

Reduced reproductive success of hatchery coho salmon in the wild: insights into most likely mechanisms

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Abstract

Supplementation of wild salmonids with captive-bred fish is a common practice for both commercial and conservation purposes. However, evidence for lower fitness of captive-reared fish relative to wild fish has accumulated in recent years, diminishing the apparent effectiveness of supplementation as a management tool. To date, the mechanism(s) responsible for these fitness declines remain unknown. In this study, we showed with molecular parentage analysis that hatchery coho salmon (*Oncorhynchus kisutch*) had lower reproductive success than wild fish once they reproduced in the wild. This effect was more pronounced in males than in same-aged females. Hatchery spawned fish that were released as unfed fry (age 0), as well as hatchery fish raised for one year in the hatchery (released as smolts, age 1), both experienced lower lifetime reproductive success (RS) than wild fish. However, the subset of hatchery males that returned as 2-year olds (jacks) did not exhibit the same fitness decrease as males that returned as 3-year olds. Thus, we report three lines of evidence pointing to the absence of sexual selection in the hatchery as a contributing mechanism for fitness declines of hatchery fish in the wild: (i) hatchery fish released as unfed fry that survived to adulthood still had low RS relative to wild fish, (ii) age-3 male hatchery fish consistently showed a lower relative RS than female hatchery fish (suggesting a role for sexual selection), and (iii) age-2 jacks, which use a sneaker mating strategy, did not show the same declines as 3-year olds, which compete differently for females (again, implicating sexual selection).

Keywords: captive breeding, parentage analysis, reproductive success, salmonids, sexual selection, supplementation

Received 20 January 2010; revision received 14 January 2011; accepted 18 January 2011

Introduction

Hatchery programs have been used for decades to compensate for worldwide declines in salmonid populations. Despite years of supplementation, there is scarce evidence that such programs have helped the recovery of, or increased the long-term productivity of wild salmonid populations (Waples *et al.* 2007; Fraser 2008). Instead,

recent studies have provided convincing evidence that hatchery fish have lower reproductive success (RS) than wild fish when both breed in the wild. This difference is detectable after as little as one or a few generations spent in the hatchery environment (review in Araki *et al.* 2008; Recovery Implementation Science Team 2009). However, most hatchery/wild fitness comparisons to date have been limited to studies using steelhead (*Oncorhynchus mykiss*). Thus, it is still unclear whether the same phenomenon occurs in other salmonid species.

Fitness differences between hatchery and wild salmonids can be genetically based (Araki *et al.* 2007, 2009) or could result from carry-over effects of the environment

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experienced in the hatchery (Fleming *et al.* 1997). Genetic differences are hypothesized to result from relaxed natural selection and/or domestication selection in the captive environment (Ford 2002; Araki *et al.* 2008). There are two main stages in the life cycle where genetic effects of hatchery rearing are expected. These are adult reproduction and juvenile survival. Because adults are spawned artificially in the hatchery, there can be relaxed natural selection or inadvertent artificial selection on adult reproductive traits (McLean *et al.* 2005). For example, hatchery breeders do not allow mates to choose, compete, find suitable spawning sites, defend nests, and so on. Alternately, viability selection (survival) could act on juveniles in the hatchery, or later in life on phenotypic variation generated in the hatchery (Reisenbichler *et al.* 2004). To date, the actual causal genetic and/or environmental mechanisms responsible for the fitness differences remain unclear.

Most studies comparing the relative reproductive success of hatchery and wild fish involve salmonid species that spend one-third to one-half of their lifetime in the hatchery (e.g. steelhead, coho salmon *O. kisutch*, Atlantic salmon *Salmo salar*, brown trout *S. trutta*) prior to release as smolts (i.e. juvenile fish undergoing osmoregulatory changes necessary to migrate from fresh water to the marine environment). Time spent in a captive environment typically ensures higher juvenile survival rates when compared to that of the wild. On the other hand, the relatively long time spent in the hatchery presents substantial opportunities for domestication selection and for environmental influences that can carry over into adulthood. In this study, we compared the relative fitness of two types of coho salmon hatchery fish: those that were created in the hatchery, but released into the wild as unfed fry, vs. their siblings that were raised for a year in the hatchery and released as smolts.

Reproductive success is measured as the number of adult offspring produced per adult individual. Coho only spawn once; therefore, fitness is measured over one full generation from returning adult to returning adult (thus including components of adult reproduction and offspring survival) and this is a true measure of lifetime fitness. Unfed fry came from the same mating pairs as the smolts, but were released upon emergence without being fed in the hatchery. They experienced the same environmental conditions and high mortality (i.e. opportunity for selection) as wild fish, except for the circumstances of their fertilization and their incubation as eggs and newly hatched fry. We hypothesize that if selection on juvenile traits in the hatchery is the principal mechanism causing the decline of hatchery fish fitness, then reproductive success of unfed fry releases should be the same as that of wild fish. On the other

hand, if selection (or lack thereof) acts on some other stage in the life cycle in the hatchery (e.g. adult reproductive behaviours), then the success of unfed fry (that return to spawn) should also be lower than that of wild spawners. Therefore, the goals of this study were to: (i) determine whether the phenomenon of lower fitness of hatchery fish in the wild that was principally documented in steelhead (Araki *et al.* 2007, 2008, 2009) also occurs in coho salmon (a congener with a number of significant life-cycle differences), and (ii) test the prediction that any fitness difference between hatchery and wild coho results from selection or environmental effects during the hatchery juvenile phase of the life cycle.

Methods

Sampling design

Hatchery programs were conducted on the Umpqua River basin sporadically from 1948 to 1980, using both local and non-local brood stocks. The North Umpqua River hatchery coho program was started in 1980, and began to use only North Umpqua fish as brood stock in 1983. This integrated hatchery program used local North Umpqua brood stock which included 30% natural fish (non-adipose fin-clipped) and 70% hatchery-born fish recaptured (adipose-fin clipped), to produce smolts that are used to supplement the natural-spawning population. Each smolt released from the hatchery was adipose-clipped and/or coded-wire tagged (CWT) to ensure the designation 'hatchery fish'. This program mainly produced smolts, but was also part of the Salmon and Trout Enhancement Program (STEP) that released non adipose-clipped unfed fry in tributaries throughout the Umpqua basin. Stream releases of both fry and smolt ended in 2006. In 2001, 2002 and 2003 ODFW (Oregon Department of Fish and Wildlife) collected 100 CWT/adipose-clipped adult coho salmon of each sex at Winchester Dam on the North Umpqua. These fish were designated hatchery-reared brood stock, H. They had at least one generation of hatchery ancestry, and possibly more. Over the same time period 94, 100, and 102 non-CWT/non-adipose-clipped adult coho salmon of each sex were collected and designated as wild-born brood stock, W. In 2001, the W brood stock was collected at Winchester Dam, while for 2002 and 2003, W fish were taken from the Nonpareil Dam at Calapooya Creek, a tributary of the Mainstem Umpqua (Fig. 1). For each of the 3 years, males and females were randomly paired within each group (H × H and W × W) and spawned at ODFW's Rock Creek hatchery facility using single-pair mating (i.e. each male and female was used only once). Hatcheries methods in

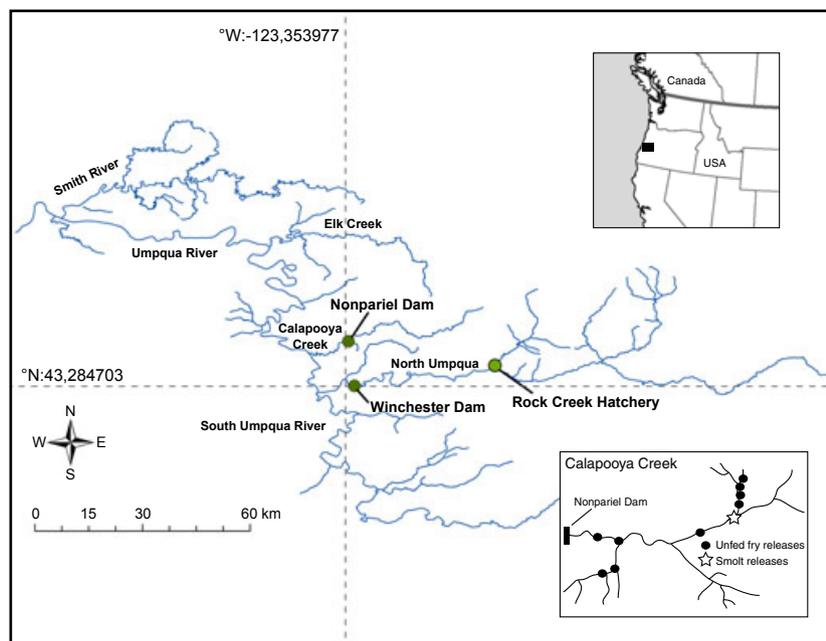


Fig. 1 Map of the study area showing sampling sites of brood stock (Winchester and Nonpareil Dam) and the location of Rock Creek hatchery. All offspring resulting from hatchery crosses were released in Calapooya Creek. The inset shows specific locations of release for unfed fry and smolts in Calapooya Creek.

Oregon vary from a random matrix design (many males with many females) to a random single-pair design, as we used in the present study. Thus, our hatchery method is representative of what can be found in a 'real' supplementation program. The progeny from these single-pair crosses are referred to as F1 hatchery fish. Note that while W is designated as wild-born, W fish may have hatchery ancestry owing to past mating of hatchery fish in the wild. Moreover, W fish collected as brood stock from Nonpareil Dam could also be composed of unfed fry returns, that is, fish born in the hatchery but released upon emergence as part of the STEP program. Calapooya Creek was supplemented in 2000 and 2001 with unfed fry, which returned as adults in 2002 and 2003. Based on analysis of returns from 2004 to 2006 in Calapooya Creek (Thériault *et al.* 2010 and the present study), we estimated the proportion of the run composed of unfed fry to be on average 15% (range 6–25%). Thus, if some W fish are in fact unfed fry returns from previous stocking, they likely form a small proportion of the brood stock. We thus assume that the majority of the W fish used in our study were born and reared in the wild, and that W fish had at least one less generation of hatchery rearing as juvenile than the H fish. Note that hatchery ancestry in W fish will just reduce our ability to detect differences between H and W.

The progeny of each pair was split at the eyed-egg stage. A portion of the offspring was transferred to

hatchboxes and mixed upon emergence to be randomly released as unmarked unfed fry at nine sites along the Calapooya Creek in spring of 2002, 2003 and 2004 (Fig. 1). The remaining eggs of each pair were mixed when ponded as fry in rearing tanks and were released as smolts in spring of 2003, 2004 and 2005 at one specific location on the Calapooya Creek (Fig. 1). These two release strategies resulted in two groups with different times spent in captivity. We released an average of 438 332 fry and 22 160 smolts per year. As expected, fish released as smolt experienced much higher survival from release to adulthood (2.4% relative to 0.05% for fish released as unfed fry, Thériault *et al.* 2010). See Moyer *et al.* (2007) and Thériault *et al.* (2010) for additional methodological details.

Most coho salmon migrate to the ocean after spending a year in freshwater. Some of the males spend only one summer at sea and return to freshwater the following fall as small but sexually mature males that are two years of age (termed jacks). The majority of males and all females spend two summers in the ocean before their fall migration to freshwater spawning habitat at three years of age. Therefore, unfed fry and smolts released in Calapooya Creek from 2002 to 2005 returned as F1 2-year-old males (hereafter 'jacks') and 3-year-old males (hereafter 'males') and 3-year-old females, alongside their naturally born counterparts, from 2003 to 2006 (designated as brood year, BY, Fig. 2). Note that spawning migration starts in fall and continues until

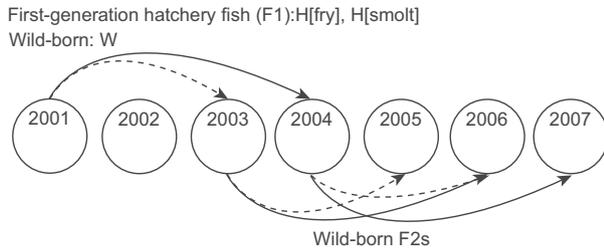


Fig. 2 Sampling design of the study. Illustrated is the sampling design for 2001, but the same scheme applies to brood stock collections in 2002 and 2003. Circles represent the brood year (BY), which correspond to the year (fall) at which the first fish enters the river during the spawning migration. For example, the first generation of hatchery fish (F1) was created from paired matings in the hatchery in the fall of 2001 and released as unfed fry (H[fry]) in spring 2002 and as smolts (H[smolt]) in spring 2003. Fish released as unfed fry and as 1-year-old smolts return on the same schedule, alongside their wild-born counterpart (W). These hatchery and wild fish returned as age-2 males (jacks; dotted line) and age-3 males and females (solid line) in BY 2003 and 2004 and mated in the wild, mixing together. F2s resulting from these matings returned as jacks and age-3 fish from BY 2005 to 2007.

February. Thus, the 'brood year' in which an adult returns is actually the fall calendar year before the year in which their offspring were born (Fig. 2). At the fish trap at the base of Nonpareil Dam, all returning coho were captured, measured, checked for fin-clips, sexed by visual identification, aged (for males only, age-2 or age-3 based on size) and had tissue samples taken via a caudal clip for genetic identification. All the fish were released above the dam after handling and allowed to spawn naturally. The offspring resulting from mating by F1s in the wild were termed F2, and returned as jacks and age-3 males and females at Nonpareil Dam from BY 2005 to 2009 (Fig. 2). All F2 returns were also caudal-clipped for genetic identification, which allowed us to match them back to their F1 parents (see parentage analysis below).

Parentage analysis

DNA was extracted from fin tissue, amplified via PCR and scored from all brood stock (BY 2001–2003), F1 and F2 progeny returning to Calapooya Creek (BY 2003–2009) at 10 microsatellites loci (*OTS519*, *OTS520*, *ONE111*, *P53*, *OTS3*, *ONEμ2*, *OCL8*, *OTS215*, *ONEμ13*, *OMY1011*) following the methods of Moyer *et al.* (2007).

The F1 returns were allocated to either a hatchery parental pair ($H \times H$ or $W \times W$) or a wild pair using the allocation method described in Thériault *et al.* (2010). Briefly, we used the software PAPA 2.0 that uses a maximum-likelihood allocation approach, to

allocate F1 returns to hatchery pairs (Duchesne *et al.* 2002). We then used PASOS 1.0 that combines a maximum-likelihood approach followed by exclusion, to allocate F1 returns to wild pairs or single parents (Duchesne *et al.* 2005). Note that these likelihood methods do not rely on significance thresholds. Therefore, our estimates of difference in reproductive success among groups should not suffer from the bias identified by Ford & Williamson (2010) (i.e. groups having a lower effective size, such as hatchery fish, wind up with an under-estimated relative RS). The following notation will be used hereafter: H[fry] and H[smolt] designate F1 fish born in the hatchery and released as unfed fry or smolts, respectively, while W designates fish born in the wild. The F2 returns were allocated to their F1 parents using PASOS 1.0. Allocation was performed for each cohort separately; F2 jacks and age-3 returns from BY 2005 to 2009 were reallocated to their F1 parents, which returned in BY 2003, 2004, 2005 and 2006 (see Fig. 2). Simulations were conducted to estimate the number of missing spawners for each BY of the F1 (see Duchesne *et al.* 2005 for description of simulation and Thériault *et al.* 2007 for an example). The accuracy of our allocations was then assessed via further simulations. Simulated offspring were produced from sampled parents used in this study and from artificially created parents based on the number of missing spawners estimated above. Simulated offspring were then allocated to known sampled parents, and the percentage of correctly assigned individuals was assessed. All the simulations were performed using the simulation procedure implemented in PASOS.

Estimation and comparison of individual reproductive success

Using parentage analysis, we estimated lifetime individual RS of F1 fish that bred in the wild (number of returning F2 mature offspring produced per individual). Mean RS was estimated separately for females, males and jacks as well as for brood stock type ($W \times W$ and $H \times H$) and stocking strategy (unfed fry and smolt). Three years of F1 mating in the wild were available for jacks (BY 2003, 2004 and 2005), and for males and females (BY 2004, 2005 and 2006). For each gender type of fish (males, females and jacks) we estimated relative reproductive success (RRS) in each year by dividing average RS of hatchery fish by average RS of W fish of the same type. The broodstock type ($H \times H$ and $W \times W$) did not seem to affect later reproductive success (see below) so we pooled those two types for the comparison of H vs. W. To test for statistical significance between RS of H and W fish within each year and fish type, we used two-tailed permutation

tests. Numbers of offspring assigned to each parent were permuted 10 000 times and the probability of obtaining a smaller or larger difference than the observed value was evaluated using an algorithm as applied in PERM 1.0 (Duchesne *et al.* 2006). We evaluated the power of our analysis by calculating the minimum difference in RS we could have detected with 80% and 95% probability. The minimum difference was obtained from distribution of RS differences in the permutation tests.

We then used a three-factor ANOVA to test the effects of gender type (females, males, jacks), brood stock type ($W \times W$, $H \times H$) and stocking strategy (unfed fry, smolt), as well as their two and three-way interactions, on the yearly RRS values. Here the data points are the individual RRS values calculated as above. We used the log values of RRS to conform to the homoscedasticity and normality assumptions of the ANOVA.

Results

Parentage analysis

Details of parentage allocation results of F1 fish to the parental generation can be found in Thériault *et al.* (2010). The number of F2 fish (progeny of F1 spawning events) returning each year over the course of this study is shown in Table 1. The proportion of returns (jacks and age-3 males and females combined) that was assigned to a parental pair or a single parent was 76%, 90%, 94% and 97% for BY 2003, 2004, 2005 and 2006 respectively. A few reasons (not mutually exclusive) can explain the unassigned fish: these include genotyping errors, errors in data recording and processing, or failure to collect parents (as a result of individuals straying from another system or spawning below the dam). The correctness rate (i.e. the percentage of fish allocated to the correct parents according to simulations) was 91%, 96%, 96% and 97% for BY 2003, 2004, 2005 and 2006 respectively.

Table 1 Numbers of F1 fish spawning in the wild in brood year (BY) 2003 to 2006 used in parentage analysis and corresponding number of F2 fish returning in BY 2005–2009

F1 hatchery/ wild (BY)	F2 total returns	F2 age-2 jacks returns (BY)	F2 age-3 adults returns (BY)	Not used	Unresolved
66/93 (2003)	616	78 (2005)	538 (2006)	4	150
428/796 (2004)	569	86 (2006)	483 (2007)	8	52
937/625 (2005)	1427	104 (2007)	1323 (2008)	9	87
935/406 (2006)	2057	120 (2008)	1937 (2009)	1	53

The numbers not used correspond to F2 missing genotype information. 'Unresolved' are F2 fish left unassigned by parentage analysis. In BY 2003, F1 are only composed of age-2 males (first F1 age-3 fish returned in BY 2004). In BY 2006, F1 are only composed of age-3 fish.

Relative reproductive success

Point estimates of RRS and a comparison between RS of H[fry] relative to wild fish via permutation tests are shown for each gender-type (jacks, males, females,) in Table 2. For females, RRS was less than 1.00 in all 3 years comparisons (range from 0.76 to 0.91) but none of the comparisons was significant (Table 2, Fig. 3a). For males, RRS was also less than 1.00 in all three years (range from 0.48 to 0.74) and was significant in 2005 and 2006, and for the 3 years combined (Table 2,

Table 2 Reproductive success in the wild of fish stocked as unfed fry relative to wild fish (RRS) for each brood year (BY)

BY	N (fry/wild)	RRS*	P-value	Power [†] (80%/95%)
Females				
2004	26/358	0.91	0.80	0.63 / 0.45
2005	97/352	0.76	0.12	0.81 / 0.70
2006	201/218	0.87	0.21	0.87 / 0.81
Overall female		0.84	0.26	
Males (3-year-old)				
2004	41/384	0.74	0.29	0.80 / 0.61
2005	101/277	0.48	0.001	0.87 / 0.75
2006	172/188	0.68	0.005	0.85 / 0.78
Overall male		0.62	<0.001	
Jacks (2-year-old)				
2003	5/93	2.13	0.30	2.17 / 3.67
2004	7/54	2.09	0.26	2.53 / 11.17
2005	18/66	1.49	0.24	1.55 / 2.10
Overall jack		1.75	0.24	

N is the number of F1 fish that spawned in the wild used in the analysis. P-values are based on two-tailed permutation tests (see Methods for details).

*RRS is calculated as the RS of hatchery fish stocked as fry over RS of wild fish. Overall RRS was estimated using weighted geometric means and the according P-values were calculated on the basis of Fisher's combined probability.

[†]Statistical power represents the minimum effect size (displayed as RRS) detectable with 80% and 95% power.

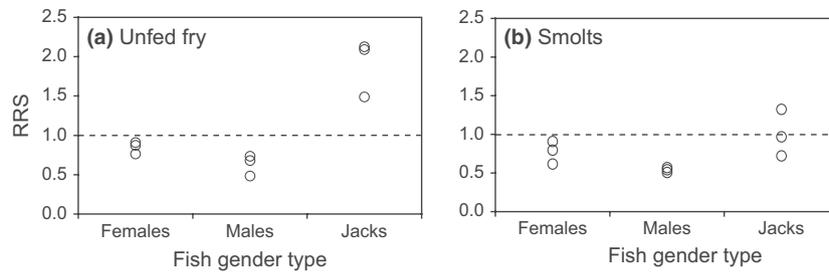


Fig. 3 Relative reproductive success (RRS, reproductive success of F1 fish of hatchery origin relative to wild fish) for each gender type (females, males, jacks) for (a) fish released as unfed fry and (b) fish released as smolts. Fish of $W \times W$ and $H \times H$ origin were pooled as brood stock type had no effect (see 'Results' section). Each point is the yearly RRS estimate. The dotted line represents the value where reproductive success of hatchery fish is equal to reproductive success of wild fish ($RRS = 1$).

Fig. 3a). For jacks, sample size limited the power of our analysis but RRS was greater than 1.00 (range from 1.49 to 2.13) for all comparisons, although not significantly so in any BY (Table 2, Fig. 3a).

Point estimates of RRS and a comparison between RS of H[smolt] relative to wild fish via permutation tests are shown in Table 3. For females, RRS was less than 1.00 for all comparisons (range from 0.62 to 0.91) and was significant in 2005 and 2006, and for the 3 years combined (Table 3, Fig. 3b). For males, RRS was also significantly less than 1.00 in all three years (range from

0.51 to 0.57, Table 3, Fig. 3b). For jacks, RRS ranged from 0.72 to 1.33 and was not significantly different than 1.00 in any comparison (Table 3, Fig. 3b).

If we pool both types of hatchery fish (H[fry] and H[smolt]), their RRS over all three years was significantly less than 1 for females and for males ($RRS = 0.77$ and 0.56 respectively, both p -value two-tailed <0.001). For jacks, RRS was not significantly different than 1 ($RRS = 1.11$, P -value two-tailed = 0.68).

The overall ANOVA testing the effects of gender type, brood stock type and stocking strategy on RRS was significant (F ratio = 3.48 , $P = 0.0051$), with the result clearly driven by the main effect of gender type ($P < 0.001$) (Table 4; Fig. 3). A post hoc Tukey HSD test revealed that RRS for jacks differs from males and females, but that the latter two were indistinguishable at the 0.05 significance level. None of the other main effects or interactions were significant, although the effect of stocking type (H[fry] vs. H[smolt]) was close ($P = 0.065$). Here females and males that were released as unfed fry did slightly better than those released as smolts, and the difference was even larger for jacks

Table 3 Reproductive success in the wild of F1 fish stocked as smolts relative to wild fish (RRS) for each brood year (BY)

BY	<i>N</i> (smolt/wild)	RRS*	<i>P</i> -value	Power† (80%/95%)
Females				
2004	135/358	0.91	0.54	0.82/0.73
2005	361/352	0.62	<0.001	0.88/0.81
2006	295/218	0.80	0.03	0.88/0.81
Overall female		0.75	<0.001	
Males (3-year-old)				
2004	156/384	0.57	0.001	0.83/0.75
2005	287/277	0.54	<0.001	0.87/0.81
2006	267/188	0.51	<0.001	0.88/0.81
Overall male		0.53	<0.001	
Jacks (2-year-old)				
2003	61/93	1.33	0.35	1.48/2.00
2004	63/54	0.97	1	0.47 / 0.21
2005	73/66	0.72	0.35	0.64/0.49
Overall jack		0.94	0.65	

N is the number of F1 fish that spawned in the wild used in the analysis. *P*-values are based on two-tailed permutation tests (see Methods for details).

*RRS is calculated as the RS of hatchery fish stocked as smolts over RS of wild fish. Overall RRS was estimated using weighted geometric means and the according *P*-values were calculated on the basis of Fisher's combined probability.

†Statistical power represents the minimum effect size (displayed as RRS) detectable with 80% and 95% power.

Table 4 Three-way analysis of variance (ANOVA) testing the effect of fish gender type (females, males, jacks), brood stock ($W \times W$, $H \times H$) and stocking strategy (unfed fry, smolt) on RRS (RS of hatchery fish over wild fish). The log values of RRS were used

Term	d.f.	Sum of squares	<i>F</i> ratio	<i>P</i>
Fish gender	2	4.123	13.915	<0.001
Brood stock	1	0.0007	0.005	0.946
Stocking	1	0.553	3.732	0.065
Fish gender \times brood stock	2	0.226	1.528	0.228
Fish gender \times stocking	2	0.540	1.824	0.183
Brood stock \times stocking	1	0.226	1.53	0.228
Fish gender \times brood stock \times stocking	2	0.023	0.078	0.925

(Fig. 4). Interestingly, H fish of the two brood stock types ($H \times H$ vs. $W \times W$) were indistinguishable ($P = 0.946$).

Discussion

Lower fitness of hatchery fish in the wild

The first important result of this study is the finding that age-3 hatchery coho exhibit a lower fitness in the wild than wild coho. This result confirms the phenomenon documented for steelhead (Araki *et al.* 2007), which is the only other published study that estimated lifetime reproductive success in the natural environment for local-origin hatchery brood stock. Thus, for both steelhead and coho, only a single generation of integrated hatchery rearing (i.e. where local wild-born brood stock was used for supplementation) is sufficient to decrease the RS of those hatchery fish in the wild. Surprisingly, we observed no difference between hatchery fish of $H \times H$ parents and those of $W \times W$ parents. Thus, the additional generation of hatchery rearing had minimal effect, which is in stark contrast to results from steelhead in which the fitness of second-generation hatchery fish was much lower than that of first-generation fish (Araki *et al.* 2007).

The second major finding of this study is that although the RS of age-3 hatchery fish released as unfed fry was slightly higher than that of hatchery fish released as smolts (Fig. 4), it was still substantially less than that of the wild fish. This trend was seen in both age-3 males and in females (Fig. 3; Tables 2 and 3). We hypothesized that if selection or environmental effects of hatchery rearing occurred during the captive juvenile phase, then hatchery fish released as unfed fry would have similar RS to that of wild fish, with both having higher RS than hatchery fish released as smolts. These results, therefore, suggest that the causal mechanism for lowered fitness is not associated only with the juvenile stage of the life cycle, but also involves some effect during the adult mating phase or during incubation of eggs or newly hatched fry (see also Berjikian *et al.* 2009).

Where in the life cycle?

Incubation and homing. Given these data that, in part, point away from the juvenile phase, it is worth reconsidering other potential stages in the life cycle that make hatchery and wild fish different. First, the incubation environment was different (i.e. hatch box/tray vs. natural gravel bed). Kihlsinger & Nevitt (2006) found a larger cerebellum (part of the brain involved in spatial recognition and social learning) for emerging fry that were incubated in tanks with structural complexity (stones) relative to fry incubated in tanks without stones. Thus, it is possible that some environmental effects experienced as early as incubation can initiate a developmental trajectory that facilitates survival in the local environment. How this might translate into reproductive success differences later in life is still unclear.

Second, our study did not take into account individuals straying to rivers other than the Calapooya. If the straying rate was higher for the wild-born descendants of F1 hatchery fish (the F2s) when compared to descendants of wild-born fish, then, this could bias our results and explain the observed lower RS. However, it is unclear why hatchery F2s would have a higher straying rate because they were reared under the same environmental conditions as their wild counterparts where olfactory learning and imprinting take place (Dittman & Quinn 1996). Moreover, these fish are not from a highly domesticated strain, where many generations in captivity could potentially alter homing abilities, but rather from an integrated hatchery program designed to minimize domestication selection. The lower RS of F1 hatchery fish could be associated with the result of fine scale homing to spawning locations where they were released within the Calapooya River if those locations are of poorer quality than locations where wild fish spawn. While information on spawning locations is lacking, that hatchery and wild fish frequently mated with each other (data not shown) suggests they are actually abundant on the same spawning grounds.

Breeding stage. The other portion of the life-cycle that is shared between unfed fry and smolt releases is the

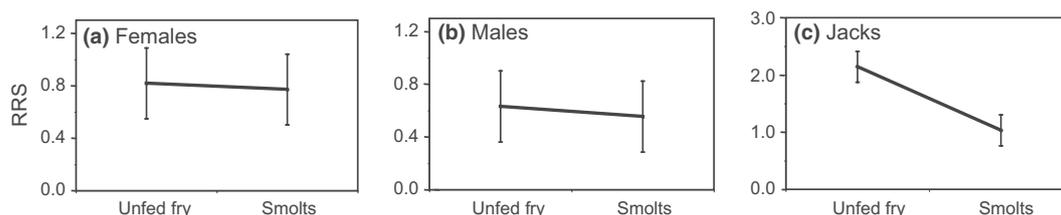


Fig. 4 Relative reproductive success (RRS, reproductive success of F1 fish of hatchery origin relative to wild fish) for fish released as unfed fry and fish released as smolts for (a) females, (b) males and (c) jacks. Each point represents the least squares means over 3 years. Error bar represents one standard error.

breeding stage. Hatchery breeding, where there is no opportunity for mate choice, intra-sexual competition, redd construction, or any other type of natural breeding behaviour, differs substantially from breeding in the wild (Wedekind 2002). During the course of this study, fish released as unfed fry and as smolts both returned to the river earlier than wild fish (Thériault *et al.* 2010). The difference in return time was attributable to the unintentional collection of earlier spawners that were used as brood. An earlier run time (a life history trait shown to be heritable in salmon; Carlson & Seamons 2008) could be associated with a lower RS providing a possible explanation for the observed lower RS of hatchery fish. However, linear and quadratic relationships between run time and RS (data not shown) were not significant or consistent over years suggesting that the magnitude of the difference in RS could not be explained by the unintentional selection of hatchery brood.

Although we cannot entirely exclude environmental effects that could happen as early as the incubation period (see above), we suggest that the absence of sexual selection, owing to artificial breeding in the hatchery, is a plausible explanation for some of the lower RS observed in fish released both as unfed fry and as smolts. In particular, three pieces of evidence in our dataset implicate selection (or lack thereof) on adult reproductive traits as a contributing cause of fitness declines in hatchery fish: (i) unfed fry release have a lower RS than wild fish (discussed above), (ii) males consistently show a more severe decrease in RRS than females, and (iii) jacks show striking divergence in RRS from age-3 males.

We observed that 3-year-old hatchery males averaged 56% of the RS of wild fish, which was 21% less than that observed in females (77%). Studies on reproductive behaviour have found hatchery fish, particularly males, to be competitively inferior to wild fish, showing less aggression and more submissive behaviour (Fleming & Gross 1993; Fleming *et al.* 1996; Berejikian *et al.* 2001). This competitive disadvantage translated into lower RS, with males more severely affected than females (Fleming & Gross 1993; Fleming *et al.* 1996). Competition among males for spawning females is usually more intense than intra-sexual female competition for spawning territories and nest defence (Fleming & Gross 1992; Quinn & Foote 1994). Thus, intense selection for breeding opportunities among males may magnify the differences in behaviour (and consequently breeding success) between hatchery and wild fish. By circumventing sexual competition that takes place on the natural spawning grounds, artificial breeding could allow less competitively fit fish to reproduce (e.g. less aggressive and more submissive fish that would otherwise be

evicted by dominant males). Agonistic behaviour has been shown to have a significant additive genetic component in salmonids, and can thus be inherited (Rose-nau & McPhail 1987; Swain & Riddell 1990; Vollestad & Quinn 2003). Therefore, the offspring resulting from artificial mating in the hatchery could show an inherited behaviour that subsequently proves to be less fit in a natural spawning context.

Hatchery jacks did not show the same fitness decline as age-3 hatchery males, suggesting that the absence of natural selection or inadvertent artificial selection on adult reproductive traits was a causal mechanism for reduced RS of hatchery fish in the wild. Jacks favour a different reproductive tactic – they stay in the periphery of the dominance hierarchy established by older males and sneak into the nest at the time of egg deposition (Gross 1985). Despite the low sample size for jacks, the pattern is very different than what we found for age-3 males. The mechanism that is causing the discrepancy in fitness between wild and hatchery age-3 males does not seem to affect jacks in the same way or as severely. Interestingly, a similar result has been observed in hatchery Atlantic salmon. Hatchery adult males have been found to have a poor fertilization success relative to wild males due to their reduced aggressiveness (Fleming *et al.* 1996) while hatchery precocious parr (males that mature younger without seawater migration) were found to be more successful, in part because they showed more aggression and dominated their wild counterparts (Garant *et al.* 2003). These results suggest that the relative level of aggression can change with life-stage and age (e.g. Berejikian *et al.* 1996) or that the genetic basis of reproductive behaviour differs between the two life-histories. Another explanation for our observed discrepancy between jacks and age-3 males could be related to the proximate mechanisms leading to the adoption of alternative mating tactics. Jacking is associated with higher growth rates as juveniles (Vollestad *et al.* 2004). Higher growth rate is often linked to higher level of aggression through the increased level of growth hormone (Jönsson *et al.* 1998; Fleming *et al.* 2002). Therefore, if we assume that the lower RS of hatchery age-3 males is due to their reduced competitiveness and aggressiveness, it is possible that this does not show up in jacks because they are among the faster growers, and hence more aggressive fish of their cohort.

Summary

Here we have shown that age-3, first-generation hatchery coho have lower RS than wild fish, with age-3 males doing worse, on average, than females. Furthermore, the same effect was observed in hatchery fish that were released as fry and in those released as smolts.

Interestingly, hatchery jacks did at least as well as wild fish in all comparisons. Finally, we observed no effect of broodstock type ($H \times H$ vs. $W \times W$) on the performance of hatchery fish, which suggests that the additional year(s) of captive rearing in the hatchery had minimal effect. The actual causal mechanism(s) responsible for the fitness decline of hatchery fish are currently unknown. Although our study only provides indirect evidence, we suggest that differences in spawning behaviour resulting from the absence of sexual selection through artificial breeding may contribute to the problem. It is not clear whether this potential mechanism would apply to all salmonids and all systems or could be extended to non-salmonids. Furthermore, selection on juvenile traits could still occur in concert with relaxation of selection on adult behaviours, especially in hatchery programs where fish are grown quickly to a size at age suitable for migration, a treatment that does not necessarily correspond to their natural life-cycle (for example steelhead, where fish are commonly released at age-1 despite the age-2 migration pattern typical in nature).

Our findings have important management and conservation implications. We suggest that releasing unfed fry may not be the panacea for circumventing negative hatchery effects. On the one hand, released unfed fry do survive to return as adults to the wild populations, and they are phenotypically more like wild fish as adults than those hatchery juveniles released as smolts (Caroffino *et al.* 2008; Thériault *et al.* 2010). But on the other hand, our results indicate that fish released as unfed fry also exhibit reduced RS once they return as adults to reproduce in the wild. If that reduced RS has a genetic origin and is carried over generations (e.g. Araki *et al.* 2009), then even unfed fry releases have the potential to negatively impact wild populations.

Finally, our results support the finding outlined by others that even contemporary hatchery practices (e.g. using wild brood stock, pairwise matings) can produce fish that have lowered reproductive success in the wild. This evidence suggests that hatcheries may need to consider how to replicate the intricacies of natural breeding behaviours if they are to produce fish for supplementation programs that truly help recover endangered populations.

Acknowledgements

Field support was provided by D. Meyer, J. Muck, L. Jackson and their staff and volunteers (Oregon Department of Fish and Wildlife). Funding was provided by the Oregon Watershed Enhancement Board to M.A. Banks and V. Thériault and by the Umpqua Fishery Enhancement Derby, ODFW Restoration and Enhancement Program, and the Oregon Wildlife Heritage

Foundation. We thank R. Bellinger, D. Jacobson, M. Moyer, and E. Murphy who provided laboratory assistance, S. Hampton for providing Umpqua basin map, and Oregon Department of Fish and Wildlife (Roseburg) for providing the Calapooya Creek release sites map. We would also like to thank K. Kostow who was instrumental in the initial discussions and design of this project. P. Duchesne provided helpful advice and comments on the parentage analysis procedure, and we thank A. Bass for valuable suggestions improving previous versions of this manuscript.

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