Hatchery Stocking for Restoring Wild Populations: A Genetic Evaluation of the Reproductive Success of Hatchery Fish vs. Wild Fish

Hitoshi Araki

Department of Zoology, Oregon State University
Corvallis, Oregon 97331, USA

Eawag, Swiss Federal Institute of Aquatic Science and Technology
Center of Ecology, Evolution and Biogeochemistry
Department of Fish Ecology and Evolution
6047 Kastanienbaum, Switzerland

E-mail: hitoshi.araki@eawag.ch

Potential impacts of hatchery programs on wild populations have long been discussed, and of particular interest is the reproductive success of hatchery-born fish in natural environments. Here I summarize our recent studies, in which DNA fingerprinting and genetic parentage analyses were used to estimate adult-to-adult reproductive fitness of steelhead trout (*Oncorhynchus mykiss*) in the Hood River, Oregon (USA). We found: (1) Hatchery fish left fewer adult offspring per parent than wild fish, but supplementation hatchery fish (from local, wild broodstock; H_{supp}) left larger numbers of offspring than traditional hatchery fish (from nonlocal, multi-generation hatchery broodstock; H_{trad}); (2) The reproductive fitness of H_{supp} declined unexpectedly fast (~40% per generation) when H_{supp} were reused as broodstock in a hatchery, suggesting that the negative effects of hatchery rearing are cumulative and heritable; (3) Effective population size was mainly restricted by variance in reproductive success among individuals, rather than by biased sex ratio and temporal fluctuation of population sizes; (4) H_{trad} showed particularly large variance in reproductive success, indicating another negative effect of traditional programs. Our case studies suggest that using local, wild broodstock reduces negative effects of hatchery rearing, but the repeated use of H_{supp} as broodstock should be minimized for efficient supplementation.

KEYWORDS   hatchery stocking; supplementation; effective population size; reproductive success; parentage analysis

1. Introduction

Traditionally, hatchery programs have focused on a single mission: producing fish for harvest. The primary goal remains the same for the majority of the current hatchery programs. Fish culture technologies have been greatly improved in the last 100 years, and many of the current fisheries depend on hatchery fish (especially those for economically valuable species such as salmon, Leber *et al.* 2004; Williams 2006). Ecologically, however, there remain many uncertainties about the effects of hatchery fish on wild populations. For example, released or escaped hatchery fish might threaten wild populations by competing with wild fish for natural resources such as food, territory and mates (Jonsson and Jonsson 2006). Profound effects of hatchery stocking on wild populations and reproductive interactions between hatchery and wild fish have been predicted (Ryman and Laikre 1991; Waples and Do 1994; Lynch and O'Hely 2001; Ford 2002; Duchesne and Bernatchez 2002; Hutchings and Fraser 2008). Some studies suggest that traditional hatchery fish, which are typically non-local fish and maintained multiple generations in hatcheries, have low reproductive success in the wild, often only ~10% of reproductive success in wild fish (Berejikian and Ford 2004; Araki *et al.* 2008, for reviews). If the level of fitness decline in hatchery fish is common, the potential impacts of reproductive interactions between hatchery and wild fish may be large because humans release billions, if not trillions, of hatchery fish into the natural ecosystems annually (Heard 1995). Nevertheless, empirical evidence is not yet compelling enough to induce widespread changes in the design of hatchery operations for many fish species.

The risk of extinction and declines in wild populations of fish species have lead to an increasing number of hatcheries adopting a new set of goals: supplementing fish for the restoration and conservation of wild populations (Cuenco *et al.* 1993; Waples and Drake 2004). Although there are many kinds of supplementation programs, a common idea behind them is to create hatchery fish from local broodstock (parents of hatchery fish), rear hatchery fish in well-protected captive environments, and stock them to 'supplement' wild populations. Main goals of the supplementation program are to have a large number of wild-born fish back to the population as a consequence of reproduction by hatchery fish in natural environments, and eventually to reestablish self-sustainable wild populations. Unlike traditional hatchery programs for harvest, therefore, having large numbers of *hatchery*-born fish in the populations itself is not a goal of the supplementation programs (although some hatchery programs have a hybrid goal, for conservation and harvest, at the same time).

An essential assumption of supplementation program is reasonably high reproductive success of hatchery-born fish in natural environments. However, it is largely untested whether supplementation hatchery stocks really reproduce better in the natural environments than do traditional hatchery stocks. In this review, I summarize our recent studies on this issue, in which we evaluated reproductive success of steelhead trout (*Oncorhynchus mykiss*) in the Hood River, Oregon (Araki *et al.* 2007a–d). Although these studies are based on an anadromous species in one system, they provide valuable information about genetic effects of hatchery rearing. Therefore I believe that the majority of conclusions are applicable to many other hatchery programs, including those for marine species.

2. The Study System

Steelhead trout is a sibling species of Pacific salmon (*Oncorhynchus* spp., Crespi and Fulton 2004), and an anadromous (sea-run) form of rainbow trout. Steelhead typically
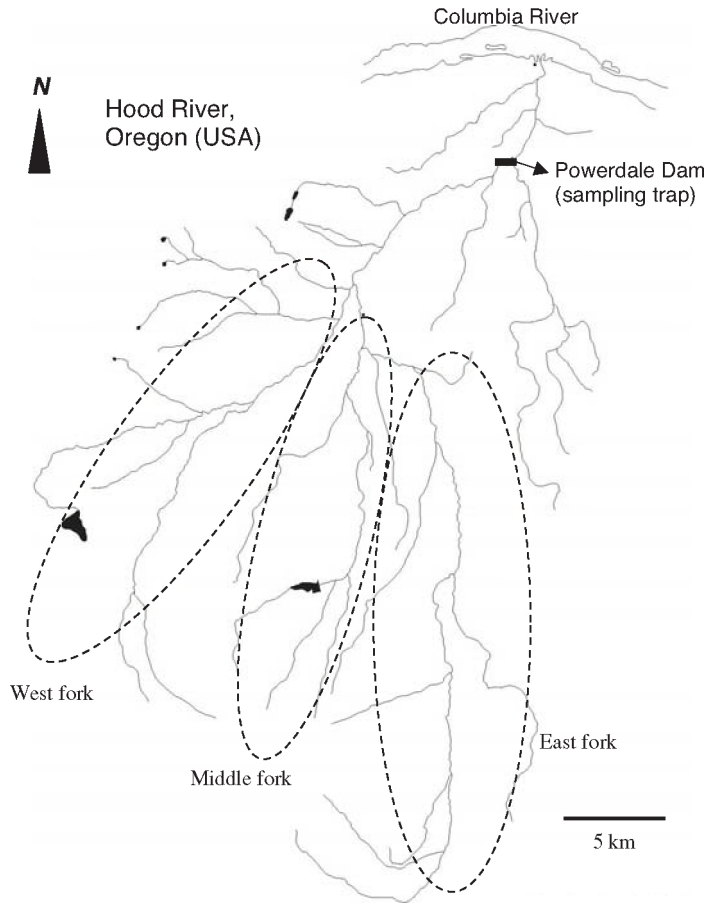
Fig. 1.   Map of the Hood River, Oregon. The Hood River is a tributary of the Columbia River in Pacific Northwest, USA. The sampling trap, which is built in the Powerdale dam, is located at the mouth of the Hood River (4 river miles from the mouth). Dotted circles indicate three major branches of the Hood River and the locations of spawning grounds for steelhead trout (East fork for summer-run population and Middle and West forks for winter-run population). This map is kindly provided by Erik Olsen (ODFW).

return to the river for reproduction at age of 2–6 after 1–4 years of journey in the ocean. In contrast, rainbow trout remain in freshwater throughout their life cycle. We used DNA fingerprinting techniques and genetic parentage assignments (below) on the returning adult steelhead for three generations to evaluate adult-to-adult reproductive success of hatchery fish and the effective population size in the wild.

Two factors make the Hood River steelhead one of the best systems for this kind of genetic evaluations. First, genetic samples are available for almost all steelhead that returned and spawned in the river for last 16 years. The number of genetic samples used in our studies is more than 15,000. The whole population sampling was possible because the sampling trap is built in a dam on the mouth of the river (Fig. 1), and only fish

captured at the sampling trap and released above the dam can reach their spawning grounds in the river. The sampling has been operated by the Hood River conservation team in the Oregon Department of Fish and Wildlife (ODFW) since 1991 on a daily basis. The quality and quantity of biological and genetic data have been proven to be crucial for the fine-scale parentage and following analyses (Araki and Blouin 2005).

Second, Hood River was stocked with juvenile steelhead from traditional and supplementation hatchery programs during this period. Because both traditional hatchery fish (H_{trad}) and supplementation hatchery fish (H_{supp}) were allowed to pass the dam and spawn in the river, we could evaluate the effects of these hatchery programs in the same system. H_{trad} were created from non-local hatchery stocks and maintained in a hatchery for many generations (~10 generations), whereas H_{supp} were created from local wild stocks and maintained only one or two generations in a hatchery in the Hood River. In this article I use the term "wild fish" to refer to fish born and reared in natural environments (regardless of parentage), and the term "hatchery fish" to refer to fish that were born and raised in a hatchery through the juvenile stage before being released. In the Hood River programs, hatchery fish are released as yearling (Appendix for details).

3. DNA Fingerprinting and Parentage Assignments

DNA fingerprinting is a powerful tool for population studies. Polymorphic genetic markers, such as microsatellite (DNA repeat sequences in which the repeat number varies among individuals) are highly polymorphic and specific to individuals, and contain sufficient information to identify individuals (Jeffreys et al. 1985a, b). In addition, DNA is inherited by progeny from their parents. Therefore genetic markers provide pedigree information on the basis of Men-

delian inheritance. DNA fingerprinting was first applied to genetic identification of individuals humans (Jeffreys et al. 1985a, b), but the technique is now used for a variety species and for many purposes, including parentage analyses for reproductive success of individuals in wild populations (e.g., Avise 2004; Bishop et al. 2004). Genetic parentage analyses require temporal sample sets and many polymorphic markers, but once they are obtained, researchers can gain powerful insights regarding mating systems, population dynamics and natural selection in wild populations.

For Hood River steelhead, we surveyed 8 microsatellite markers after DNA extraction from sampled scales or fin clips (Araki et al. 2007a). These markers were highly polymorphic (average heterozygosity ~90%) and provided enough power for identifying parentages (Exclusion probability > 0.9996, Araki et al. 2007a). The 15000+ samples included fish from two steelhead populations (summer-run and winter-run), which are considered to be reproductively isolated from each other (Appendix). In the following studies, we treat them as two independent population samples. 96% of the samples were successfully examined for more than 6 microsatellite loci. Using the DNA fingerprinting and fine-scale parentage assignments, we reconstructed a two-generation pedigree for summer-run population and a three-generation pedigree for winter-run population (Araki et al. 2007a, b). The obtained pedigree information was used to estimate the relative reproductive success (*RRS*) of hatchery fish to wild fish and effective population size.

4. Reproductive Success of Hatchery Fish

RRS is defined as the ratio of average numbers of wild-born offspring from one type of parents (e.g., hatchery fish) to those from the other (e.g., wild fish) that returned in the

Table 1.   Number of fish returned to the Hood River (updated from Araki *et al.* 2007a)

Run year of the parents	Winter-run population				Summer-run population			
	Parents		Offspring	RS	Parents		Offspring	RS
	Wild-born	Hatchery-born			Wild-born	Hatchery-born		
1991	716	292 (T)	273	0.27	—	—	—	
1992	408	5	304	0.74	537	1677 (T)	87	0.04
1993	382	2	212	0.55	240	1108 (T)	128	0.09
1994	203	6	298	1.43	199	1652 (T)	199	0.11
1995	276	185 (S)	1237	2.68	132	518 (T)	212	0.33
1996	242	283 (S)	995	1.72	182	1310 (T)	615	0.41
1997	226	199 (S)	901	2.12	83	447 (T)	494	0.93
1998	299	220 (S)	628	1.21	134	4	271	1.96
1999	920	267 (S)	520	0.44	182	0	283	1.55
2000	1013	657 (S)	466	0.28	208	0	>191	—
2001	1025	684 (S)	>362	—	491	115 (S)	>144	—
2002	725	413 (S)	>390	—	641	482 (S)	>50	—
2003	625	535 (S)	>17	—	241	189 (S)—	—	—
2004	352	242 (S)	—	—	217	148 (S)—	—	—
2005	502	295 (S)	—	—	195	128 (S)—	—	—
2006	493	350 (S)	—	—	208	164 (S)—	—	—
Total	8407	4635	6603	—	3890	7942	2674	—

Parents: Number of wild-born fish or hatchery-born fish returned and passed above the dam for spawning in each run year (Appendix for definition). (T): Traditional hatchery fish; (S): Supplementation hatchery fish. Offspring: Number of fish born in the wild in that specific run year and returned in the subsequent run years as adults (typically after 2–6 years). The years of birth of the returned fish were estimated from year rings on scales by ODFW. RS: an absolute reproductive success (= number of offspring per total number of parents) among all the potential parents for each run population. Data as of August 24, 2007 from ODFW.

same year (Appendix). We used *RRS* to evaluate reproductive success of hatchery fish because absolute values of reproductive success fluctuated considerably among years (Table 1). Analyses on *RRS* in the Hood River steelhead revealed a rapid fitness decline of hatchery fish as they are released in the wild after the hatchery rearing for more than one generation (Araki *et al*. 2007a, b). One extreme example is H_{trad}. The broodstock of H_{trad} in the Hood River were reared in a hatchery for ~10 generations. On the basis of the pedigree information, we found that the overall *RRS* of H_{trad} to wild fish was only 8% for winter-run and 33% for summer-run (Fig. 2). Although non-local origins of H_{trad} might also affect the reproductive success due to the lack of local adaptation, these results indicate that domestication through the generations of hatchery rearing has a large potential to reduce reproductive fitness of hatchery fish in the wild.

The first generation of H_{supp} (H_{supp} created from wild broodstock) reproduced better than H_{trad} in the wild, but generally worse than wild fish (Fig. 2). The difference in reproductive success between first generation H_{supp} and wild fish was not statistically significant in the first three run years (winter-run 1995–1997, Araki *et al*. 2007a), but it was significant when another three run years of data are included (Overall *RRS* of first generation H_{supp} to wild fish in winter-run 1995–2000 = 0.85, $P < 0.001$, Araki *et al*. 2007b). These results suggest that when H_{supp} are created from wild parents, hatchery fish can leave 85% of offspring as do wild fish (in contrast to only 8–33% *RRS* in H_{trad}). The difference in *RRS* between H_{supp} and H_{trad}
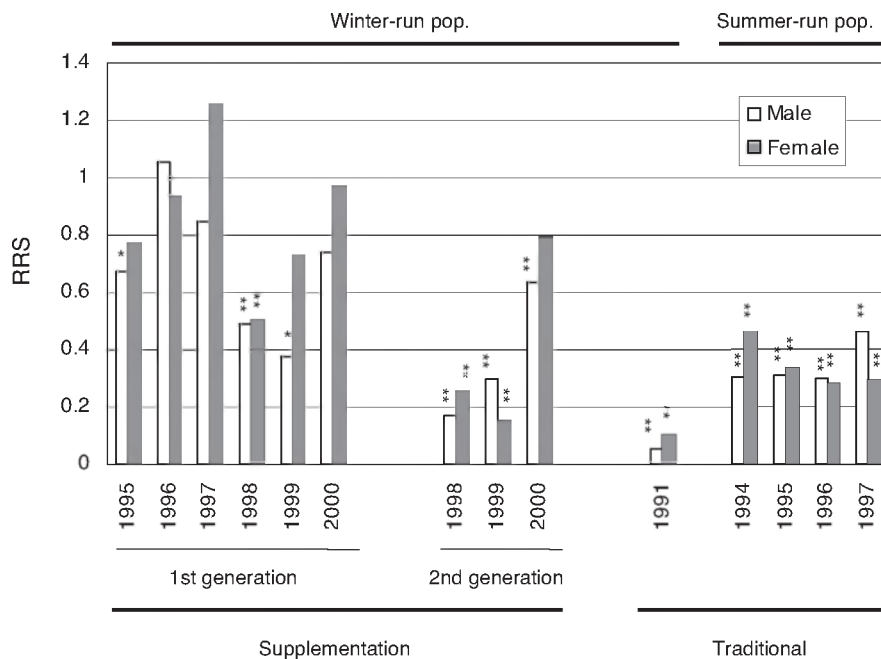
Fig. 2. Relative reproductive success (*RRS*) of hatchery fish to wild fish in the Hood River steelhead. Original data are shown in Araki *et al.* (2007a, b). *RRS* = 1 (average reproductive success of hatchery fish is equal to that of wild fish in the same run year) was used as a null hypothesis of the permutation test. *RRS* was estimated for fish from two types of hatchery programs operated during 1991–2000. In winter-run population, overall *RRS* of hatchery fish to wild fish was 0.848 for the first generation of supplementation hatchery fish, 0.379 for the second generation, and 0.079 for the traditional hatchery fish. In the summer-run population, overall *RRS* was 0.334 for the traditional hatchery fish (P < 0.01 in all four cases above). Reproductive success of supplementation hatchery fish in summer run population was not evaluated because enough number of offspring from the summer-run supplementation hatchery has not returned yet (Table 1). Angling of hatchery fish was allowed above the dam between run years 1992 and 1997 but ignored in this table because the correction for harvest had only minor effects on our conclusions (see Araki *et al.* 2007a). *$P < 0.05$, **$P < 0.01$.

indicates that the supplementation hatchery practice indeed improves the effectiveness of hatchery program for the restoration of wild population, at least in a short term. Whether the 15% decrease in reproductive fitness compared to wild fish is acceptable or not and what that means to the long term (evolutionary) consequences of wild populations remain open questions.

In the second generation of the supplementation program (winter-run 1995–1999), the hatchery managers started using the returning first generation H_{supp} as broodstock

to create more hatchery fish. Although [hatchery × hatchery] broodstock cross was avoided, both [hatchery × wild] and [wild × wild] broodstock crosses were allowed in the hatchery (Fig. 3). By reconstructing parentages between second generation H_{supp} and their wild-born offspring, the reproductive success of the second generation H_{supp} in the wild was evaluated. *RRS* of the second generation H_{supp} from the [hatchery × wild] crosses to wild fish was significantly lower than 1.0 (Fig. 2, Overall *RRS* = 0.38, $P < 0.001$, Araki *et al.* 2007b).
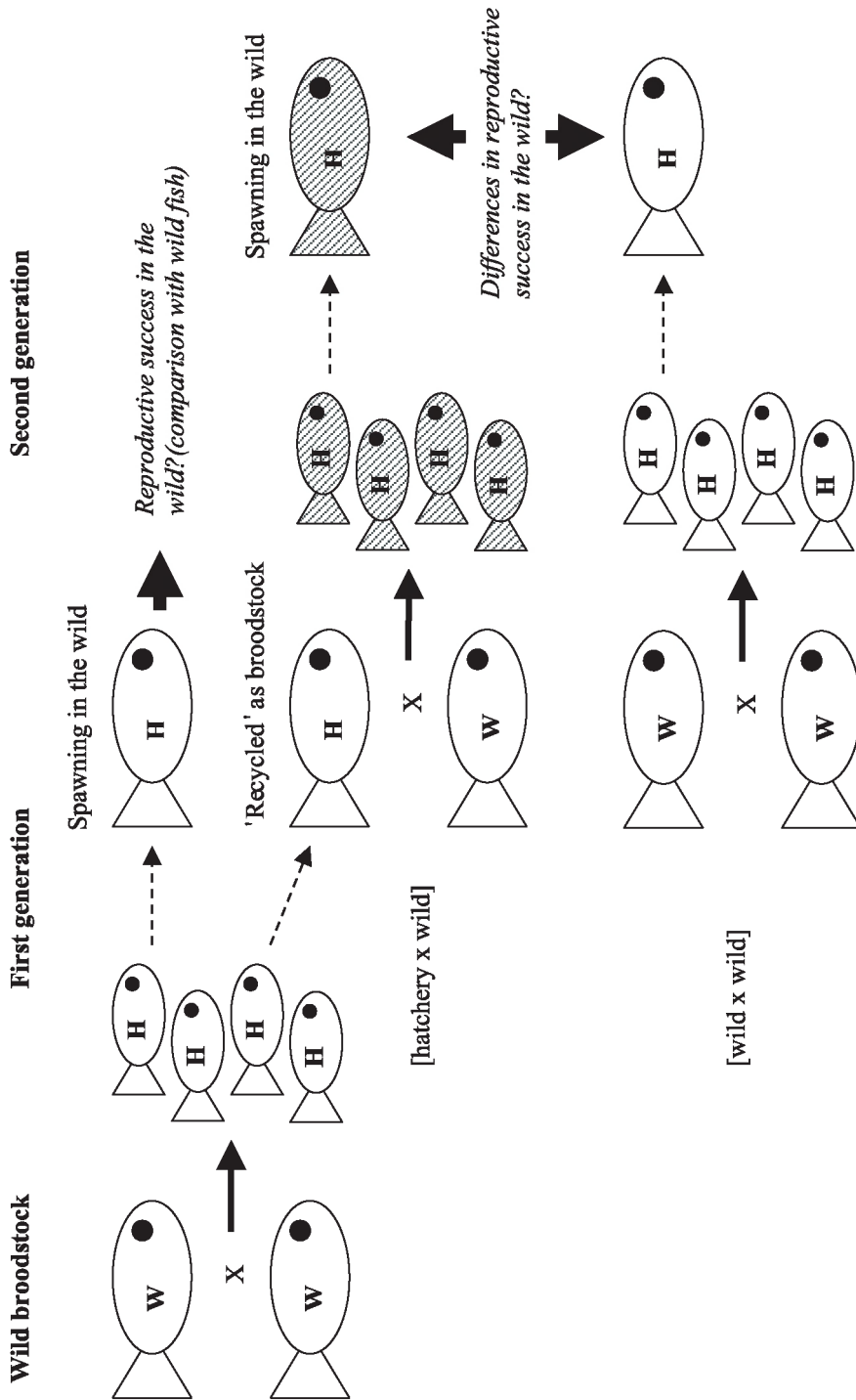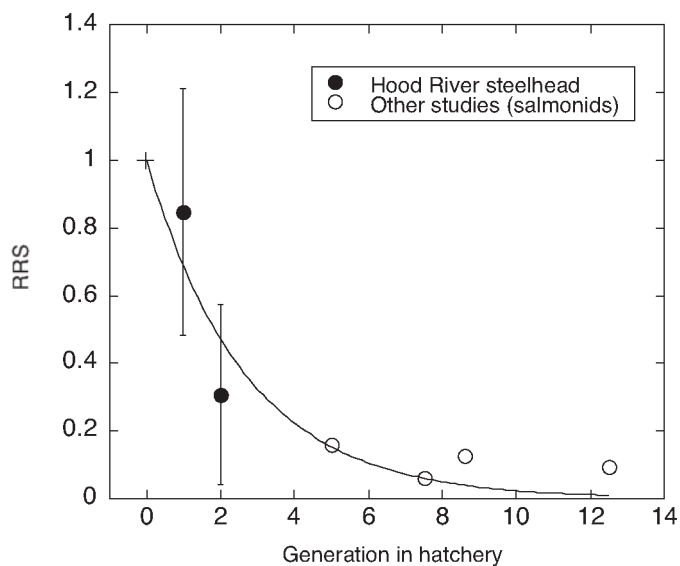
Fig. 3. Conceptual diagram for the evaluation of natural reproductive success in supplementation hatchery fish. Shaded fish represent hatchery fish from a hatchery parent and a wild parent, which are compared with hatchery fish from two wild parents.

Fig. 4. Relative reproductive success of salmonid hatchery fish to wild fish plotted against generation time in captivity (cited from Araki *et al.* 2007b). Closed circles represent the estimates from the Hood River steelhead (weighted geometric means from Fig. 2). The open circles represent four other estimates from studies on local hatchery stocks of various salmonid species (See text for details). The exponential regressions suggest a 37.5% fitness decline per captive reared generation ($y = e^{-0.375x}$, correlation coefficient = 0.962).

We also performed a meta-analysis including data available for other salmonid hatchery stocks that have local origins. In this analysis, we combined the *RRS* estimates from the Hood River steelhead with those from four other studies, two on steelhead, one on Atlantic salmon, and one on brown trout (Salmon Recovery Science Review Panel 2004; Araki *et al.* 2007b and references therein). These data fit very well on an exponentially declining curve (Fig. 4), despite the fact that these data include *RRS* estimates using different species and methods. The clear decline in reproductive success as a function of the number of generation in hatchery suggests: The reproductive fitness of hatchery fish in the wild can decline very quickly when the broodstock have been reared for multiple generations in a hatchery. Reproductive fitness decline can be as high as 37.5% per generation in the hatch-

ery (Fig. 4); The cause of the fitness decline is cumulative through captive-reared generations, indicating that genetic changes in the hatchery rearing are largely responsible for the fitness decline. Our results are consistent with those from other studies, in which genetic degradation of fish in hatchery is suggested to have a large effect on reproductive traits of hatchery fish (Reisenbichler and McIntyre 1977; Reisenbichler and Rubin 1999; but also see Dahl *et al.* 2006; Ford *et al.* 2006). The genetic effects of hatchery rearing are further discussed in the next section.

5. Genetic Effects of Hatchery Rearing

One problem for determining effects of hatchery rearing is the confounding effects of genetic and non-genetic factors. Hatchery

environments differ radically from natural environments, thus the reproductive success of hatchery fish might be directly affected by environmental (non-genetic) factors. While the cumulative fitness effects of hatchery rearing indicate that the major cause of the decline is heritable (and hence genetically-based), we cannot completely exclude the effects of the different rearing environments in the comparisons between hatchery and wild fish. In addition, H_{trad} had non-local origins in the Hood River steelhead, and the difference in their genetic origins also confounded this comparison.

A major advantage of the second generation study on Hood River steelhead (Araki *et al*. 2007b) is that we could eliminate the environmental factors. As mentioned above, we had two types of hatchery fish in the second generation (hatchery fish from the [hatchery × wild] cross and those from the [wild × wild] cross, Fig. 3). Comparison of the reproductive success of the two types of hatchery fish, instead of the comparisons between hatchery and wild fish above, was used to directly evaluate the genetic effect of hatchery rearing for one additional generation. Both types of hatchery fish were reared in the same hatchery at the same time, and shared the same local origin. The only difference between these hatchery fish was the number of generations for which half of the genome was exposed to the hatchery environment. Our data show that the overall *RRS* of H_{supp} from [hatchery × wild] to H_{supp} from [wild × wild] was only 55% ($P = 0.009$, Araki *et al*. 2007b), suggesting that H_{supp} from a hatchery parent and a wild parent had lower reproductive success than H_{supp} from wild parents. This result also suggests that the extended hatchery rearing for only one generation can lower reproductive fitness of the next generation in the wild, and that the negative effect is most likely genetically-based.

6. Effective Population Size

In the above sections we focused on the average reproductive success of hatchery fish in the wild. The individual-based parentage analysis also provides the direct estimate of variance in reproductive success, which is another key factor to understanding how genetically effective the individuals are in producing the next generation (Crow and Morton 1955). In this section, I briefly summarize a study on effective population size (N_e) of the Hood River steelhead, on the basis of parentage assignments and the estimates of the variance in reproductive success (Araki *et al*. 2007c). Although I focus on the demographic estimates of N_e, it is noteworthy that empirical comparisons of N_e estimates using different methods in the Hood River steelhead also provided an important finding that a temporal estimation of N_e (a genetic method) contains a systematic bias when reproductively inferior groups (e.g., hatchery fish) are included in the estimation (Araki *et al*. 2007d).

N_e can be defined as the size of an ideal population that has the same rate of genetic change as the population in question. An ideal population is one in which mating is occurring at random, population size is constant and the sex ratio is 1:1 (Wright 1931; Crow and Kimura 1970). N_e is a fundamentally important parameter in evolutionary and conservation biology because effectiveness of natural selection and the level of genetic variation within a population depend on the effective population size (Crow and Kimura 1970; Frankham *et al*. 2002). N_e is generally smaller than census population size (N) because the conditions for the ideal population are seldom, if ever, met in real populations (e.g., Frankham *et al*. 2002; Turner *et al*. 2002).

In Hood River steelhead, we found that overall N_e of steelhead per generation in the river was not small ($N_e \sim 1000$–1500 per

generation, Araki *et al*. 2007c). This result is consistent with the results in the previous sections because natural selection is effective only when N_e is large, and we expect large variance in reproductive success when natural selection is strong (Crow and Kimura 1970). However, the N_e/N ratio was only 0.17–0.40, with large variance in reproductive success among individuals being the primary cause of small N_e/N (Araki *et al*. 2007c). The small N_e/N indicates that the level of population genetic diversity could be low in this population even if there are large numbers of individuals in the population (e.g., Frankham *et al*. 2002). In our case, biased sex ratio (male:female = ~2:3) and temporal fluctuation in population size within generation had relatively minor effects on N_e/N. Interestingly, H_{trad} showed large variance in reproductive success among breeding parents, whereas H_{supp} showed no sign of increased variance when compared with wild fish (Araki *et al*. 2007c). It suggests that H_{trad}, but probably not H_{supp}, impose a genetic risk of reducing N_e on the wild population.

7. Genetic Compensation between Life-History Forms

Another interesting result in the effective population size study (Araki *et al*. 2007c) is a novel function of reproductive interaction between life-history polymorphisms. As mentioned earlier, *O. mykiss* has two major life-history forms, anadromous (steelhead) and resident (rainbow trout). They have distinct morphologies at the adult stage, but they interbreed in natural environments when both forms coexist sympatrically (Zimmerman and Reeves 2000). Although we could not obtain genetic samples from the resident population, the near-complete sampling from anadromous population enabled us to infer when and how much the resident fish contributed to the production of the anadromous population (Araki *et al*. 2007a, c). We dis-

covered that the reproductive interaction between life-history forms can work as a genetic buffer, by which the relatively large effective population size is stably maintained: According to the parentage analyses, inferred reproductive contribution of resident parents to anadromous offspring was larger when fewer anadromous parents had returned to spawn in the Hood River (Araki *et al*. 2007c). It means that resident fish provided a maximum support for the reproduction of anadromous fish when the anadromous population was at the risk of genetic bottleneck. In fact, theoretical prediction suggests that the effective population size could have decreased 40–60% if there was no contribution from resident population (unpublished data). If such a genetic buffering between life-history forms is common, a conservation plan must be designed carefully so that the wild populations do not lose the buffering function via losing life-history polymorphisms in the same system. In the case of steelhead, unfortunately conservation plans often focus strongly on saving anadromous population and rarely take its resident counterpart into account.

8. Discussion

The most striking result from the studies of Hood River steelhead is the rate of fitness declines of hatchery fish—perhaps 30–40% per captive-reared generation (Fig. 4). The rate of fitness decline implies that hatchery fish rapidly lose their reproductive capabilities in the wild, presumably as a consequence of adaptation to hatchery environments (i.e., domestication). Strong influence of domestication is not generally expected for supplementation hatchery programs because intentional artificial selection is usually avoided in this type of hatchery program. However, quantitative genetics theory suggests that rapid domestication is not impossible if domestication selection works on multiple traits at the same time, or very strong selection

Table 2. Hypothetical stocking effects on population size in the next generation.

$N_{[wild]}$	$N_{[stocked]}$	$\%_{[stocked]}$	$N_{[offspring]}$ (No. of generation in hatchery)					
			0	1	2	3	5	10
100	10	9.1	110	106	103	102	101	100
100	100	50.0	200	163	139	124	110	101
100	1000	90.9	1100	725	491	344	195	109
100	10000	99.0	10100	6350	4006	2541	1054	191

This table illustrates expected demographic consequences of different scales of hatchery stocking into a hypothetical wild population with $N = 100$ ($N_{[wild]}$). $N_{[stocked]}$: Number of hatchery-born fish stocked into the population. $\%_{[stocked]}$: a percentage of hatchery-born fish in the stocked population. $N_{[offspring]}$: Number of wild-born offspring in the next generation. $N_{[offspring]}$ is a product of N after stocking (=$N_{[wild]}$ + $N_{[stocked]}$) and the average reproductive fitness of the parents (W). For simplicity, absolute reproductive fitness of wild fish = 1, 37.5% fitness decline of stocked fish per hatchery-reared generation (see text), and no influence of the carrying capacity are assumed to calculate W. This table shows, for example, when ten fish are stocked into the wild population with $N = 100$ (first line in the table), the expected number of wild-born fish in the next generation is 110 if there is no fitness decline, but is 106, 103, 102, 101 and 100 if hatchery stock is from the first, second, third, fifth, and tenth generations in a hatchery, respectively.

works on a single trait both in captivity and in the wild and the trait under selection is highly heritable between generations (Araki *et al*. 2008). Another possible mechanism is an accumulation of deleterious mutations due to relaxation of natural selection, which would be certainly occurring in well-protected hatchery environments. However, mutation accumulation is unlikely to explain such a rapid fitness decline *per se*, while it might have fitness consequences in a long span of hatchery stocking (Lynch and O'Hely 2001).

The rapid fitness decline also implies that the repeated use (i.e., recycling) of hatchery broodstock to produce the next generations can result in a large loss of reproductive fitness in hatchery fish. The most obvious consequence of the low fitness of hatchery fish could be a failure of population size recovery with hatchery stocking. A simple calculation demonstrates the effects of fitness decline on the population size in the next generation: Let's assume that a current population is well under the carrying capacity and that there is no density dependence in population growth for simplicity. Then the number of wild-born offspring ($N_{[offspring]}$) after hatchery stocking can be calculated simply as $N_{[offspring]} = NW$, where N is the total number of parents in a population and W is the average (absolute) reproductive fitness of the parents. Table 2 shows some of the demographic consequences of hypothetical stocking of hatchery fish, which have low reproductive fitness due to up to 10 generations of hatchery rearing. In these examples 37.5% fitness decline per captive-reared generation was assumed (from Fig. 4). It is clear that supplementation hatchery programs lose the potential for increasing the size of wild population very quickly as they use more heavily recycled hatchery stock. For instance, under the scenario of 50% stocking (i.e., when stocking intends to double the population size), population size increases only 39%, 10%, and 1% in the next generation if hatchery stocks are maintained for two, five, and ten generations in captivity, respectively. These percentages would be even lower if mortality of the stocked fish is higher than that of wild fish in natural environments

before reproduction. Of course larger scales of hatchery stocking increase the population size more in a short term (in the case of no restriction due to carrying capacity), but such a heavy stocking might reduce fitness of the wild population in a long term, considering the fact that hatchery fish have genetic differences that reduce reproductive fitness in the wild.

At this point, it is unclear whether hatchery programs are generally helping or harming the wild populations. However, evidence from the Hood River research suggests that hatchery programs, even the supplementation programs, produce reproductively inferior fish than wild fish. It means hatchery fish are not genetically or reproductively equivalent to wild fish. Especially, the negative effects of H_{trad} (small average and large variance in reproductive success) suggest that the hybridization between H_{trad} and wild fish should be carefully avoided in order to protect the wild population. Our data show that the use of local, wild fish has an advantage over the traditional hatchery programs for recovering the wild population. However, the cumulative effect of the hatchery rearing on the fitness of H_{supp} also suggests that the recycling of hatchery broodstock imposes a high risk both genetically and demographically. Genetic changes of hatchery fish occur rapidly, and the reuse of hatchery fish should be considered as a final option (e.g., when wild populations are strongly depressed and a collection of new wild broodstock is impossible). Given the fitness concerns, alternative conservation plans such as habitat restoration or control of over-fishing might be more effective in restoring wild populations in some cases.

Understanding the actual mechanism of fitness decline and the trait(s) under selection pressure will hopefully guide us to solutions for mitigating the negative effects of hatchery rearing. Previous studies show that many traits can be associated with fitness declines, such as egg size, growth rate, and feeding and mating behaviors (Johnsson and Abrahams 1991; Arendt 1997; Berejikian et al. 2000; Einum and Fleming 2000; Heath et al. 2003; Reisenbichler et al. 2004; Tymchuk et al. 2007). However, none of these has yet been proven to be responsible for the observed rate of fitness decline to date. Monitoring and evaluating the effects of hatchery programs on wild populations are important for improving the designs of the hatchery and stocking programs. It is also noteworthy that the most appropriate design might be different among programs because it often depends on the goal of the program, conditions of wild populations, and species of interest.

The reproductive success of *wild* fish that have hatchery-born ancestors is another important issue for self-sustainable wild populations. Given that the fitness decline of hatchery fish is genetically-based, it is possible that even wild fish suffer low reproductive success after generations of supplementation due to introgression of 'hatchery' genes. However, it is also possible that one or a few generations of exposure to the natural environments are enough to eliminate the maladapted genes from the wild population. In the latter scenario, surviving wild fish from hatchery ancestors might show no difference in reproductive success when compared with wild fish from wild parents. Again, the DNA fingerprinting and the genetic parentage analysis are powerful tools to evaluate these possibilities, and the evaluation study is currently ongoing on the Hood River steelhead (Araki et al. in prep.).

Results from recent studies show that we are in a new era, in which we can directly test the effects of hatchery stocking on wild populations using various molecular techniques. I believe that we can reach to general conclusions on the effectiveness of hatchery stocking and more sophisticated means of hatchery/wild stock management—hopefully before too many wild populations go extinct.

Acknowledgements

References

Araki H, Blouin MS. Unbiased estimation of relative reproductive success of different groups: evaluation and correction of bias caused by parentage assignment errors. *Mol. Ecol.* 2005; **14**: 4097–4109.

Araki H, Ardren WR, Olsen E, Cooper B, Blouin MS. Reproductive success of captive-bred steelhead trout in the wild: evaluation of three hatchery programs in the Hood river. *Conserv. Biol.* 2007a; **21**: 181–190.

Araki H, Cooper B, Blouin MS. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. *Science* 2007b; **318**: 100–103.

Araki H, Waples RS, Ardren WR, Cooper B, Blouin MS. Effective population size of steelhead trout: influence of variance in reproductive success, hatchery programs, and genetic compensation between life-history forms. *Mol. Ecol.* 2007c; **16**: 953–966.

Araki H, Waples RS, Blouin MS. A potential bias in the temporal method for estimating N_e in admixed populations under natural selection. *Mol. Ecol.* 2007d; **16**: 2261–2271.

Araki H, Berejikian BA, Ford MJ, Blouin MS. Fitness of hatchery-reared salmonids in the wild. *Evol. Appl.* 2008; **1**: 342–355.

Arendt JD. Adaptive intrinsic growth rates: an integration across taxa. *Quarterl. Rev. Biol.* 1997; **72**: 149–177.

Avise JC. *Molecular Markers, Natural History, and Evolution.* 2nd ed. Sinauer Associates, Sunderland. 2004.

Berejikian BA, Tezak EP, LaRae AL. Female mate choice and spawning behavior of chinook salmon under experimental conditions. *J. Fish. Biol.* 2000; **57**: 647–661.

Berejikian B, Ford MJ. A review of the relative fitness of hatchery and natural salmon. National Oceanic and Atmospheric Administration (NOAA) Draft Processed Report, NMFS-NWFSC-61. Northwest Fisheries Science Center, Seattle. 2004.

Bishop JM, Jarvis JU, Spinks AC, Bennett NC, O'Ryan C. Molecular insight into patterns of colony composition and paternity in the common mole-rat Cryptomys hottentotus hottentotus. *Mol. Ecol.* 2004; **13**: 1217–1229.

Crespi BJ, Fulton MJ. Molecular systematics of Salmonidae: combined nuclear data yields a robust phylogeny. *Mol. Phylogenet. Evol.* 2004; **31**: 658–679.

Crow JF, Morton NE. Measurement of gene frequency drift in small populations. *Evolution* 1955; **9**: 202–214.

Crow JF, Kimura M. *An Introduction to Population Genetics Theory.* Harper & Row, New York. 1970.

Cuenco ML, Barkman TWH, Mundy PR. The Use of Supplementation to Aid in Natural Stock Restoration. *In*: Cloud JG, Thorgaard GH (eds). *Genetic Conservation of Salmonid Fishes.* Plenum Press, New York. 1993; 269–294.

Dahl J, Petersson E, Dannewitz J, Järvi T and Löf AC. No difference in survival, growth and morphology between offspring of wild-born, hatchery and hybrid brown trout (*Salmo trutta*). *Ecol. Freshw. Fish.* 2006; **15**: 388–397.

Duchesne P, Bernatchez L. An analytical investigation of the dynamics of inbreeding in mult-generation supportive breeding. *Conserv. Genet.* 2002; **3**: 45–58.

Einum S, Fleming IA. Highly fecund mothers sacrifice offspring survival to maximize fitness. *Nature* 2000; **405**: 565–567.

Ford MJ. Selection in captivity during supportive breeding may reduce fitness in the wild. *Conserv. Biol.* 2002; **16**: 815–825.

Ford MJ, Fuss H, Boelts B, LaHood E, Hard JJ *et al.* Changes in run timing and natural smolt production in a naturally spawning coho salmon (*Oncorhynchus kisutch*) stream after 60 years of intensive hatchery supplementation. *Can. J. Fish. Aquat. Sci.* 2006; **63**: 2343–2355.

Frankham R, Briscoe DA, Ballou JD. Introduction to conservation genetics. Cambridge University Press, Cambridge, UK; New York. 2002.

Heard WR. An estimate of total 1992 hatchery releases of the North Pacific Ocean and adjacent seas. North Pacific Anadromous Fish Commission Doc. 154, Auke Bay Fisheries Laboratory, Alaska Fisheries Science Center (NMFS). National Oceanic and Atmospheric Administration (NOAA), Juneau. 1995.

Heath DD, Heath JW, Bryden CA, Johnson RM, Fox CW. Rapid evolution of egg size in captive salmon. *Science* 2003; **299**: 1738–1740.

Hutchings JA, Fraser DJ. The nature of fisheries- and farming-induced evolution. *Mol. Ecol.* 2008; **17**: 294–313.

Jeffreys AJ, Brookfield JF, Semeonoff R. Positive identification of an immigration test-case using human DNA fingerprints. *Nature* 1985a; **317**: 818–819.

Jeffreys AJ, Wilson V, Thein SL. Hypervariable 'minisatellite' regions in human DNA. *Nature* 1985b; **314**: 67–73.

Johnsson JI, Abrahams MV. Interbreeding with domestic strain increases foraging under threat of predation in juvenile steelhead trout (*Oncorhynchus mykiss*)—an experimental study. *Can. J. Fish. Aquat. Sci.* 1991; **48**: 243–247.

Jonsson B, Jonsson N. Cultured Atlantic salmon in nature: a review of their ecology and interaction with wild fish. *ICES J. Marine Sci.* 2006; **63**: 1162–1181.

Leber KM, Kitada S, Blankenship HL, Svåsand T. *Stock Enhancement and Sea Ranching*. Blackwell Scientific Press, Oxford. 2004.

Lynch M, O' Hely M. Captive breeding and the genetic fitness of natural populations. *Conserv. Genet.* 2001; **2**: 363–378.

Olsen EA. Hood River and Pelton ladder evaluation studies (Annual report 2000–2001). The Oregon Department of Fish and Wildlife, Portland. 2003.

Reisenbichler RR, McIntyre JD. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, Salmo gairdneri. *Can. J. Fish. Res. Board.* 1977; **34**: 123–128.

Reisenbichler RR, Rubin S. Genetic changes from artificial propagation of Pacific salmon affect the productivity and viability of supplemented populations. *ICES J. Mar. Sci.* 1999; **56**: 459–466.

Reisenbichler RR, Rubin S, Wetzel L, Phelps S. Natural selection after release from a hatchery leads to domestication in steelhead, *Oncorhynchus mykiss*. *In*: Leber KM, Kitada S, Blankenship HL, Svåsand T (eds). *Stock Enhancement and Sea Ranching*. Blackwell Scientific Press, Oxford. 2004; 371–384.

Ryman N, Laikre L. Effects of supportive breeding on the genetically effective population size. *Conserv. Biol.* 1991; **5**: 325–329.

Salmon Recovery Science Review Panel. Report for the meeting held August 20–September 2, 2004. Northwest Fisheries Science Center, Seattle. 2004.

Turner TF, Wares JP, Gold JR. Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, Estuarine-dependent marine fish (*Sciaenops ocellatus*). *Genetics* 2002; **162**: 1329–1339.

Tymchuk WE, Sundstrom LF, Devlin RH. Growth and survival trade-offs and outbreeding depression in rainbow trout (*Oncorhynchus mykiss*). *Evolution* 2007; **61**: 1225–1237.

Waples RS, Do C. Genetic risk associated with supplementation of Pacific salmonids: captive broodstock programs. *Can. J. Fish. Aquat. Sci.* 1994; **51**(suppl. 1): 310–329.

Waples RS, Drake J. Risk/benefit considerations for marine stock enhancement: A Pacific salmon perspective. *In*: Leber KM, Kitada S, Blankenship HL, Svåsand T (eds). *Stock Enhancement and Sea Ranching*. Blackwell Scientific Press, Oxford. 2004; 260–306.

Williams RN. *Return to the River: Restoring Salmon to the Columbia River*. Elsevier Academic Press, Amsterdam; Boston. 2006.

Wright S. Evolution in Mendelian populations. *Genetics* 1931; **16**: 97–159.

Zimmerman CE, Reeves GH. Population structure of sympatric anadromous and nonanadromous *Oncorhynchus mykiss*: evidence from spawning surveys and otolith microchemistry. *Can. J. Fish. Aquat. Sci.* 2000; **57**: 2152–2162.

Appendix: Terminology

The Hood River hatchery programs for steelhead trout: Traditional hatchery programs for the Hood River steelhead had been operated since mid 1900s until supplementation programs took place in 1990s. The Hood River supplementation programs of winter-run and summer-run steelhead (definitions below) started in 1991 and 1997, respectively (Olsen 2003). In the supplementation programs, broodstock (parents of hatchery fish) are collected from the Hood River each year. They are crossed at the Parkdale Hatchery on the Middle Fork of the Hood River, and hatchery fish are reared to yearling smolts. Smolt is juvenile fish that changed phenotypes from freshwater form to ocean form through a developmental process known as smoltification (e.g., growing silver scales). The smolts are first acclimated in the Parkdale Hatchery before release or are released directly into the Hood River. All hatchery fish are adipose-fin clipped before release, and fish without adipose fin are identified as hatchery fish at the sampling dam trap when they return as adults. The returning supplementation hatchery fish have been allowed to pass the Powerdale Dam since 1995 for winter-run and 2001 for summer-run (Table 1).

Run populations: The Hood River supports two run populations of steelhead (summer-run and winter-run), named for the time of year the most adults return to freshwater. Both runs breed in the spring. However, summer-run returns to the river up to one year earlier than the spawning, whereas winter-run returns to the river shortly before the spawning. They distribute to different forks of the river (Fig. 1). Our parentage analysis confirmed that there is little or no hybridization between them (Araki *et al.* 2007c).

Run year: Run year X of the parents represents a parental group that returned to the river between the year X and $X + 1$ and spawned in the year $X + 1$. For example, fish in the winter-run 1991 returned between 1991 and 1992, and spawned in 1992.

RRS: Relative reproductive success. *RRS* can be defined as a ratio of the average numbers of offspring from two types of parents. For example, if hatchery fish left one wild-born offspring per parent whereas wild fish left two, the *RRS* of hatchery fish to wild fish is 50% (1:2). We used an unbiased estimate of *RRS*, in which systematic bias due to errors in the genetic parentage assignments is corrected (Araki and Blouin 2005). The bias in the *RRS* estimation is inevitable especially when genetic markers are highly polymorphic, there are null alleles, and a part of the parents are not sampled (Araki and Blouin 2005). Overall *RRS* is a geometric mean of *RRS* between two groups of parent (e.g., hatchery fish and wild fish) over run year and sex. Each point estimate was weighted by number of parents. Geometric mean was used to summarize *RRS* because each estimate was obtained as a ratio of the absolute reproductive success.