxed selection and domestication to result in differentiation from the natural component of the population. This increases the genetic risk posed to natural spawners if hatchery-origin fish were to interbreed with natural-origin fish, but this risk is mitigated by the segregation between the two spawning habitats. On the other hand, an integrated program maintains natural selection pressures in the broodstock through the inclusion of natural-origin fish, which reduces the likelihood of substantial genetic differentiation between hatchery- and natural-origin fish. Hatchery-origin fish still pose genetic risks for the population as a whole, but these risks are reduced relative to those from the hatchery-origin fish produced by a segregated program (Mobrand et al. 2005; CHSRG 2012).

Consistent with these different strengths and weaknesses, recent reviews of hatchery practices in the United States (USA) by the Hatchery Scientific Review Group (HSRG) have concluded that both strategies can be viable options to minimize the genetic influences of hatcheries and promote local adaptation of natural salmon populations (e.g., HSRG 2004, 2009). However, the *California Hatchery Review Report* reached a different conclusion; "the California HSRG asserts that a truly segregated anadromous fish hatchery program is not possible in California, and we are therefore generally unsupportive of the concept" (CHSRG 2012). This conclusion follows from the nature of a segregated program; due to the high likelihood of genetic differentiation and hatchery adaptation, if all hatchery-origin fish from cannot be reliably removed from the natural spawning grounds, negative genetic effects are likely to be substantial. When this is the case, an integrated hatchery is a better option to minimize these negative effects on natural spawners.

Complete removal of hatchery-origin spawners from natural habitats is unrealistic in the majority (if not all) of the Pacific salmon populations enhanced by SEP. This suggests that the use of integrated programs is likely the most appropriate option for hatcheries operated or supported by SEP, which is consistent with existing genetic management guidelines (DFO 2016).

Collection of broodstock relative to escapement size

The level of hatchery production (i.e., hatchery size) is perhaps the most overarching factor impacting the influence of the hatchery on an integrated population, as it has a fundamental effect on the relative numbers of hatchery- and natural-origin salmon in the population. Not surprisingly, evaluation of hatchery size, marking of hatchery releases and selective removals of hatchery-origin spawners clearly concluded that adjusting hatchery size (defined by the number of broodstock) was the most effective management action to control hatchery selective influences within an integrated population (Withler et al. 2018).

Hatchery size is ultimately determined by production and broodstock collection targets, which are decided as an integrated management decision in SEP. These decisions account for a myriad of factors, including program objectives, habitat status and multiple risk factors (DFO 2018). Current genetic management in SEP (DFO 2016) provides general guidelines for broodstock collection to maintain natural influences within enhanced populations:

1. The number of collected broodstock should not exceed one third (33%) of the escapement.
2. For conservation and rebuilding populations, hatchery-origin fish should not exceed 50% of the spawners on the natural spawning grounds.
3. In conservation populations, the number of collected broodstock may reach up to 50% of the escapement.

Considerations regarding conservation populations are considered later in this report.

Each of guidelines 1 and 2 are independently logical approaches to limit hatchery influences within an integrated population, but as pointed out in Withler et al. (2018), the two guidelines are likely inconsistent. If hatchery-origin spawners have egg-to-adult survival rates that exceed twice that of natural-origin spawners, then using 33% of the escapement as broodstock will rapidly produce greater than 50% hatchery-origin spawners in the natural habitat. Despite highlighting this discrepancy, the guidelines above were not considered further in Withler et al. (2018). Instead, Withler et al. (2018) established a framework of biological designations to manage hatchery influences in populations enhanced by SEP that make use of HSRG's metrics of proportionate natural influence (*PNI*), proportion hatchery-origin spawners on the natural spawning grounds (*pHOS*) and proportion natural-origin spawners in the broodstock (*pNOB*). Together, after simplifications that are generally valid for populations integrated with a hatchery, these metrics can approximate the relative gene flow between the natural and hatchery components of a Pacific salmon population (Equation 1):

$$PNI = \frac{pNOB}{pHOS + pNOB} \tag{1}$$

PNI values range from 0 to 1 with higher values indicating relatively higher gene flow from the natural habitat into the hatchery (i.e., higher natural influences in the integrated population), and lower genetic risks to the adaptive state of the population overall. For integrated populations, Withler et al. (2018) published three biological designations: integrated-wild (PNI ≥ 0.80), integrated-transition (0.80 > *PNI* ≥ 0.50) and integrated-hatchery (0.50 > *PNI*) (Table 1). These designations can be used to manage gene flow in integrated populations: the integrated-wild designation maintains at least 50% wild fish (as defined by the Wild Salmon Policy; DFO 2005) on the natural spawning grounds, the integrated-transition designation maintains net gene flow from the natural habitat into the hatchery, and the integrated-hatchery designation allows relatively high hatchery influences in the population (Withler et al. 2018). Thus, these designations can be utilized to manage genetic risks within integrated populations across different enhancement objectives, as the trade-off between high production and high hatchery influence can be balanced appropriately. Furthermore, the *PNI* designations allow not only assessment of the current status of an integrated population, but also creation of genetic planning targets for future levels of hatchery production. Guidelines based on the Withler et al. (2018) designations are clear refinements and improvements of the relatively simple guidelines above (#1–3), and where relevant the biological designation framework should supersede previously existing guidelines regarding gene flow between the hatchery and natural environments. SEP is currently developing enhanced contribution guidelines for different enhancement objectives making use of these biological designations. However, to assess *PNI*, *pHOS* and *pNOB* there must be sufficient monitoring, and marking or tagging of hatchery-origin salmon, which is not the case for all enhanced populations. Thus, there remains utility in simplified guidelines such as numbers 1 and 2 above. Here we consider these guidelines in the context of the biological designations for enhanced populations established in Withler et al. (2018).

First, it is likely that a general guideline based on *pHOS* alone (i.e., guideline 2 above) is no longer consistent with best practices for genetic management of integrated populations. This becomes readily apparent by examining the *pHOS* thresholds for different biological designations for enhanced populations (Table 1).

Table 1. Biological designations of hatchery influence in populations of Chinook and other Pacific salmon with integrated enhancement (from Withler et al. 2018).

Designation	$pHOS_{census}$[a]	$pHOS_{eff}$[a]	*pNOB*	*PNI*	*pWILD*[b]
Integrated-wild	≤ 0.23	≤ 0.19	≥ 0.77	≥ 0.80	≥ 0.50
Integrated-transition	≤ 0.53	≤ 0.47	< 0.77 ≥ 0.47	< 0.80 ≥ 0.50	< 0.50 ≥ 0.13
Integrated-hatchery	> 0.53	> 0.47	< 0.47	< 0.50	< 0.13

[a] $pHOS_{census}$ is *pHOS* as estimated from the estimated numbers of hatchery- and natural-origin spawners on the natural spawning grounds, whereas $pHOS_{eff}$ is the effective *pHOS* after correcting the $pHOS_{census}$ to reflect the lower relative reproductive success of hatchery-origin spawners compared to natural-origin spawners.

[b] *pWILD* is the expected proportion of wild salmon in the spawning population as defined in the Wild Salmon Policy (DFO 2005).

In Table 1, $pHOS_{census}$ is the *pHOS* based on census estimates of the numbers of hatchery- and natural-origin spawners. Hatchery-origin spawners typically have lower reproductive success in natural spawning than their natural-origin counterparts, and the effective *pHOS* ($pHOS_{eff}$) in the context of genetic contribution to the next generation adjusts $pHOS_{census}$ based on the relative reproductive success of hatchery- and natural-origin spawners (HSRG 2015; Withler et al. 2018). In this report, *pHOS* is considered as $pHOS_{census}$, which is conservative as the relative reproductive success of hatchery- and natural-origin spawners in populations enhanced by SEP is not well-defined (Withler et al. 2018). This is also consistent with the assessment and application of *pHOS* in SEP. For integrated-wild, integrated-transition or integrated-hatchery designations, the acceptable limits for *pHOS* are ≤ 0.23, ≤ 0.53 or > 0.53, respectively. Thus, a guideline based on a single *pHOS* limit cannot account for the biological designation that is the goal for the enhanced population. For example, a guideline of *pHOS* < 0.50 would be too liberal for an integrated-wild target, whereas it would be unnecessarily conservative for an integrated-hatchery target. Additionally, accurate monitoring of *pHOS* requires marking of hatchery-origin fish, suggesting direct application of the biological designation framework from Withler et al. (2018) is preferable to placing a limit specifically on *pHOS* independently.

In contrast, managing the proportion of the escapement that is removed for broodstock does not require marking or tagging of hatchery-origin fish, and it may be possible to revise the current limit of 33% (guideline 1 above) to be consistent with the biological designations from Withler et al. (2018). To demonstrate this possibility, we apply simplified life-stage population assumptions to assess the effects of different proportions of escapement removals for broodstock on *PNI*, *pHOS* and *pNOB*. This approach is similar to reasoning presented in Withler et al. (2018) and described above that suggested a 33% limit on broodstock collection was likely inconsistent with the 50% limit on *pHOS*. Prior to reporting these analyses, we again emphasize that broodstock collection and management should be guided directly by the biological designation framework in Withler et al. (2018) in populations with marking of hatchery-origin fish.

In any integrated population of Pacific salmon, there will be a number of spawning adults in the escapement in a given year (*S*). The number of potential hatchery and natural spawners (S_H and S_N, respectively) can then be determined by the proportion of the escapement removed for broodstock (*pB*):

$$S_H = S \times pB \tag{2}$$

$$S_N = S \times (1 - pB) \tag{3}$$

Depending on the species of Pacific salmon being enhanced, the offspring from these spawners will return distributed across several return years generally 2–6 years later. However, we can consider the total number of hatchery- and natural-origin returns (*HOR* and *NOR*, respectively) expected among those offspring by utilizing the expected recruits per spawner for the S_H and S_N (R_H and R_N, respectively):

$$HOR = S_H \times R_H = S \times pB \times R_H \tag{4}$$

$$NOR = S_N \times R_N = N \times (1 - pB) \times R_N \tag{5}$$

It is important to acknowledge that R_H and R_N can vary, potentially substantially, across brood years and hatcheries, but they each have a single value for any given year and hatchery, and therefore R_H can be expressed as a multiple of R_N for any given cohort of spawners:

$$R_H = \alpha \times R_N \tag{6}$$

where α is the ratio of recruits per hatchery spawner to recruits per natural spawner. Equation 6 can be substituted into Equation 4 such that both *HOR* and *NOR* are expressed as functions of *S*, *pB*, R_N and *α*.

For integrated populations without identifiable hatchery-origin fish, Equations 4 to 6 can be used to assess the effects of the proportion of escapement removed for broodstock (*pB*) on *pHOS*, *pNOB* and *PNI* in the subsequent generation. For this analysis, two simplifying assumptions are necessary:

1. *pHOS*, *pNOB* and *PNI* are calculated as if the offspring are an independent cohort of returns; and
2. *pNOB* is equivalent to the proportion of natural-origin spawners on the natural spawning ground (*pNOS*).

The first of these assumptions is not valid for all Pacific salmon, but this simplification is often employed in life-stage modeling (e.g., Withler et al. 2018) and it should not invalidate the average impact of a value for *pB* applied across brood years contributing to mixed age classes of returns. The second assumption is valid for populations without selection of broodstock based on hatchery marks following from SEP's current genetic management guidelines, "collected broodstock should as far as possible be randomly selected" (DFO 2016). After these assumptions, *pHOS*, *pNOB* and *PNI* can be expressed as below (see Appendix B for further details):

$$pHOS = \frac{pB \times \alpha}{(pB \times \alpha) + (1 - pB)} \tag{7}$$

$$pNOB = \frac{(1 - pB)}{(pB \times \alpha) + (1 - pB)} \tag{8}$$

$$PNI = \frac{(1 - pB)}{(pB \times \alpha) + (1 - pB)} \tag{9}$$

Note that Equations 8 and 9 show *pNOB* equals *PNI*, which is a certain outcome when *pHOS* and *pNOB* sum to one (i.e., when broodstock are randomly selected from the escapement and *pNOB* equals *pNOS*). The utility of Equation 9 is that *PNI* can be plotted as a function of the proportion of the escapement removed for broodstock (*pB*) in a single generation for any value of the ratio between the recruits per hatchery spawner and the recruits per natural spawner (α) (Figure 1).

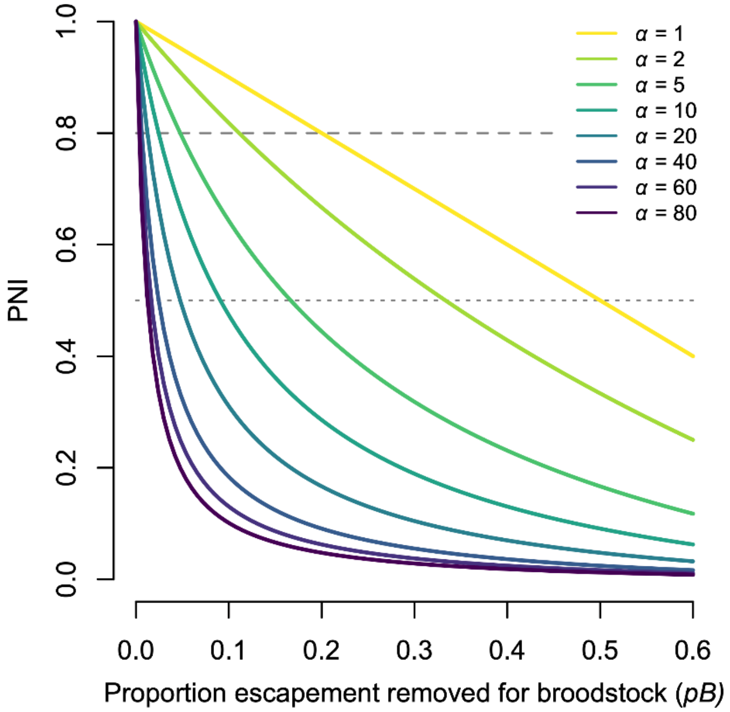

Figure 1. Proportionate natural influence (PNI) as a function of the proportion of escapement removed as broodstock (pB) for a range of ratios of recruits per hatchery spawner to recruits per natural spawner (α). Colours display curves for different values of α. Grey dashed and dotted lines display the threshold PNI values for the biological designations of integrated-wild, integrated-transition and integrated-hatchery for enhanced populations (PNI = 0.80 or 0.50, respectively).

Figure 1 displays the relationship between *PNI* and *pB* for *α* values ranging from 1 to 80, which spans scenarios where the recruits per spawner are equivalent between the hatchery and natural environment to scenarios where the recruits per hatchery spawner substantially exceed those per natural spawner (e.g., when hatchery production is high to support a harvest objective resulting in density dependence and low recruits per natural spawner). An immediate conclusion from Figure 1 is that the relationship between *PNI* and the proportion of escapement removed for broodstock (*pB*) is highly sensitive to the ratio of recruits per spawner between the two environments (*α*). Models parameterized using information from East Coast Vancouver Island Chinook populations suggest *α* values of approximately 4 may be plausible (Withler et al. 2018). Several SEP production lines are consistent with this estimate (*α* ≈ 4-5), but there can be substantial variation among populations and years (Angus Straight, DFO, pers. comm.). Consequently, it is challenging to identify accurate limits for broodstock collection that are consistent with the biological designations from Withler et al. (2018) in general (see Table 1), and expected PNI should only be predicted from proportional escapement removals for populations where *α* can be estimated with a high degree of confidence. Despite this limitation, even at low *α* values, proportional escapement removals consistent with the integrated-wild designation (*PNI* ≥ 0.80) are less than 0.33, and for all but the lowest *α* values this is also the case for the integrated-transition designation (0.80 > *PNI* ≥ 0.50) (Table 2).

Table 2. Predicted proportionate natural influence (PNI) values for combinations of the proportion of escapement removed as broodstock (B) and ratios of recruits per hatchery spawner to recruits per natural spawner (α). Green shading with an asterisk (*) indicates PNIs consistent with the integrated-wild population designation, and yellow shading with a circumflex (^) indicates PNIs consistent with the integrated-transition population designation; all unshaded PNIs are consistent with the integrated-hatchery population designation.

		α							
		1	**2**	**5**	**10**	**20**	**40**	**60**	**80**
B	**0.1**	0.90*	0.82*	0.64^	0.47	0.31	0.18	0.13	0.10
	0.2	0.80*	0.67^	0.44	0.29	0.17	0.09	0.06	0.05
	0.3	0.70^	0.54^	0.32	0.19	0.10	0.06	0.04	0.03
	0.4	0.60^	0.43	0.23	0.13	0.07	0.04	0.02	0.02
	0.5	0.50^	0.33	0.17	0.09	0.05	0.02	0.02	0.01
	0.6	0.40	0.25	0.12	0.06	0.03	0.02	0.01	0.01
	0.7	0.30	0.18	0.08	0.04	0.02	0.01	0.01	0.01
	0.8	0.20	0.11	0.05	0.02	0.01	0.01	0.00	0.00
	0.9	0.10	0.05	0.02	0.01	0.01	0.00	0.00	0.00

The lowest value of *α* considered in Table 2 is one, as if *α* is less than one, collecting an adult for hatchery spawning would effectively reduce the number of that adult's offspring that would be expected to return to spawn compared to allowing the adult to spawn in the natural habitat. This runs counter to the majority of SEP's program objectives and is an unlikely scenario in SEP's hatchery lines. When originally identifying the potential inconsistency between the 33% broodstock collection limit and the 50% hatchery-origin among natural spawners limit, Withler et al. (2018) identified that a two-fold increase in the egg-to-adult survival for hatchery spawners compared to that of natural spawners (i.e., $\alpha = 2$) was the maximum increase that was consistent with both guidelines. In contrast, several hatcheries operated or supported by the SEP have *α* values of approximately 4–5 (Angus Striaght, DFO, pers. comm.). Thus, to illustrate the potential application of *α* in determining the proportion of the escapement used as broodstock we calculate thresholds of proportional removal at $\alpha = 1$, 2 or 5 that are consistent with the three biological designations for integrated populations (Table 3). Note, as discussed above, the utility of these thresholds is dependent on the uncertainty associated with the estimated *α* value.

Table 3. Biological designations for enhanced populations with the acceptable ranges of PNI established in Withler et al. (2018) and consistent proportions of escapement removal for broodstock (pB) assuming α = 1, 2 or 5.

Biological designation	**Range of *PNI***	**Range of *pB* (*α* = 1)**	**Range of *pB* (*α* = 2)**	**Range of *pB* (*α* = 5)**
Integrated-wild	≥ 0.80	≤ 0.20	≤ 0.11	≤ 0.05
Integrated-transition	< 0.80	> 0.20	> 0.11	> 0.05

Biological designation	Range of *PNI*	Range of *pB* ($\alpha = 1$)	Range of *pB* ($\alpha = 2$)	Range of *pB* ($\alpha = 5$)
	≥ 0.50	≤ 0.50	≤ 0.33	≤ 0.17
Integrated-hatchery	< 0.50	> 0.50	> 0.33	> 0.17

Despite the utility of the simplified approach above to address whether a proportional escapement removal for broodstock is consistent with a population's targeted biological designation, there are clear limitations. Perhaps the most important of these is the inability to extend the analysis beyond a single generation or cohort, which also prevents consideration of long-term fitness effects on an integrated population directly. Yet, maintaining an integrated-wild designation safeguards against negative fitness effects associated with hatchery production (Withler et al. 2018), and therefore the analysis above likely has indirect relevance to long-term fitness effects as well.

To further consider potential fitness consequences of the maximum proportion of escapement used for broodstock, in this report we employ the detailed life-stage model developed by Withler et al. (2018) to evaluate the relative impact of different management actions on genetic risk indicators (e.g., *PNI*) for Chinook Salmon. In brief, smolt production from the natural system is estimated with a Beverton-Holt function, and the rates of survival to adulthood and harvest are determined to maintain a sustainable population given current exploitation levels. Modeled broodstock are collected from the river, hatchery survival and fecundity reflect average values from four east coast Vancouver Island facilities, and fitness effects associated with hatchery production are modeled following the approaches of the HSRG (HSRG 2009; see Withler et al. 2018 for additional details).

For the analysis presented in this report, broodstock and hatchery size in the model was the proportion of the return to the river collected for hatchery spawning. Independently, this addition results in a runaway population size as the hatchery size in the model increases to levels that are far beyond those feasible in a real-world hatchery (e.g., spawners greater than 10^{16}). This initial phase is then followed by population collapse as negative fitness effects are substantial at these unrealistic hatchery sizes. To prevent these dynamics, we also placed a "cap" on the maximum broodstock collected that was determined by a proportion of the equilibrium abundance of the natural spawners. Taken together, these minor modifications to the Withler et al. (2018) model produce two scenarios for broodstock collection:

1. when the escapement is small enough that the broodstock collected under the proportional limit does not exceed the cap and the proportional limit sets the broodstock size; and
2. when the escapement is large enough that the proportional broodstock limit exceeds the cap and the cap sets the broodstock size.

This approach approximately reflects the current application of the 33% guideline for escapement removal as broodstock in SEP's production planning process (Angus Straight, DFO, pers. comm.). Model runs were conducted over 100 simulated generations for proportional broodstock limits from 0.05–0.50 of the escapement at increments of 0.05, and for hatchery caps from 0.1–0.5 of the equilibrium number of natural spawners.

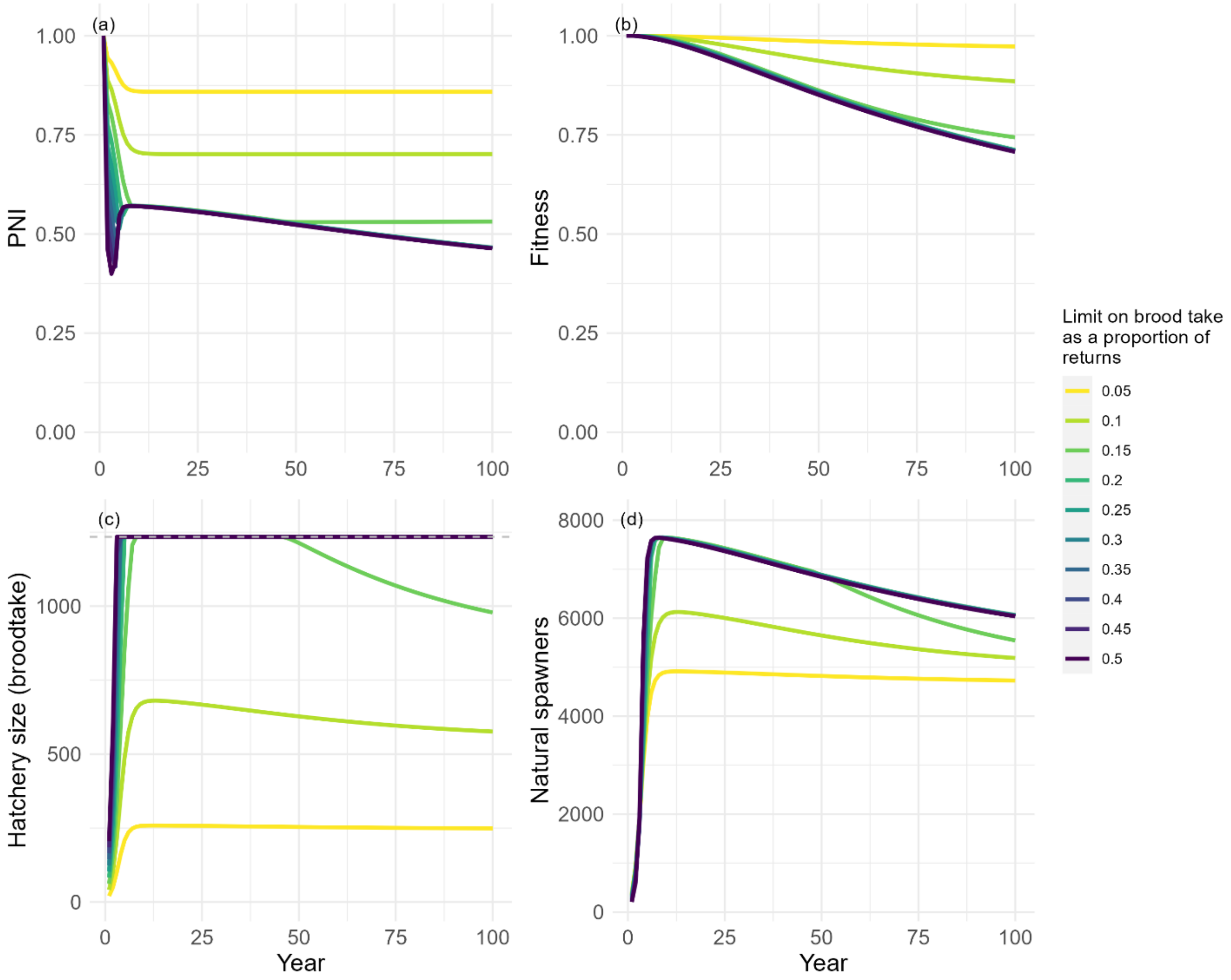

Figure 2. Modeled proportionate natural influence (PNI) (a), population-level fitness (b), hatchery size (c) and natural spawner abundance (d) over 100 simulated generations with a maximum hatchery size (i.e., maximum number of broodstock) of 30% of the equilibrium abundance of natural spawners in the population. Colours display curves for different limits on broodstock collection determined by the proportion of the escapement; note curves for proportional brood takes greater than 0.15 essentially overlap.

To illustrate the overall results from the models, Figure 2 displays *PNI* and population-level fitness with a hatchery cap set at 30% of the equilibrium number of natural spawners (similar figures for hatchery caps of 10, 20, 40 and 50% are available in Appendix C). Long-term *PNI* and fitness appear to be affected by the limit on broodstock collection as a proportion of the escapement, and as the broodstock collection limit increases, both PNI and fitness decrease consistent with increasing hatchery influence in the integrated population. However, after the initial five to ten generations of enhancement, these patterns are only observed at the lowest broodstock collection limits considered (0.05–0.15). At these low limits, the population size does not increase to the point that the hatchery cap (30% of the equilibrium abundance of natural spawners) becomes the more restrictive limit on broodstock collection. Therefore, the maximum hatchery size is dependent on the proportion limit of escapement used for broodstock from 0.05–0.15, whereas at proportional limits greater than or equal to 0.20 the maximum hatchery size reaches the cap and is independent of the proportional limit of escapement collection (note the limits at which this transition occurs depend on the percentage of the equilibrium natural spawners used to establish the cap; see Figures C1 to C4 in Appendix C). In other words, the long-term effects of enhancement on *PNI* and fitness are most tightly tied to hatchery size in all cases, and the broodstock limits based on the escapement proportion are only relevant when they determine the size of the hatchery.

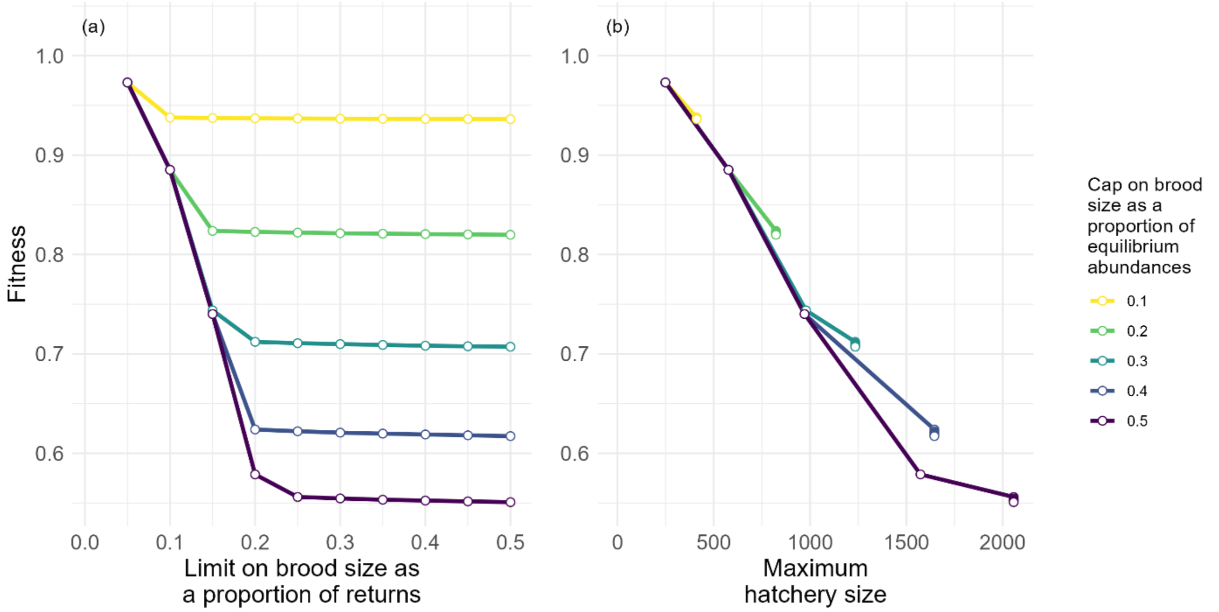

Figure 3. Modeled population-level fitness after 100 generations of enhancement plotted against the limit on broodstock collection as a proportion of the escapement (a), or the maximum hatchery size as defined by the maximum number of broodstock spawners (b). Colours display curves for different hatchery caps set as proportions of the equilibrium abundance of natural spawners from 0.1–0.5.

Across hatchery size caps from 10 to 50% of the equilibrium abundance of natural spawners, the dependence of long-term fitness outcomes on the proportional limit for broodstock collection is only evident if the limit is sufficiently low that the cap is not reached (Figure 3a). This occurs at proportional limits below 0.10 to 0.25 depending on the maximum hatchery size, because lower caps are reached with lower proportions of the escapement removed as broodstock. In contrast, fitness outcomes after 100 generations closely track the maximum hatchery size across all of the modeled scenarios (Figure 3b). Moreover, for any of the five cap values evaluated, there are negligible differences in long-term fitness outcomes for all proportional limits on broodstock collection that allow the cap to be reached. For example, if the hatchery size cap is set at 30% of the equilibrium abundance of natural spawners, all limits on the proportion of the escapement used as broodstock greater than 0.15 result in a proportional long-term fitness loss of slightly less than 0.30. This suggests that in the early generations, before the hatchery cap becomes the more stringent limit on broodstock size, the proportion of the escapement removed as broodstock does not substantially impact long-term fitness outcomes. However, this outcome should be interpreted with some caution as the Withler et al. (2018) model is deterministic without random or natural variation, and after initiation of enhancement at generation 0, the population predictably increases in size until the hatchery cap is reached over a relatively small number of generations.

Selection of broodstock based on hatchery marks or tags

The relative contribution of hatchery- and natural-origin spawners in an integrated population can be managed beyond the proportion of the escapement used as broodstock if hatchery-origin fish are marked. As emphasized above, in this situation, direct application of the biological designation framework from Withler et al. (2018) is preferred to the use of more simplified guidelines. However, SEP's existing genetic management guidelines include one guideline for situations when hatchery-origin spawners are dominant in the escapement (i.e., when *pHOS* > 0.5):

> "Where externally identifiable hatchery fish predominate in the portion of the escapement accessible for broodstock collection, the proportion of hatchery fish utilized should be roughly the inverse of their proportion in the sample to ensure adequate representation of wild fish (e.g. 70% hatchery, 30% wild in the sample – broodstock should be comprised of about 30% hatchery fish and 70% wild)."

Note that (1) this guideline predates the Wild Salmon Policy (DFO 2005) and "wild" above is equivalent to natural-origin using current definitions, and (2) the proportional representation of hatchery- and natural-origin fish described by the guideline is after accounting for the marking rate of fish released from the hatchery. It is also worthwhile to clarify that this guideline does not accurately utilize the "inverse" of the proportions of hatchery- and natural-origin fish in the escapement. Instead, if *pHOS* is the proportion of hatchery-origin spawners in the escapement (or sample), and *pNOS* is the proportion of natural-origin spawners in the escapement, then this guideline suggests the proportion of hatchery-origin fish in the broodstock (*pHOB*) should be 1-*pHOS*, and the proportion of natural-origin fish in the broodstock (*pNOB*) should be 1-*pNOS*. Note that *pHOS* and *pNOS* sum to one by definition, and consequently, this guideline can be more accurately described with current terminology as the *pNOB* equals *pHOS* guideline. In turn, this means *PNI* can be calculated as below:

$$PNI = \frac{pNOB}{pHOS + pNOB} = \frac{pNOB}{pNOB + pNOB} \tag{10}$$

Use of the *pNOB* equals *pHOS* guideline for broodstock selection necessarily results in a *PNI* of 0.50. For populations experiencing high hatchery influences such as *pHOS* greater than 0.50, it is likely that this guideline would increase the natural influence within the integrated population. However, if the overall broodstock size remains at the level that produced *pHOS* above 0.50, then it is likely *pHOS* in the subsequent generation will remain above 0.50 without additional management actions. Repeated application of the *pNOB* equals *pHOS* guideline will maintain a *PNI* of 0.50, but it is worth highlighting that 0.50 is the minimum *PNI* required for an integrated-transition population and precautionary management would target *PNI* greater than this minimum. Moreover, a targeted designation of integrated-wild requires a minimum *PNI* of 0.80, which will never be achieved through application of this guideline alone.

Taken together, the *pNOB* equals *pHOS* guideline does not increase natural influence above a *PNI* of 0.50, and requires identification of hatchery-origin fish at the time of broodstock selection by marks or tags, which would also allow application of the full biological designation framework of Withler et al. (2018) directly. In this context, the utility of this guideline is minimal in the context of current genetic management of populations enhanced by SEP.

Selection of broodstock with respect to trait variation

As the majority of hatchery programs collect a relatively small proportion of the escapement for use as broodstock and the offspring of these broodstock tend to be over-represented in the subsequent generation, biased collection or spawning of broodstock may generate artificial selection pressures on the population that drive trait distributions away from those evolved in the natural environment. To maintain the natural adaptive state of enhanced populations it is critical to minimize the induction of this artificial selection (Neff et al. 2011), which can be achieved by maintaining a high-level of natural influence in the population (i.e., high *PNI*) and by, as much as possible, collecting broodstock from the entirety of the annual run timing randomly with respect to all traits and characteristics of the population (e.g., McLean et al. 2005; CHSRG 2012; DFO 2016).

Random broodstock collection and spawning protocols have been described as “genetically benign spawning protocols” (Campton 2004), because randomizing the use of broodstock avoids the deliberate imposition of directional selection (i.e., a genetically benign or do no harm approach). However, a challenge to this stance is the clear evidence that natural salmon spawning is not random with sexual selection favouring some traits over others (Quinn 2005b; Hankin et al. 2009). Random spawning protocols remove these selection pressures experienced on the natural spawning grounds, and thus these protocols create an inadvertent directional “lack” of selection relative to natural spawning (Quinn 2005a).

This phenomenon can perhaps be best demonstrated by considering the size of potential spawners. Generally in Pacific salmon, large adults have a competitive advantage on the natural spawning grounds both in the defense of high-quality spawning areas and in direct competition for potential mates (e.g., access to larger, more fecund, females) (Quinn et al. 2001; Hankin et al. 2009; Berejikian et al. 2010). Size on the spawning ground is related to the age of return, meaning these competitive spawning advantages are balanced against additional exposure to predation in the ocean and failure to return to spawn at all (Quinn et al. 2001; Berejikian et al. 2010). However, once adults successfully migrate to fresh water and are collected as broodstock, current hatchery spawning practices remove the majority of spawning advantages associated with large size. In turn, this improves the relative spawning success of smaller individuals, which may inadvertently select for smaller body sizes and earlier ages of return (Quinn 2005b; Hankin et al. 2009), and similar logic can extend to other fitness-related traits in Pacific salmon (e.g., egg size). As such, overall trends for earlier returns at smaller sizes in many Pacific salmon populations have led to concerns regarding randomized hatchery spawning practices (Hankin et al. 2009; Ohlberger et al. 2018; Oke et al. 2020; Devlin et al. 2021), particularly as smaller adults are relatively low fitness spawners, are less desirable in fisheries, and are poorer prey items for predators of high conservation concern (e.g., Southern Resident Killer Whales) (Oke et al. 2020).

Hankin et al. (2009) modeled a Chinook Salmon population to mimic the fall run in the Elk River, Oregon, USA, and explored the consequences of three different hatchery spawning protocols on the size and age distributions of the population: (1) random spawning, (2) random spawning with the exclusion of jacks, and (3) spawning such that within mate-pairs male length is always greater than or equal to female length. The first two of these protocols resulted in selection for younger age of return (smaller sizes overall), whereas the third protocol produced a distribution of age of return that was similar to that observed in natural populations. These outcomes are consistent with known heritability of size, age of return and other fitness-related traits in Pacific salmon (e.g., Hankin et al. 1993; Withler and Beacham 1994b; Silverstein and Hershberger 1995; Heath et al. 2002; Funk et al. 2005; Devlin et al. 2021; McKinney et al. 2021; Gamble and Calsbeek 2023), and led to a recommendation that spawning protocols that emulate natural spawning should replace random spawning in hatcheries (Hankin et al. 2009).

Spawner size is clearly one important factor involved in natural spawning success; however, the recommendation of Hankin et al. (2009) is based on a modeled population rather than empirical data, and many other traits are likely also affected by sexual selection in the natural habitat (Auld et al. 2019). There is consensus that random spawning protocols do not accurately reflect natural spawning in salmon, but it is likely that hatchery spawning cannot recapitulate spawning in the natural habitat regardless of the protocol employed (Campton 2005; Quinn 2005a). Moreover, despite demonstrations of heritable variation in fitness-related traits, such as size and age of return, in Pacific salmon (Hankin et al. 1993; Withler and Beacham 1994b; Silverstein and Hershberger 1995; Heath et al. 2002; Funk et al. 2005; Devlin et al. 2021; McKinney et al. 2021; Gamble and Calsbeek 2023), relatively little is known about the genetic architecture underlying variation in these traits. For instance, recent studies have identified y-chromosome haplotypes with substantial effects on the age of maturity in male Chinook Salmon (McKinney et al. 2020, 2021), suggesting selective spawning protocols would be more effective based on specific genetic loci rather than on size directly. McKinney et al. (2021) further cautioned that selective spawning protocols based on single traits may have unintended consequences if the genetic loci underlying variation in the selected trait also affect variation in other fitness-related traits (i.e., pleiotropy). Furthermore, selective spawning to counter putatively maladaptive trends in a trait distribution (e.g., decreases in size or age of maturity; e.g., Oke et al. 2020) requires a complete understanding of the selective pressures responsible for the change in trait distribution, and this level of confidence may rarely be achieved. With respect to decreasing size and age of return, hatcheries may be a contributing factor, but these trends have also been observed in populations without hatchery enhancement (Ohlberger et al. 2018), and other factors, such as fishery-mediated selection and environmental change, may be important (Bromaghin et al. 2011; Oke et al. 2020). Indeed, some authors have suggested that the closest correlation with smaller sizes and younger ages of return in Chinook Salmon is the recovery of Northern Resident Killer Whales which preferentially prey upon the largest salmon (Ohlberger et al. 2019). This could imply that these putatively maladaptive trait trends in Pacific salmon have adaptive value in some contexts.

Taken together, there is potential value in modification of hatchery spawning protocols such that they more closely mimic spawning in the natural habitat. Yet, the in-depth understanding of natural spawning required to mitigate the risks associated with hatchery-mediated selection is, at least currently, unavailable. Given this, the California HSRG did not endorse a change from random spawning to size-selective spawning (CHSRG 2012). Instead, they recommended an experimental test of size-selective spawning at Coleman National Fish Hatchery to assess this possibility further; no recommendations were published for the design of this test (CHSRG 2012). To the author's knowledge, the recommended test has not been conducted, or at least no report of the test has been published. It is possible that an equivalent experimental test could be carried out at a SEP hatchery, and preliminary results from a conceptually similar effort at the Quinsam River Hatchery suggest heritability of size is sufficiently strong in Chinook Salmon that offspring from crosses between large females and males return at larger sizes than offspring from crosses between small females and males (William Woods, DFO, pers. comm.). If a full experimental test of size-selective spawning were explored, we would recommend the use of an enhanced population with a low *PNI*, such that changes in hatcheries protocols would likely be evident in the enhanced population. In this case, a key concern would be the use of a population with low stray rates to reduce genetic risks of possible strays into recipient populations. Regardless, in the absence of empirical evidence to suggest otherwise, the precautionary approach is to maintain the use of random spawning protocols that maximize genetic diversity among hatchery-origin offspring and minimize intentional artificial selection in enhanced populations (e.g., HSRG 2004; Campton 2005; CHSRG 2012). This approach is not only consistent with SEP's current genetic management guidelines (DFO 2016), but also with the biological designation framework developed by Withler et al. (2018) in which natural selective influences are maintained in enhanced populations such that the natural environment is the dominant factor underlying adaptation.

Use of jack males in broodstock

One possible exception to the random use of spawners in broodstock is the extent to which "jacks" are incorporated into the brood relative to their occurrence in the escapement. This exception is a rare case in which the effects of a trait on spawning dynamics are sufficiently well understood that modification of hatchery spawning protocols may be justified. Jacks are males that migrate back to fresh water one year younger than the youngest returning females (e.g., Berejikian et al. 2010). These males display an alternate life history and spawning strategy referred to as a “sneaker” strategy. Jacks participate in spawning opportunistically by sneaking into spawning events between females and larger males (Gross 1991; Fleming and Gross 1994). This male life-history strategy is an important natural occurrence in the majority of Pacific salmon species, and can create a vital avenue for gene flow among cohorts in species that otherwise have a strict age of return (e.g., Coho Salmon; Smith et al. 2015). The main advantage of the jack life history is the reduction in exposure to mortality in the ocean before migrating to the spawning ground. However, jacks typically have reduced spawning success compared to larger and older males (Berejikian et al. 2000; Quinn et al. 2001), and are undesirable in fisheries due to their small size (Heath et al. 1994). There is evidence that jacks experience negative frequency-dependent selection (Berejikian et al. 2010; DeFilippo et al. 2019). In other words, when there are few jacks in a population, jacks are relatively successful in spawning by avoiding high competition for mates between larger males, but when there are many jacks in a population, there is higher competition among jacks for opportunistic spawning chances, and jacks are relatively unsuccessful as they are outcompeted by the relatively few larger males (Berejikian et al. 2010). These selection dynamics are thought to contribute to the persistence of the jack life history in natural populations despite the reduced spawning success of jack individuals overall (Berejikian et al. 2010; King et al. 2023).

As discussed above, hatchery spawning removes many of the dynamics of natural spawning, including the disadvantage experienced by jacks compared to larger males. High heritability has been demonstrated for the jack life history (Heath et al. 2002; Devlin et al. 2021), but both genetic and environmental factors underlie the tendency of a male to return as a jack (Carlson and Seamons 2008). Hatcheries can increase the likelihood of jack males both through genetic mechanisms via the over-representation of jacks among successful spawners, and through environmental mechanisms via the conditions juveniles experience prior to release. For instance, the tendency to return as a jack appears to be associated with an interaction between high juvenile growth rates and size-at-age thresholds early in life (Larsen et al. 2006; Dodson et al. 2013; Harstad et al. 2014) such that the high growth rates targeted by hatcheries can increase the occurrence of jacks (Larsen et al. 2013, 2019). Consequently, even if a hatchery is associated with an increase of jacks, it can be challenging to identify the practices that underlie the increase. However, high heritability of the jack life history combined with recent evidence for y-chromosome haplotypes that are strongly associated with the tendency to return as a jack (McKinney et al. 2020, 2021) suggest that genetic mechanisms play a large role. Thus, the extent to which jacks are used in broodstock is likely an important factor in the occurrence of jacks in the next generation.

Current genetic management guidelines in SEP recommend the use of jacks, “proportionally to their abundance in the escapement as these precocious males may contain genetic material important for the long-term fitness of the population” (DFO 2016). Based on the discussion above, it is likely that application of this recommendation will lead to increased frequencies of jack males in hatchery-origin offspring compared to natural-origin offspring. This suggests that jacks should be used in broodstock at a lower frequency than their proportional representation in the escapement (e.g., CHSRG 2012). However, as the current SEP guidelines indicate, jacks may be important for the long-term fitness of the population, and thus complete exclusion of jacks from broodstock is likely also not advisable (Berejikian et al. 2010; CHSRG 2012). Ideally, jacks would be incorporated into broodstock at a proportion of their representation in the escapement that reflected their relative spawning success compared to larger males (CHSRG 2012), although this value is generally unknown. One examination of relative success of Chinook Salmon jacks in experimental spawning channels determined jacks sired 20.3% of progeny despite spawning with a similar number of mates compared to the larger males in the channels (Berejikian et al. 2010). In comparison, estimates of relative jack spawning success for both Sockeye and Coho Salmon in natural systems are highly variable (3 to 93% and 23 to 96%, respectively) (Foote et al. 1997; King et al. 2023). These results highlight that, beyond the frequency-dependent nature of jack spawning success (Berejikian et al. 2010), the relative spawning success of jacks is likely variable among populations, years and environmental conditions. Thus, it is challenging to use a single estimate of jacks relative reproductive success to validate their proportional use in broodstock.

The California Hatchery Review Report provided three specific guidelines for the incorporation of jacks into broodstock (Guidelines 1.10.1, 1.10.2 and 1.10.3; CHSRG 2012):

- “For Chinook Salmon, the number of jacks to be incorporated into broodstock should not exceed the lesser of: 1) 50 percent of the total number of jacks encountered at the hatchery, and 2) 5 percent of the total males used for spawning.”
- “For Chinook and Coho Salmon, when the number of males available as broodstock is less than or equal to 50, or when less than or equal to 50 broodstock are used to accomplish specific program objectives, the acceptable number of two-year-olds is unlimited.”
- “For Coho Salmon, the number of jacks to be incorporated into broodstock should not exceed the lesser of: 1) 50 percent of the total number of jacks encountered at the hatchery, and 2) 10 percent of the total males used for spawning.”

Note, the second of these guidelines is relevant in situations with limited numbers of broodstock, such as programs with conservation objectives in SEP, which will be considered in more detail later in this report; this guideline removes the restriction on the use of jacks primarily to maximize the number of spawners and the effective population size. The guidelines above are a refinement of previous HSRG recommendations, which proposed a maximum rate of use for jacks of 10% in general and a minimum of 10% in Coho Salmon (HSRG 2004). The main distinction between Coho Salmon and other species is the increase in the percent of total males that can be jacks in Coho Salmon spawning. This increase reflects the relatively strict age of return in Coho Salmon with the vast majority of spawners returning to fresh water at age three (Van Doornik et al. 2002). As a result, jacks are the major route of gene flow between Coho Salmon cohorts, and a higher rate of inclusion in broodstock is justified to avoid artificial differentiation among the three spawning cohorts (Smith et al. 2015).

In the absence of information regarding the relative spawning success of jacks within a specific population, the guidelines proposed by the California HSRG are a practical approach to safeguard against over-representation of the progeny of jacks in hatchery-origin offspring relative to natural-origin offspring while maintaining jack males in hatchery broodstocks to a reasonable extent.

Broodstock spawning procedures

Beyond the collection and selection of broodstock, other spawning procedures must also be conducted in a genetically conscious manner to minimize the genetic risks associated with enhancement activities. Of primary concern is equalization, to the maximum extent possible, of the contributions of individual broodstock spawners to the subsequent generation (Allendorf 1993; Neff et al. 2011; Fisch et al. 2015). This equalization provides several genetic benefits including minimizing inadvertent selection among spawners and maximizing genetic diversity among the released juveniles. Moreover, this practice avoids exacerbating the over-representation of individual hatchery spawners in the next generation relative to natural spawners, and thus reduces the negative impacts on the effective population size of the integrated population and the likelihood of inbreeding, particularly when the hatchery size is relatively large compared to the natural spawning component of the population (Anderson et al. 2020).

The simplest method to equalize the contributions of individual spawners is achieved by mating females and males in a 1:1 spawn or in an equivalent spawning design where the contribution of each spawner is equivalent to that in a 1:1 spawn (HSRG 2004; CHSRG 2012; Fisch et al. 2015). For example, the eggs from two females (A and B) can be divided equally into two lots each, and then the milt from two different males (E and F) can independently be used to fertilize one lot from each of the females (Table 4). This practice is known as matrix spawning (in this example with a 2x2 matrix). In a matrix with an equal number of females and males, the contributions of each spawner are equivalent to those in a 1:1 spawning design (i.e., each male fertilizes the eggs from the equivalent of one female), but a greater number of families contribute to the pool of offspring.

Table 4. An example of matrix spawning with a 2x2 matrix.

		Females	
		A	**B**
Males	**E**	AE	BE
	F	AF	BF

Four families are generated in the spawning design shown in Table 4. If the same spawners were used in a straight 1:1 spawning design, two families would be generated (e.g., AE and BF continuing from Table 4) and these families would be approximately twice the size of the families in the 2x2 matrix spawning example. Considering the full pools of potential offspring, in the 1:1 spawn each offspring would be a full sibling of 50% of the pool, whereas in the 2x2 matrix spawn each offspring would be a full sibling of 25% of the pool and a half siblings of 50% of the pool. Despite the increase in sibling relationships overall in the progeny of the matrix spawn, the decrease in the proportion of full siblings results in increased genetic diversity among the offspring, decreased relatedness through time, and a higher effective population size (Engström et al. 1996; Fiumera et al. 2004; Dupont-Nivet et al. 2006; Busack and Knudsen 2007).

Matrix spawning can be logistically burdensome relative to 1:1 spawning protocols, particularly in large hatchery production lines, but matrix spawning will essentially always produce higher genetic diversity among the offspring of the spawners compared to a 1:1 spawning. Therefore, two key questions are: (1) what are the best practices for matrix spawning, and (2) when are the benefits of matrix spawning worth the extra logistical challenges in the hatchery?

The increases in genetic diversity and effective population size gained as a result of matrix spawning become greater as the dimensions of the matrix increase. In other words, for example, an 8x8 matrix produces greater genetic diversity than a 4x4 matrix, and a 4x4 matrix produces greater genetic diversity than a 2x2 matrix (Busack and Knudsen 2007). Table 5 shows the results of a 4x4 matrix to illustrate this effect in an over-simplified fashion through comparison with the outcome of a 2x2 matrix shown in Table 4.

Table 5. An example of matrix spawning with a 4x4 matrix.

		Females			
		A	**B**	**C**	**D**
Males	**E**	AE	BE	CE	DE
	F	AF	BF	CF	DF
	G	AG	BG	CG	DG
	H	AH	BH	CH	DH

In the 4x4 matrix each offspring is a full sibling of 6.25% of the pool of progeny and a half sibling of 37.5% of the pool. To directly compare this to the 2x2 matrix design it is necessary to consider two 2x2 matrices such that the number of spawners is four females and four males in both cases. In the outcome of two 2x2 matrices, each offspring is a full sibling of 12.5% of the pool of progeny and a half sibling of 25% of the pool. The decrease in full siblings again is sufficient that genetic diversity is increased among the progeny of the 4x4 matrix. Although this will continue as the matrix dimensions increase, higher dimension matrices quickly become unfeasible in a real-world hatchery situation. Beneficially in this regard, genetic diversity does not increase linearly with the matrix size such that there are diminishing returns of increasing the dimensions of the matrix (e.g., Fiumera et al. 2004; Busack and Knudsen 2007).

With respect to the effective number of breeders contributing to the effective population size, Busack and Knudsen (2007) modeled the benefit of matrix size relative to a full factorial mating of an entire broodstock of up to 120 females and 120 males (i.e., when all spawners are mated with every spawner of the opposite sex). A 5x5 matrix spawning design achieved up to 80.0% of the full factorial benefit, whereas a 10x10 design only resulted in a relatively small improvement from 80.0% to 92.8%. This suggests that matrix spawning with dimensions greater than 5x5 is likely not of sufficient benefit to merit the logistical challenges associated with higher dimensions. Indeed, the current SEP genetic management guidelines propose a maximum of four females in a matrix for practical purposes (DFO 2016), suggesting even a matrix involving five females is a substantial challenge. In this context, it is important to note that Busack and Knudsen (2007) also demonstrated that utilization of even a 2x2 matrix spawning design achieved up to 45.6% of the full factorial benefit to the effective number of breeders in the broodstock, indicating the smallest matrix possible with equal numbers of females and males can produce a meaningful increase in effective population size.

Despite the consistent benefits of matrix spawning for genetic diversity relative to 1:1 spawning even at small matrix sizes, for hatcheries that enhance large populations of Pacific salmon (e.g., SEP hatchery lines with harvest objectives) the benefit of any gain in genetic diversity associated with matrix spawning may be relatively minor (CHSRG 2012), particularly factoring in the additional challenges associated with matrix spawning when the broodstock is large. Demonstration of this point requires a more detailed consideration of the random loss of genetic variants given an effective population size. There are three broadly accepted breakpoints in effective population size (N_e) with respect to the maintenance of genetic diversity: (1) N_e above 50 to avoid inbreeding depression and prevent an “extinction vortex”, (2) N_e above 500 to maintain additive genetic variation, and (3) N_e above 5,000 to maintain quasi-neutral variation and permit establishment of novel mutations (Frankel and Soulé 1981; Lande 1995; CHSRG 2012). Indeed, the “50/500 rule” is a well-established principle in conservation management (e.g., Franklin 1980; Jamieson and Allendorf 2012, although see Frankham et al. 2014). There is not a straightforward way to estimate the N_e of a salmon population from the census population size (N_c), and N_e can be substantially less than N_c for wild populations (Frankham

1995; Ferchaud et al. 2016). Genetic methods can estimate the effective number of breeders (N_b) within an escapement (see Waples 2006; Waples and Do 2008; Wang 2009; Waples 2024), and the effective population size can be calculated by:

$$N_e = g \times N_b \tag{10}$$

where g is the average age at spawning or the generation time (Waples 1990). Alternatively if N_b estimates are available annually over the generation time, N_e can be calculated as the sum of the N_b estimates (Withler et al. 2014). Regardless, genetic estimates of N_b are not available for many populations enhanced by SEP, and estimates of the relationship between N_e and N_c are typically highly variable in salmonids. For instance, in Rainbow Trout (*O. mykiss*), estimates of N_e/N_c range from 0.17 to 0.40 in an Oregon population (Araki et al. 2007b), and are 0.53 or 0.73 in a Washington population depending on methodology (Ardren and Kapuscinski 2003). Alternatively, a study across ten populations of Atlantic Salmon (*Salmo salar*) assessed the relationship between genetically estimated N_e values and census population size estimates (Ferchaud et al. 2016), and found the average N_e/N_c across populations was 0.22. However, again this value was highly variable among populations (0.11 to 0.54) with 19% variance in N_b/N_c estimates among years alone. Taken together, these results suggest a relatively similar range of N_e/N_c values across studies (~0.1–0.5), but as the variation across populations ranges from 2 to 5 fold among studies, it would be inaccurate to naively apply these ratios to an unknown population of Pacific salmon. Yet, even the lower estimates of this ratio, suggest that in many cases the N_e of large populations experiencing hatchery enhancement are likely sufficiently above the $N_e > 500$ breakpoint that 1:1 hatchery spawning designs do not pose a substantial risk to the maintenance of additive genetic variation in the population.

Individual male spawners can also become over-represented among hatchery offspring as a result of the protocols through which milt is combined with the eggs. The milt collected from different males does not have equal performance with regard to fertilization success due to sperm competition and other factors (Gharrett and Shirley 1985; Withler 1988; Withler and Beacham 1994a; Gile and Ferguson 1995; Campton 2004). Consequently, if milt from several males is added to the eggs of several females, which at least historically has been a common practice (Campton 2004), it is highly likely that sperm from a relatively small number of males will fertilize the majority of eggs from all the females. This dominance is consistently observed whether milt of different males is added simultaneously or sequentially to a pool of eggs (Withler and Beacham 1994a). Thus, it is best if the milt from only a single male is added to the eggs of a female (or lot of eggs in matrix spawning) (HSRG 2004; DFO 2016). The possibility of cryopreservation of milt can produce similar concerns if milt from a male is both used directly in spawning and cryopreserved for later use; progeny of the male become over-represented across brood years, relatedness increases overall, and half siblings may mate if there are overlapping return years among offspring from the different brood years. For hatchery lines with large production targets it may be challenging to keep the eggs from different females separated prior to the addition of milt. In these cases, it is possible to avoid male dominance in fertilization by pooling the eggs from a group of females, dividing the pool into an equivalent number of lots equal to the number of females contributing eggs to the pool, and fertilizing each lot with the milt of a different male (see the current SEP genetic management guidelines; DFO 2016). This protocol does not invalidate any of the principles underlying the best practices described above.

One concern regarding the use of only a single male to fertilize a group of eggs is the complete loss of the group due to male infertility (Fiumera et al. 2004; Busack and Knudsen 2007). This concern may be minimized by strict application of best spawning techniques in the hatchery. Yet, an alternative that is utilized in some cases is the sequential addition of milt from two males. The current SEP genetic management guidelines include a recommended strategy for this practice to minimize the potential negative effects with respect to male dominance, which includes guidance that milt from a different male should always be used first for any group of eggs (DFO 2016). This recommendation is both logically and scientifically sound if a sequential protocol is deemed necessary, but it should be stressed that these minor protocol modifications do not change that best genetic practices avoid mixing milt during spawning. Based on descriptions in SEP's Fish Health Management Plans, some hatcheries have developed intriguing alternative strategies for the addition of milt to eggs. For instance, at the Conuma River Hatchery, eggs from three Chinook Salmon females are placed in a divided bin, and then the eggs from each female are fertilized with milt from a different male. After a brief interval, the divider is removed and all the eggs and milt are mixed. These practices may provide a middle ground in which the genetic contributions of individual males remain relatively equal, but the potential consequences of male infertility are minimized. That said, the genetic outcomes of non-standard practices are unknown, and should be tested before they can be fully endorsed.

One possible challenge to maintaining equal contributions among spawners in a hatchery is if there is an unequal sex ratio in the escapement, and thus in a randomly selected broodstock. An unequal sex ratio will necessarily create unequal genetic contributions between female and male spawners unless individuals of the more abundant sex are excluded; spawners of the less abundant sex will make a proportionally greater contribution to the hatchery offspring. The existing SEP genetic management guidelines offer best practices for spawning when broodstock sex ratios are unequal (DFO 2016). These are ultimately equivalent to matrix spawning with unequal dimensions that reflect the ratio of females and males in the broodstock (see an example for a 3:2 female to male ratio in Table 6).

Table 6. An example of a matrix spawning for an unequal sex ratio. For a 3:2 female to male ratio in the broodstock a 2x3 matrix is used.

		Females		
		A	**B**	**C**
Males	**E**	AE	BE	CE
	F	AF	BF	CF

Despite the application of these guidelines, the effective number of breeders will be smaller than that of an equivalent collection of broodstock with an equal sex ratio (Frankham 1995). This loss of effective population size (N_e) is gradual until the sex ratio becomes substantially skewed. For instance, assuming random mating, a 70:30 sex ratio produces an N_e that is approximately 84% of the N_e of a 50:50 sex ratio, whereas for a 90:10 sex ratio N_e drops to 36% of the N_e for an even sex ratio (Tave 1984). Correspondingly, until the sex ratio becomes more skewed than a 70:30 ratio, there may be limited gain in any potential actions to equalize the females and males in the broodstock, but when the sex ratio is highly skewed, there is likely benefit in equalizing the representation of the sexes among hatchery spawners (Tave 1984) as long as these actions do not cause substantial increase in the sex ratio skew among natural spawners (i.e., the brood is a small proportion of the escapement).

A final important consideration with regard to equalizing the genetic contribution of broodstock among hatchery offspring is that equalizing the contributions at the time of spawning will not necessarily produce equal contributions among hatchery releases. Variation in female fecundity, fertilization success and survivorship among families will add variance in the genetic contribution after a mating design is chosen and mates are paired. As a result, the most effective approach to equalize genetic contributions of spawners among hatchery releases is to normalize family sizes at stages in the production process that are close to the release of juveniles (Neff et al. 2011). This approach may often be logistically challenging as it requires maintaining families independently throughout much of their time in the hatchery. Despite this, the reduction in risks associated with elevated relatedness among hatchery releases may be worth the logistical challenge, particularly in conservation programs when population sizes are typically small and relatively few families may make up the broodstock.

Conservation objectives and balancing competing genetic risks

Hatcheries with conservation or rebuilding objectives may often encounter competing genetic risks that pose complications for planning and managing the enhanced contribution within the integrated population. The core of these challenges is the dichotomy between the desire to maintain or increase natural spawner abundance via hatchery enhancement, and the need to maintain the natural adaptive state of the population such that after recovery the population is healthy and self-sustaining in the natural habitat. As emphasized by the HSRG, a necessary step in navigating these challenges is developing quantitative thresholds for the transition between phases of conservation and rebuilding that establish the relative weighting of different genetic (and other) risks (HSRG 2020).

The effective population size of an enhanced population with a conservation objective will play a large role in determining the best practices for broodstock collection and spawning. If N_e is less than 50, then high relatedness among spawners means inbreeding is likely (Franklin 1980). Inbreeding further increases relatedness among offspring, leading to further reductions in N_e in a positive feedback cycle. This is known as an “extinction vortex”, which results in extremely low genetic diversity leaving the population at high risk of extirpation (Gilpin and Soulé 1986). Previously abundant populations that decrease to spawner numbers consistent with an N_e of 50 or less are clearly of high conservation concern. Genetic drift (i.e., random chance) plays a substantial role in genetic change within these populations and selection is relatively ineffective (e.g., Adkison 1995). In these scenarios, the primary conservation concern is the maintenance of genetic diversity by increasing the effective population size (HSRG 2017, 2020). It is worth noting that there are many wild populations of Pacific salmon that are stable through time with numbers of spawners that are consistent with extremely low N_e. This may be the natural state of these populations, and adaptation is likely facilitated to some extent through natural straying from other populations (Slatkin 1985; Milner and Bailey 1989). However, these populations are still genetically susceptible in the face of a changing environment. At low N_e (e.g., less than 50), hatcheries can have a role that is, in some senses, the opposite of the standard risks of over-representation of hatchery spawners. Collection of a large proportion of the escapement as broodstock may result in not only a demographic boost to the population, but also an equalization of genetic contributions among spawners compared to those expected if the broodstock had spawned in the natural habitat (Hedrick et al. 2000; O’Reilly and Kozfkay 2014; Berejikian and Van Doornik 2018). Thus, hatchery production can lead to an increase in effective population size, particularly through the application of matrix spawning, at the cost of high hatchery selective influences in the population. This cost is mitigated to some extent due to the high influence of genetic drift at these population sizes regardless of the balance of hatchery and natural spawners. Importantly, this argument is not to say natural influences are irrelevant in conservation hatcheries; rather, it is only that the loss of genetic diversity and likelihood of inbreeding are the major risks to the genetics of the population. Consistent with this conclusion, the potential merits of relatively high hatchery influence in conservation programs is acknowledged within the current SEP genetic management guidelines (DFO 2016):

> “For populations in active recovery processes: … Broodstock removals may comprise up to 50% of the returning spawners particularly if habitat is very poor, resulting in poor wild production. As populations decline, from a genetic perspective, it may be preferable to use all available spawners as broodstock. However, this strategy carries the risk of catastrophic loss of the entire population if a problem is encountered in the hatchery.”

When the majority of the escapement is collected as broodstock and the effective population size of the enhanced population is low, spawning protocols to minimize inbreeding and maximize genetic diversity among offspring become even more critical. Beyond the standard approaches described earlier in this report, it is possible to genotype all spawners in conservation programs, which allows mate pairings to be informed by the pedigree of the spawners in the program (Fraser 2008; O'Reilly and Kozfkay 2014). Substantial effort may be necessary to generate and maintain this information in a timeframe that allows mate-pair selection to be informed ahead of spawning; however, a pedigree-based strategy is the most effective option to maximize genetic diversity through time (e.g., Tave 1984; Fraser 2008; O'Reilly and Kozfkay 2014), and was employed effectively by SEP in the initial phases of the Cultus Lake Sockeye Salmon recovery program in the early 2000s (Ackerman et al. 2014).

Use of cryopreserved milt in these extreme conservation scenarios has potential benefits. Despite the risk of over-representation of individual male spawners mentioned earlier in this report, cryopreserved milt may allow increased genetic diversity among spawners contributing to a brood year, particularly if the number of male spawners is limited (e.g., Bøe et al. 2021). The risks associated with over-representation can be somewhat mitigated by using genetic information to guide the selection of broodstock spawning pairs. Furthermore, cryopreservation of milt establishes a bank of genetic variation to safeguard against future loss from the population, and if milt is collected from natural-origin males or from males prior to the presence of high hatchery influence, utilizing preserved milt also has the potential to increase natural influences in a broodstock (e.g., Ackerman et al. 2014; Withler et al. 2014). However, a possible concern with incorporating cryopreserved milt after long storage periods is the introduction of genetic variation that reflects a previous state of the population that is no longer adaptive under current conditions. Adaptation from standing genetic variation can proceed rapidly, particularly in response to environmental change (Barrett and Schluter 2008), such that there is merit to this concern. Yet, in the contexts of high inbreeding risk and random loss of genetic variation at effective population sizes approaching 50, it is likely that the benefits of increasing spawner diversity outweigh the risks of introducing variation that has lost its adaptive value under current conditions.

Given the emphasis on within-population genetic diversity in populations of conservation concern, particularly at extremely low N_e (e.g., $N_e < 50$), it is tempting to consider transplants of spawners from other populations to increase both effective and census population sizes. General best practices for enhancement in Pacific salmon are clear. For example, "Broodstock should be chosen from locally adapted stocks native to the basin and with life history characteristics appropriate for the program goals" (Guideline 1.1.1. in CHSRG 2012), which is echoed by the framing of "local production objectives" and collection of "broodstock that adequately represent the entire donor population" in SEP's current genetic management guidelines (DFO 2016). Even in a conservation context focused on within-population genetic diversity, transplanting fish from other populations is probably best considered an option of last resort genetically, and it would be necessary to minimize the genetic differentiation between the population of conservation concern and the source population for the transplanted individuals. Transplanting individuals undoubtedly has the potential to increase the genetic diversity within a recipient population, but if the source population of the transplanted individuals is not very similar genetically to the recipient population, then any increase in diversity would come at the degradation of between-population variation and genetic integrity. Moreover, if transplants introduce substantially different population-level variation into the population of concern, the practice may increase the number of salmon in the habitat, but the extent to which the original genetic population is conserved may be relatively low (Anderson et al. 2014). In addition, local salmon are most likely better adapted to their local habitat than salmon from other foreign habitats would be (Fraser et al. 2011), and in theory transplants could lead to outbreeding depression (Allendorf and Waples 1996, although there is limited evidence for outbreeding depression in wild Pacific salmon; e.g., Dann et al. 2010; Lehnert et al. 2014); both of these factors could pose a threat to long-term population viability after the transplanted fish interbreed with local fish. Taken together, transplanted spawners may have utility in populations of extreme conservation concern, but the genetic risks associated with transplants are sufficiently high that a precautionary approach is warranted.

In contrast to populations with an N_e less than 50, in populations with an N_e greater than 500 inbreeding is relatively unlikely and additive genetic variation is generally stable (i.e., loss of the majority of genetic variants by chance is unlikely; Franklin 1980). In these cases selection is relatively effective, and maintaining natural selective influences in the integrated population is critical for long-term population-level fitness and productivity (Withler et al. 2018). As discussed earlier in this report, collecting broodstock at a proportion of the escapement consistent with the objectives of the hatchery program is critical, and avoiding exacerbating over-representation of a relatively small number of hatchery spawners is necessary to prevent a hatchery-mediated decrease in N_e. Between the N_e less than 50 and N_e greater than 500 situations, the types of risk present for the genetics of the population do not change, but the prioritization of those risks with regards to hatchery enhancement shifts as the dominant risk shifts from loss of within-population genetic diversity through neutral processes to loss of the natural adaptive state of the population through hatchery-mediated selection (i.e., adaptive processes).

The discussion above implies there is an effective population size between 50 and 500 at which there is a switch between the dominant genetic risk. In reality, this transition is continuous as N_e increases or decreases, such that many context-dependent factors may determine the most appropriate use of hatchery production during the population rebuilding (e.g., habitat condition, hatchery capacity and other considerations regarding population abundance). As a result, we do not prescribe a recommended threshold for the relative prioritization of different genetic risks in general, and consistent with the recommendations of the HSRG we suggest these thresholds be developed as part of an integrative management approach (HSRG 2017, 2020). Moreover, without care the “50/500 rule” can easily be misapplied as a population size that is sufficient to stabilize additive genetic variation may not be equivalent to a minimum viable population size in part due to the timescales over which genetic process occur (Jamieson and Allendorf 2012), and there is some debate on the 50 and 500 N_e thresholds with respect to long-term viability as well (Rieman and Allendorf 2001; Frankham et al. 2014; Pérez-Pereira et al. 2022). However, as an example, the HSRG provided a demonstration of a possible quantitative trigger for the transition between the recolonization and local adaptation phase of recovery—an observed N_e greater than 200 (HSRG 2020).

An important caveat to defining thresholds for the prioritization of genetic risks is an acknowledgement that there is no simple method to convert a census population size estimate to an estimate of effective population size in Pacific salmon, as discussed in an earlier section of this report. There are established genetic methods to estimate the effective number of breeders in a wild population (e.g., Waples and Do 2008), which can then be converted to an effective population size, and these methods have been applied previously with success in populations of conservation concern enhanced by SEP (e.g., Cultus and Sakinaw Lake Sockeye Salmon, and Puntledge River Summer Chinook; Ackerman et al. 2014; Withler et al. 2014; Wetklo et al. 2020). Towards a goal of establishing common approaches across hatchery programs, it is perhaps worth consistent genetic sampling and analyses to allow direct estimation of N_e across SEP hatchery lines with conservation as the primary objective.

Uncertainties, gaps and future work

- The objectives of this report were deliberately focused on genetic risks associated with hatcheries. However, production and release of Pacific salmon from hatcheries results in several other categories of risk factors (e.g., ecological and disease) that must also be taken into account to operate hatcheries in a scientifically defensible manner. For example, in extreme conservation situations, removing high proportions of the escapement for broodstock may be a sound practice genetically, but this may not always be advisable due to increases in other risks. In this example, even a technical failure could lead to a catastrophic loss of all or the majority of a spawning year, which is a consequence that should not be dismissed without consideration.
- The intention of this report was not to provide a systematic review of the scientific literature on hatcheries. Instead, the core of this report was based on the existing genetic management guidelines with SEP (DFO 2016) and specific issues raised by SEP members of the steering committee (see the Context). In most cases, the information contributing to this report built on the publications of the HSRG supplemented by targeted searches of scientific literature databases and by life-stage modeling. The results were then presented within the context of SEP. Although this approach captures the current scientific consensus on the topics covered, a future systematic approach would have the potential to identify additional relevant studies.
- This report summarizes best practices for genetic management of broodstock within integrated Pacific salmon populations. Operationalization of these practices will require a balance of genetics and logistical concerns. These are highlighted throughout the report, including where there may be flexibility with relatively minor consequences genetically. However, the majority of best practices described here are currently utilized within hatcheries. Further comment on the prioritization of genetics concerns versus other concerns is beyond the current scope.
- A foundational component of effective genetic management is clearly stated biological objectives for hatchery lines. The application of PNI population designations is currently in development and will be a key step in SEP's management of gene flow and adaptive influences in integrated populations of Pacific salmon. This may lead to widespread use of techniques allowing identification of hatchery-origin fish such that simplified guidelines based on the proportion of escapement used as broodstock are unnecessary. Where possible, the biological designation framework of Withler et al. (2018) should be used to manage enhanced contributions within SEP's hatchery lines.
- In hatchery lines with a harvest objective, there is a clear trade-off between the extent of hatchery production and the proportionate natural influence in the integrated population. This report does not consider this trade-off beyond the application of the biological designations established by Withler et al. (2018). Future consideration of genetic risks within populations and conservation units associated with high hatchery production is merited.
- Consistent with current practices, integrated populations are recommended for enhancement activities within SEP. However, under extreme scenarios, captive broods have been used to support conservation efforts (e.g., Cultus Lake Sockeye, Sakinaw Lake Sockeye and Puntledge River Summer Chinook). Although these programs have utility, they may more closely resemble segregated populations than integrated ones. In the face of continuing declines in abundance for many Pacific salmon populations, should the use of captive broods increase over the coming decades, future work to provide genetic guidance on best practices is recommended. Many of the risks described in this report for populations

with conservation objectives would become, if anything, even more important in captive broods.

- There remains considerable uncertainty in the ratio between recruits per hatchery spawner and recruits per natural spawner (α) within populations enhanced by SEP, including how variable this ratio is among facilities and brood years. It should be possible to estimate these values in many hatcheries, and addressing this gap would provide a substantial improvement to the analyses presented in this report. Furthermore, it would allow validation of the relationship between the proportional removal of escapement for broodstock and *PNI* predicted by Equation 9.
- The life-stage model developed by Withler et al. (2018), and applied in this report, relies on parameterization based on populations of Chinook Salmon from the East Coast of Vancouver Island. There is substantial uncertainty in many of these parameters, and refinement with additional data would improve the predictive capability of the model. Moreover, collecting the relevant data for hatchery populations found in other regions would allow assessment of the generality of the modeling results.
- The life-stage model from Withler et al. (2018) is deterministic, whereas Pacific salmon populations are affected by many stochastic processes. Future work to add stochasticity to the model would provide a valuable opportunity to further assess the outcomes of broodstock collection and other genetic management strategies on adaptive influences in integrated populations.
- A key assumption underlying the threshold values for *PNI*, *pHOS* and *pNOB* in the Withler et al. (2018) biological designation framework is that matings between hatchery- and natural-origin fish occur proportionally to their relative abundances on the natural spawning grounds. Research to validate this assumption is recommended.
- Intentional selection of spawners among potential hatchery broodstock is not recommended, but one potential exception to consider is where previous hatchery practices are conclusively known to have caused an artificial shift in the traits or characteristics of the enhanced population. For instance, genetic variation from Puntledge River Fall Chinook Salmon was introduced into Puntledge River Summer Chinook Salmon due to inaccurate broodstock collection historically, which was associated with a later run timing in the Summer Chinook. Consequently, enhancement efforts were focused on the early component of the run to reduce the influence of the Fall Chinook genetic variation (Wetklo et al. 2020). Under scenarios such as this example, exceptions to the general recommendations may be justified. However, this uncertainty is provided with a high degree of caution, because circumstances similar to this example will rarely be the case due to the complexity of biological traits.
- Much remains unknown about competition on natural spawning grounds and sexual selection in Pacific salmon. Although it is unlikely it will be possible to recapitulate these dynamics fully in hatchery spawning, research to better understand natural spawning could lead to improved recommendations for spawning practices in hatcheries.
- With the recent publications of the Pacific salmon genomes, there are now opportunities to address the gap in knowledge regarding the genetic architecture underlying many fitness-related traits in these species. This information will improve not only our understanding of the inter-relatedness among different traits and the potential for unintended consequences of hatchery spawning, but also open possibilities for genetic management based on trait-associated variants.

- The estimated rate of spawning success of jack males relative to other males in natural populations remains highly variable. Improving the precision of these estimates across species would allow refinement in the recommended proportional use of jack males in hatchery spawning.
- Jack males typically fertilize small numbers of eggs from many females, which is not captured in standard 1:1 spawning protocols. It is unclear if reflecting this aspect of jack spawning in hatcheries would provide a substantial benefit genetically. Population genetic models could be used to assess this possibility in the future.
- There is some uncertainty in the maximum matrix dimension that should be used in hatchery spawning. Higher dimensions are beneficial, but can be logistically challenging. A practical maximum dimension of five is supported by this report. However, this could be increased or slightly decreased depending on situations within specific hatcheries.
- The extent to which 1:1 or matrix spawning equalizes spawner contributions among the offspring released from SEP hatcheries is unclear, and could be assessed via parentage-based tagging of juvenile DNA samples collected at the time of release.
- Sequential addition of milt to eggs creates the possibility of over-representation of male spawners among hatchery offspring, but use of milt from one male may produce loss due to male infertility. Characterization of rates of male infertility in SEP broodstock would address this uncertainty.
- It is clear there is no straightforward relationship between census population size (N_c) and effective population size (N_e) in salmonids, and estimates of N_e/N_c are highly variable. Future work could characterize this relationship in populations enhanced by SEP to improve the relevance of the data available to support conservation decision making.
- A key gap in the planning process for enhanced populations with conservation and rebuilding objectives is the need to develop quantitative thresholds (e.g., effective population sizes) for transitioning between stages of recovery and balancing different genetic risks throughout these stages. Guidance for a staged process of recovery occurring across distinct phases of enhancement goes beyond the scope of this report, but merits development in the future.
- The majority of the evidence discussed in this report is drawn from studies on Chinook Salmon. However, in most cases this information and advice should be generally relevant across the Pacific salmon species (and in Steelhead, *O. mykiss*) as well.

Conclusions

In this report we review current scientific knowledge with respect to hatchery broodstock management and compare current best practices with genetic management guidelines in SEP that were originally established in the 1980s (DFO 2016). These guidelines are presented in Appendix A for reference along with commentary regarding the consistency of the guidelines with the information found in this Science Response Report. In general, the existing SEP guidelines are overwhelmingly consistent with current best practices, suggesting adherence to these guidelines over the last four decades has contributed to minimizing genetic risks associated with SEP's programming. This consistency is perhaps not surprising as the original guidelines were developed based on fundamental genetics principles regarding effective population size and adaptation that have not changed substantially through time. Past this overall consistency between current best practices and SEP's existing genetic guidelines, the evidence reviewed in this report leads to a relatively small number of potential revisions or additions to ensure genetic risks are minimized in populations enhanced by SEP. In particular:

1. The limit of one third of the escapement removed for broodstock is likely too high to be consistent with the integrated-wild designation from Withler et al. (2018) in the majority of hatcheries, and may also be inconsistent with the integrated-transition designation in some hatcheries.
2. Jack males should be included in broodstock, but their incorporation into hatchery spawning should occur at a rate below their proportional representation in the escapement to more accurately reflect their relative spawning success in natural habitats.
3. Quantitative and defensible thresholds for the prioritization of genetic risks in conservation programs should be defined and implemented; these thresholds should likely consider the genetic effective population size of the integrated population.

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Appendix A

The information presented below is an excerpt from *A Compilation of Operational and Planning Guidelines for the Salmonid Enhancement Program 2016* (DFO 2016) containing the guidelines relevant to genetic management of hatchery broodstocks that formed the foundation of this Science Response Report. Interspersed between the quoted sections from DFO (2016) are bolded lines starting with “Comment”, which provide brief statements regarding the consistency of the previous information with the updated review presented in the Analysis and Response section above.

4. Operational guidelines for broodstock collection and spawning at Salmon Enhancement Program (SEP) hatcheries

4.1. Scope of guidelines

These guidelines have been developed to guide broodstock collection and spawning of Pacific salmon at SEP hatcheries and incubation facilities. They describe production objectives and provide strategies to manage genetic resources in order to preserve as much as possible the entire range of genetic material within an existing population for any objectives. The guidelines do not apply to steelhead or cutthroat as the management of these species is a provincial responsibility.

4.2. Program production objectives

Enhancement projects are planned and implemented within an integrated planning process and involve establishing juvenile release targets and strategies that will produce the number of adults desired while considering species interactions, effects on existing stocks, harvest, habitat capacity and project capacity. This process is described in the SEP Production Planning Framework (DFO, 2012). This framework also defines five specific objectives for fish production and each group of fish considered through production planning must address at least one of these objectives. These objectives in turn influence brood stock collection and spawning strategies.

- Harvest – enhancement for fisheries that are reliant on enhanced production, and would disappear or become severely constrained in the absence of enhancement. This includes harvest opportunities for First Nations, recreational, or commercial fisheries. When the objective is to provide a targeted-fishery opportunity, production targets may be set to consider both natural spawning and harvest requirements.
- Assessment – fish produced for marking where stock assessment information contributes to Pacific region assessment priorities, such as the Pacific Salmon Treaty. The information may also contribute to assessment as defined under the regional stock assessment framework, Area stock assessment priorities and regional SEP assessment priorities i.e. those produced for program performance measurement. Fish produced for assessment generally address other objectives as well but, in a few instances, fish are produced solely for marking for assessment purposes.
- Conservation – enhancement of a stock highly at risk of extirpation or extinction, or a vulnerable stock that has been identified as a regional priority (e.g. populations which have a formal conservation/recovery strategy). This includes re-establishing locally extinct populations/CU’s and rebuilding population/CU’s at high risk of extirpation.
- Rebuilding – enhancement of a stock that is below apparent carrying capacity. This includes rebuilding depleted populations and mitigating for habitat loss.

- Stewardship and Education - small numbers of fish produced to provide a stewardship or educational opportunity. Production for these purposes is assessed based on contribution to stewardship and educational goals and not on production levels or contribution to harvest or escapement.

Comment: Although not a focus of the current report, clearly stated program and biological goals for hatchery lines are key components of assessing enhancement programs and mitigating genetic risks.

Production targets for conservation and rebuilding objectives are set at levels that re-establish the naturally spawning population but that limit the risk of changing its genetic variation by regulating the proportion of enhanced fish that spawn within the naturally spawning population. As such, release targets and strategies should be set such that salmon returns of enhanced origin do not exceed 50 percent of the target escapement. This may be exceeded in years prior to full achievement of target. Brood stock collection targets should not exceed 30 percent of the escapement.

Comment: The intents of the guidelines above are consistent with mitigating genetic risks to wild Pacific salmon; however, the limits proposed should depend on the biological designation of the population as defined in Withler et al. (2018). During at least some phases of conservation or rebuilding, it is likely that a population will have an integrated-wild or integrated-transition target designation, and the 50% and 30% limits above are unlikely to be consistent with those targets in all cases.

Where the objective is re-establishment of a locally extinct population, or where the population is the focus of a formal recovery process, such as under the Cultus sockeye conservation strategy (Cultus Sockeye Recovery Team, 2005), broodstock collection plans and enhanced contribution should be set as part of the recovery or production planning process and may exceed these limits in order to address the recovery objectives and schedules.

Comment: The section above is consistent with this report.

Exceptions may also be applied in watersheds where natural spawning habitat is severely limited, such as the Capilano River where the installation of a dam has limited fish access to much of the spawning habitat.

Comment: The section above is consistent with this report.

For production groups with a defined harvest objective, the proportion of the naturally spawning escapement that may be comprised of hatchery fish and collected for brood stock may be established as part of an endorsed integrated planning process or harvest roundtable. Such a process will link fish production with harvest planning for the target fishery and will consider hatchery and natural escapement requirements. If the proportion of the escapement to be comprised of enhanced fish or collected for broodstock is not established through an approved integrated planning process, the limits that are set for rebuilding and recovery will apply.

Comment: The section above is consistent with this report.

4.3. Genetic management – broodstock collection and spawning practices

Regardless of program production objectives, sufficient broodstock that adequately represent the entire donor population and its genetic characteristics are essential to minimize the potential for loss of variation and undesirable genetic effects. Appropriate broodstock selection and spawning practices can minimize chance genetic events and maintain genetic variability of the population. Such practices are critical for determining the genetic make-up of a population and its long-term fitness.

Comment: The section above is consistent with this report.

4.4. Broodstock collection

4.4.1. For all program objectives. The collected broodstock should as far as possible be randomly selected to represent the entire range of run timings, age groups, body sizes, etc. Key aspects are:

- maximize the effective breeding population.
- use fish from the entire run timing.
- collect broodstock randomly from the whole population to represent fish from the full range of physical characteristics, including small, or sexually precocious fish.

Comment: The three points above are consistent with this report.

  - collect jacks proportionally to their abundance in the escapement as these precocious males may contain genetic material important for the long-term fitness of the population.

Comment: Jack males do not contribute to natural spawning at a rate that matches their proportional abundance in the escapement. Jacks should be used in spawning, but at a rate lower than their proportional abundance. See the Analysis and Response section for further information.

  - avoid artificial or intentional selection of spawners in order to preserve and maintain genetic diversity, and minimize artificial selection.

- Where egg targets are small (≤ 10,000 eggs) or when weather or logistical circumstances confine broodstock collection to a short period (e.g. one weekend), strategies to improve representativeness should be employed. These could include collecting some broodstock from as many sites as possible within the river and/or collecting broodstock from a different portion of the run timing each year.

Comment: The two points above are consistent with this report.

4.4.2. Where the objective is rebuilding or conservation

a) For depleted populations that are not part of active recovery planning processes,

- Do not remove more than one third of the naturally spawning escapement for hatchery use. This may mean that production targets will not be achieved. Allow the remaining fish to spawn naturally to maintain a viable naturally spawning population.
  - When collecting brood from a fence or hatchery collection rack, collect about one third of the fish handled on each occasion, stratifying by sex.

Comment: The above point is inconsistent with current best practices based on the biological designations established by Withler et al. (2018). Proportional removals for broodstock should be determined in the context of the targeted biological designation of the population. In some cases, one third will be appropriate, whereas in others it will be either too liberal or too conservative. See the Analysis and Response section for further information.

  - When collecting broodstock without a fence or hatchery fishway (angling, seining etc.), estimating one third of the total return may be difficult. If it appears that returns are weak, broodstock collection should be conservative. Consult knowledgeable staff and consider the previous cycle year escapement.

Comment: The point above is consistent with this report if the one third limit is adjusted to reflect the biological designation of the population as mentioned in the preceding comment.

  - Where returns are weak, broodstock utilized will frequently be limited and involve small numbers of adults. Careful adherence to spawning guidelines is critical to minimize risks of genetic change.

Comment: The above point is consistent with this report.

- Where there are approximately equal proportions of wild and externally identifiable hatchery fish in the return, it is acceptable to include both groups in the broodstock at their rate of occurrence, recognizing that not all hatchery fish may have been marked at release.
- Where externally identifiable hatchery fish predominate in the portion of the escapement accessible for broodstock collection, the proportion of hatchery fish utilized should be roughly the inverse of their proportion in the sample to ensure adequate representation of wild fish. (e.g. 70% hatchery, 30% wild in the sample – broodstock should be comprised of about 30% hatchery fish and 70% wild).

Comment: The two points above as general guidelines are inconsistent with the biological designations established by Withler et al. (2018). Depending on the target biological designation of the enhanced population these points may be too liberal or too conservation. See Withler et al. (2018) for further information.

- Where hatchery fish are not externally identifiable, use broodstock collection methods that include all run timings and body sizes to provide a mix of hatchery and wild returns that represent the population.

Comment: The point above is consistent with this report.

b) For populations in formal recovery processes:

Broodstock collection plans and options should be developed in advance as part of a recovery planning process and must be reviewed and endorsed by the recovery team. General considerations are:

- As far as possible, avoid the use of identifiable hatchery fish in broodstock. However, where returns are severely depleted, inclusion of some hatchery origin fish may be necessary.
- Broodstock removals may comprise up to 50% of the returning spawners particularly if habitat is very poor, resulting in poor wild production. As populations decline, from a genetic perspective, it may be preferable to use all available spawners as broodstock. However, this strategy carries the risk of catastrophic loss of the entire population if a problem is encountered in the hatchery.

Comment: The section above is consistent with this report overall. However, the total avoidance of hatchery fish in broodstock is unlikely to be feasible in all cases, and even in integrated-wild populations pNOB can be as low as 0.77, suggesting that complete bias against hatchery fish in broodstock may be unnecessarily conservative and could impede population recovery.

4.4.3. Where the program objective is for harvest

- The proportion of the escapement removed for broodstock should be developed as part of the production planning process for the target fishery. In the absence of a planning process, the proportion removed should be the same as that used for the recovery objective i.e. no more than one third of the naturally spawning escapement.

- When the enhancement objective is a targeted fishery, broodstock are frequently collected entirely at the hatchery rack and are comprised of fish that that swim into the hatchery. Infusion of wild salmon into the hatchery broodstock (about 10%) can be beneficial. However, it is acceptable to obtain all broodstock required from fish that swim into the hatchery when broodstock populations are in excess of 100 pairs as a small proportion of the return is likely to have originated from naturally spawning fish.
- Infusion of wild broodstock from capture outside of the hatchery is not likely to have an appreciable effect when broodstock populations are in excess of 100 pairs but will be beneficial when broodstock populations are smaller.

Comment: The section above is consistent with this report overall. The incorporation of natural-origin fish into hatchery broodstock is key to avoid segregation of the hatchery salmon and to maintain natural influences in the population. However, depending on the target biological designation of the population as defined in Withler et al. (2018), it may be possible to exceed the one third limit above (e.g., integrated-hatchery).

4.5. Spawning

4.5.1. All Objectives – all broodstock population sizes

- Spawn all collected fully mature broodstock, without regard to age, size or other physical characteristics. Do not exclude any individuals for any reason except for those with overt disease symptoms or physical injuries that may compromise gamete fertility or viability.
- Use fully random mating; avoid any selection. Natural mating patterns are complex and poorly understood, and unlikely to be maintained in a hatchery environment.
- Use one male to one female except as described below. This strategy ensures that each male makes an equal genetic contribution.
- Do not mix the milt from two or more males and then add it to eggs. This practice is known as “pooling” milt and can result in milt from a single male fertilizing a disproportionate share of the eggs.

Comment: The four points above are consistent with this report.

- It is strongly advised that males not be re-used, except as part of specific spawning protocols. In a sequential protocol two males may be used sequentially per female. A given male should be used as the first male for only one female, as follows:

Figure A1. Diagram from DFO (2016) showing a sequential protocol using two males per female.

Comment: The point above is generally consistent with this report, if sequential use of males is deemed necessary; however, re-use of males is not consistent with best genetic practices.

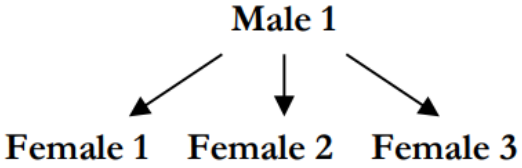
- Consult a support biologist if you are planning to re-use males in any way other than the spawning protocols identified in these guidelines.
- Generally, do not release live males that have been used for hatchery spawning back to their systems of origin. These males will already have contributed a disproportionate amount

of genetic material to the stock compared to wild fish, and, if released, would have the opportunity to contribute even more. Consult a support biologist, however, if there is a very disproportionate sex ration among natural spawners.

Comment: The two points above are consistent with this report.

4.5.2. Spawning broodstock of more than 50 pairs

- When spawning more than 50 broodstock pairs with:
  - a sex ratio of approximately 1:1, mate each female with an individual male. This helps to maintain genetic diversity.
  - more females than males, use matrix spawning (Table 1) or the following hierarchical spawning strategy , where milt from individual males is split amongst available females. Milt must not be pooled.



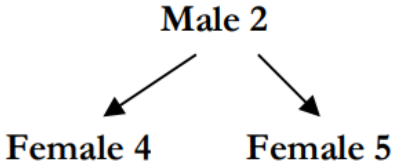


Figure A2. Diagram from DFO (2016) showing an example of a hierarchical spawning protocol with unequal sex ratios (e.g., more females than males).

- One to one spawning is most desirable. However, in spawning situations where it is logistically difficult to keep eggs from individual females separated prior to fertilization (e.g. greater than 250 independent crosses or remote field situations), factorial mating may be considered. Eggs from a number of females are pooled, then gently and thoroughly mixed. Pooled eggs are divided into equal lots in separate containers with the number of containers equal to the number of constituent females. Each lot is then fertilized with milt from a different male, as follows. Milt must not be pooled.

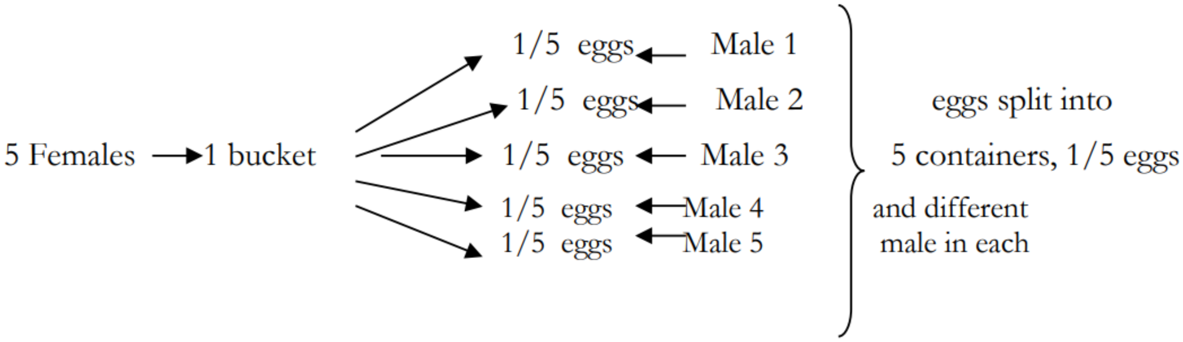


Figure A3. Diagram from DFO (2016) showing factorial mating, using five females.

Comment: The above section is consistent with this report. Note that the “Table 1” referred to above in DFO (2016) is not found in this Appendix, but it contains the same information as Tables 4 and 6 found in the Analysis and Response section.

4.5.3. Spawning broodstock of fewer than 50 pairs

- When spawning fewer than 50 pairs, regardless of sex ratio, attempt to utilize all adults in matrix type breeding to maximize genetic variation in eggs. (Table 1). In matrix spawning, eggs from each female are divided into equal lots. Each lot of an individual female must be fertilized by a different male. This strategy allows the use of all broodstock, even when the sex ratio is unequal, and maximizes genetic combinations and each parent's contribution. It also allows information on families to be tracked if required.

Comment: The point above is consistent with this report. See note regarding "Table 1" in the preceding comment.

- The matrix choice will depend on broodstock maturity, availability, and sex ratio. A minimum of two of the least available sex is recommended for each matrix and for practical purposes, a maximum of four females.

Comment: The point above is generally consistent with this report. If logistically feasible, increasing the maximum number of females to five would have merit. Increases beyond five provide additional benefit, but there are diminishing returns. See the Analysis and Response section for further information.

Appendix B

pHOS (Equation 7)

$$pHOS = \frac{HOR}{HOR + NOR}$$

$$pHOS = \frac{S \times pB \times \alpha \times R_N}{(S \times pB \times \alpha \times R_N) + (S \times (1 - pB) \times R_N)}$$

$$pHOS = \frac{pB \times \alpha}{(pB \times \alpha) + (1 - pB)}$$

pNOB (Equation 8)

$$pNOB = \frac{NOR}{HOR + NOR}$$

$$pNOB = \frac{S \times (1 - pB) \times R_N}{(S \times pB \times \alpha \times R_N) + (S \times (1 - pB) \times R_N)}$$

$$pNOB = \frac{(1 - pB)}{(pB \times \alpha) + (1 - pB)}$$

PNI (Equation 9)

$$PNI = \frac{pNOB}{pHOS + pNOB}$$

$$PNI = \frac{\dfrac{(1 - pB)}{(pB \times \alpha) + (1 - pB)}}{\dfrac{(pB \times \alpha)}{(pB \times \alpha) + (1 - pB)} + \dfrac{(1 - pB)}{(pB \times \alpha) + (1 - pB)}}$$

$$PNI = \frac{(1-pB)}{(pB\times\alpha)+(1-pB)}$$

Appendix C

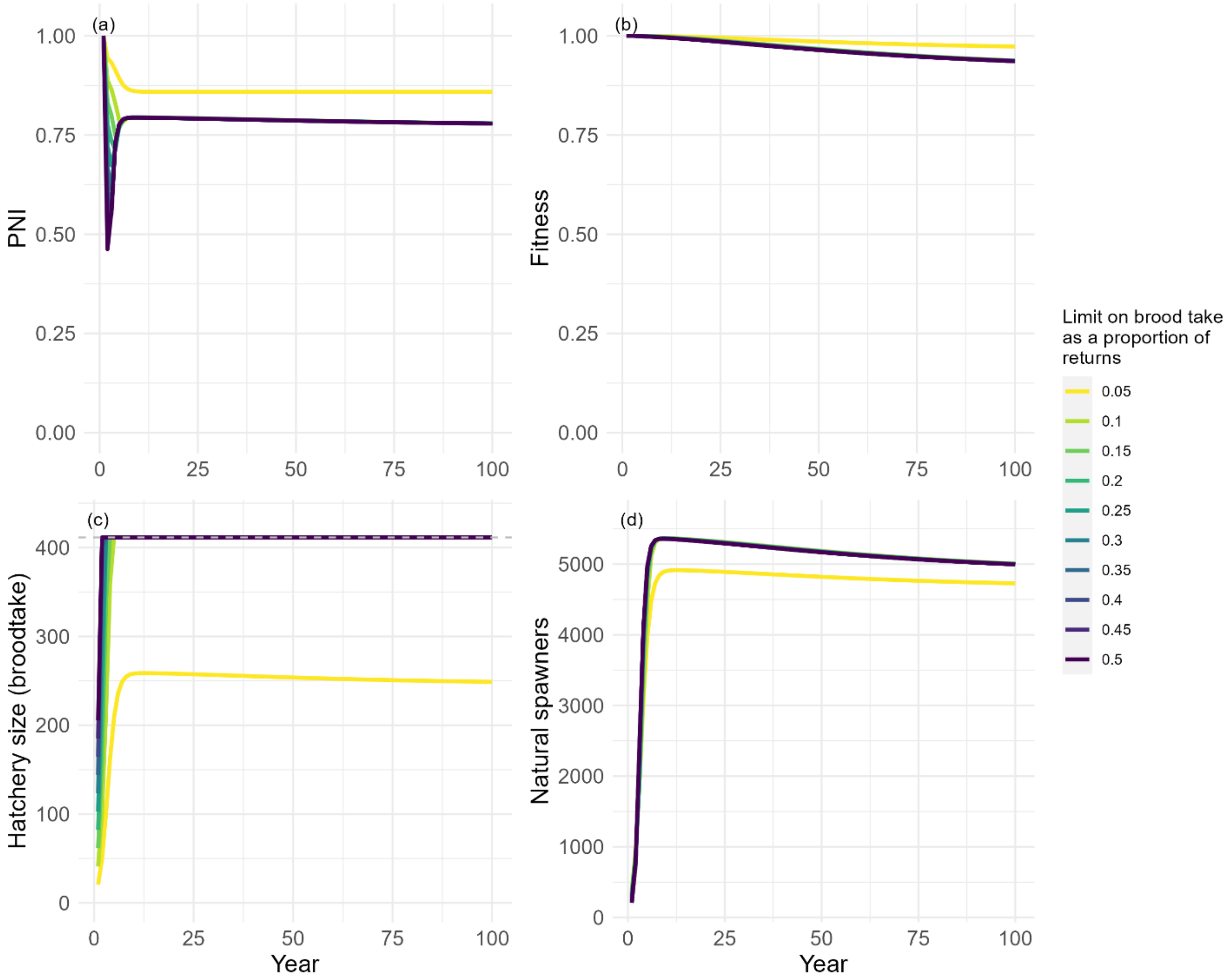


Figure C1. Modeled proportionate natural influence (PNI) (a), population-level fitness (b), hatchery size (c) and natural spawner abundance (d) over 100 simulated generations with a maximum hatchery size (i.e., maximum number of broodstock) of 10% of the equilibrium abundance of natural spawners in the population. Colours display curves for different limits on broodstock collection determined by the proportion of the escapement; note curves for proportional brood takes greater than 0.05 essentially overlap.

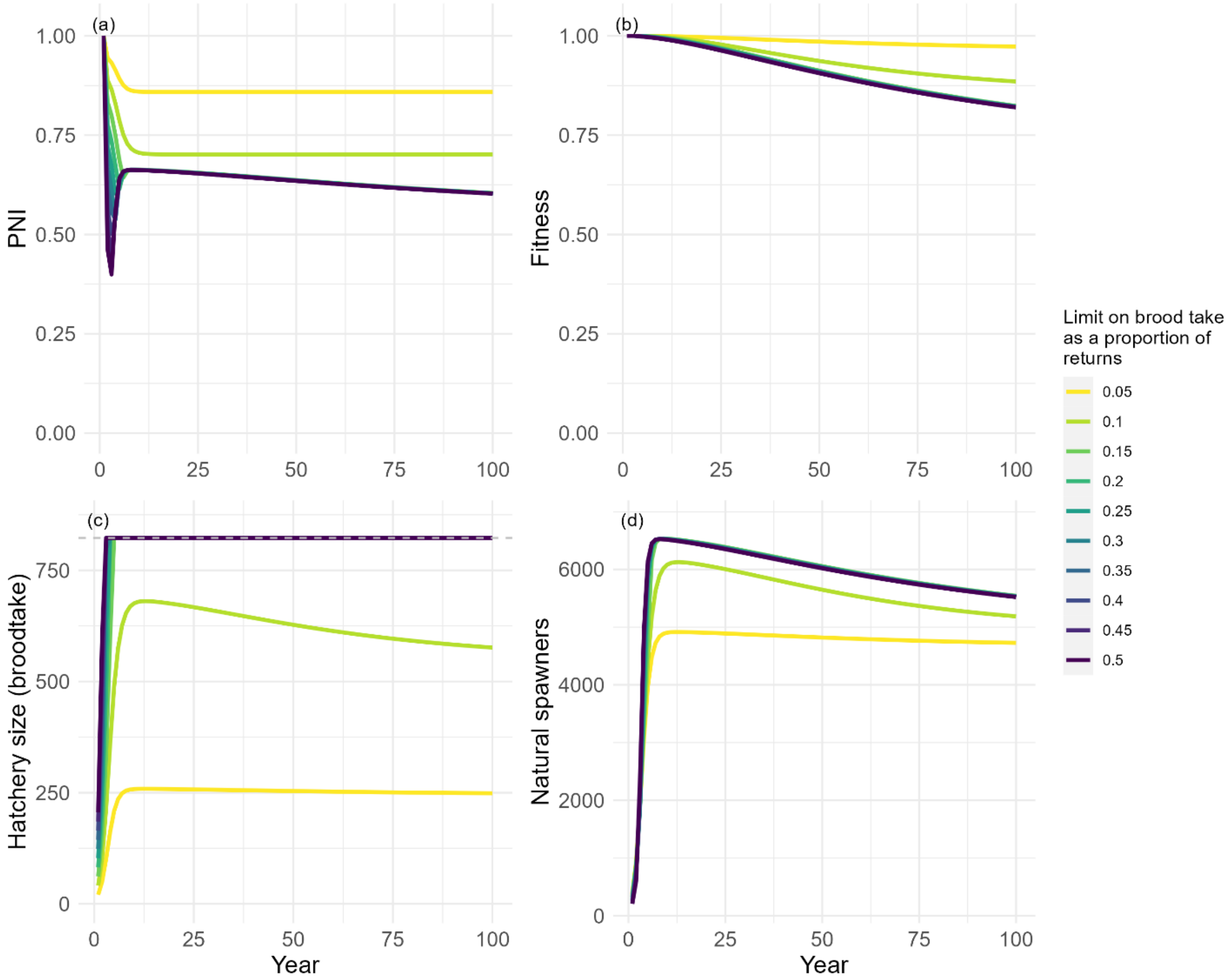


Figure C2. Modeled proportionate natural influence (PNI) (a), population-level fitness (b), hatchery size (c) and natural spawner abundance (d) over 100 simulated generations with a maximum hatchery size (i.e., maximum number of broodstock) of 20% of the equilibrium abundance of natural spawners in the population. Colours display curves for different limits on broodstock collection determined by the proportion of the escapement; note curves for proportional brood takes greater than 0.10 essentially overlap.

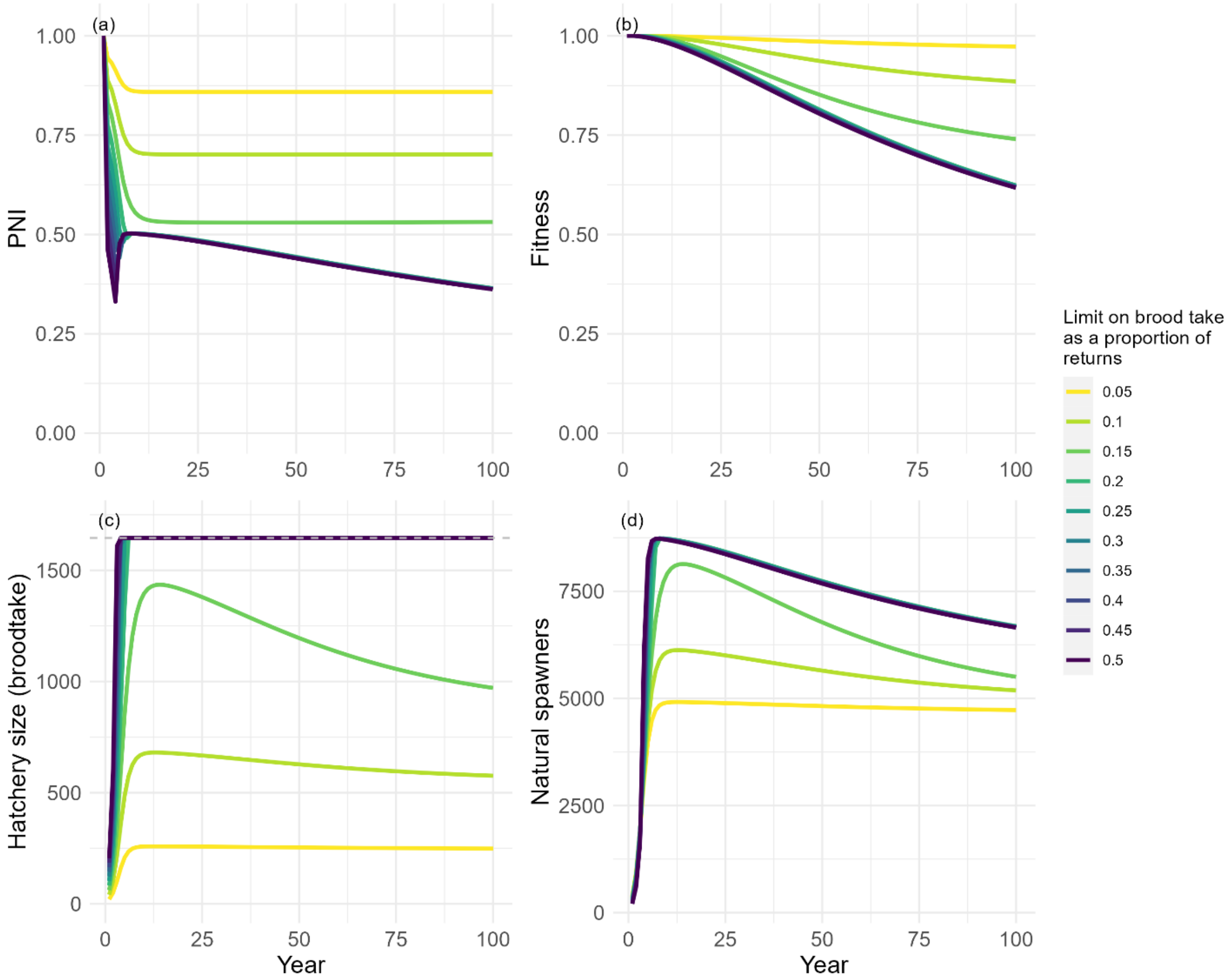


Figure C3. Modeled proportionate natural influence (PNI) (a), population-level fitness (b), hatchery size (c) and natural spawner abundance (d) over 100 simulated generations with a maximum hatchery size (i.e., maximum number of broodstock) of 40% of the equilibrium abundance of natural spawners in the population. Colours display curves for different limits on broodstock collection determined by the proportion of the escapement; note curves for proportional brood takes greater than 0.15 essentially overlap.

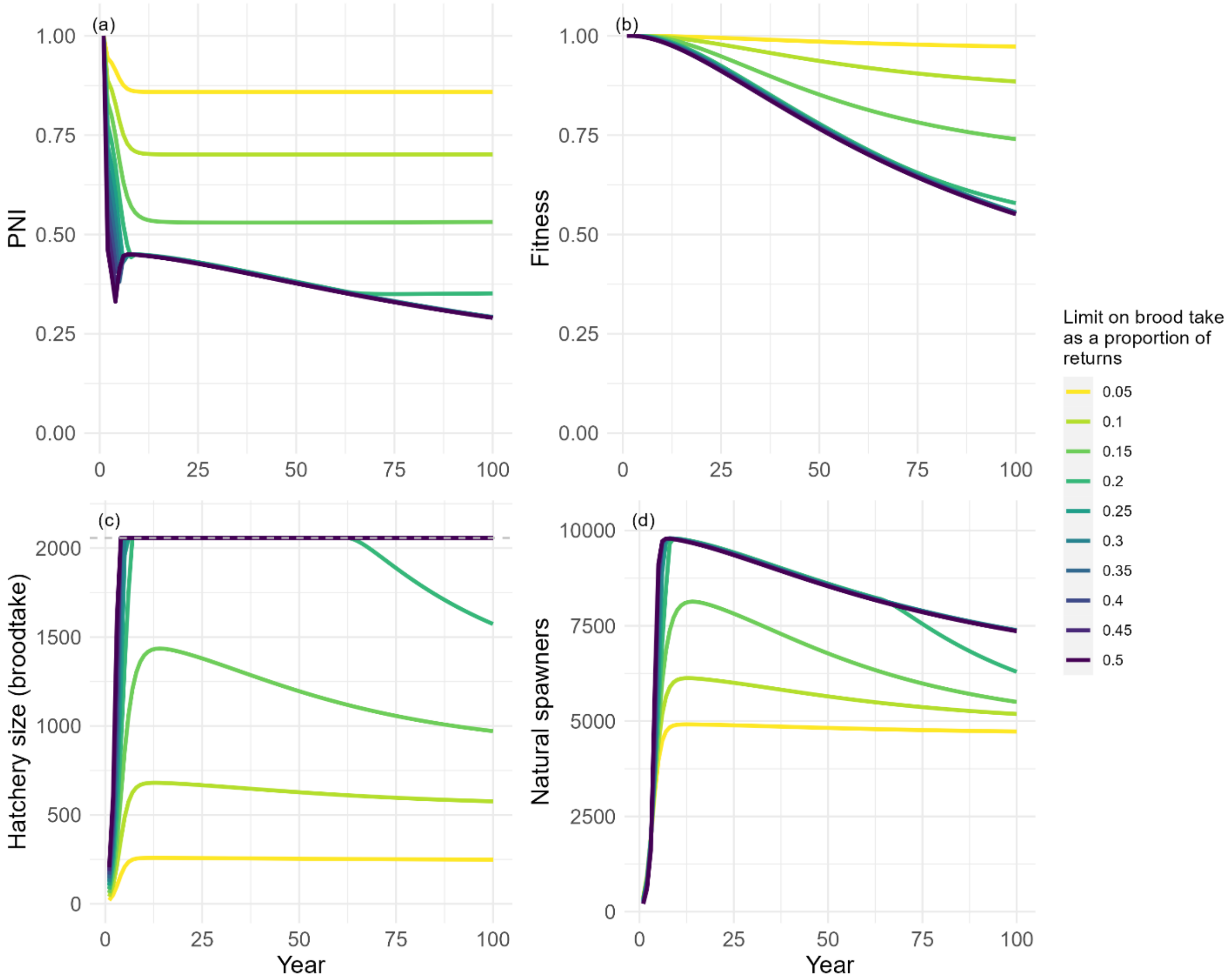


Figure C4. Modeled proportionate natural influence (PNI) (a), population-level fitness (b), hatchery size (c) and natural spawner abundance (d) over 100 simulated generations with a maximum hatchery size (i.e., maximum number of broodstock) of 50% of the equilibrium abundance of natural spawners in the population. Colours display curves for different limits on broodstock collection determined by the proportion of the escapement; note curves for proportional brood takes greater than 0.20 essentially overlap.

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