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ARTICLE

Duration in captivity affects competitive ability and breeding success of male but not female steelhead trout (*Oncorhynchus mykiss*)

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Abstract: Female steelhead trout (*Oncorhynchus mykiss*) reared for 1 year (traditional, “S1”) and 2 years (experimental, “S2”) in captivity in the Methow River Basin, Washington, produced very similar numbers of offspring in replicate spawning channels over 3 consecutive years. In contrast, S1 anadromous males outcompeted S2 males for access to nesting females and, as a result, produced significantly more offspring. Male dominance was positively associated with body mass, but body mass did not account for differences between S1 and S2 males. Much smaller precocious S2 males that matured during rearing in hatchery raceways sired offspring in all six breeding groups. Contributions from precocious males were nearly always the result of sneak spawning events, although on rare occasions, precocious males were able to stimulate females to spawn in the absence of an anadromous male. Similarities in female breeding success suggest that S1 and S2 hatchery steelhead should exhibit similar productivity under natural conditions, but S1 male steelhead may result in greater rates of gene flow from hatchery to natural populations.

Résumé : Des truites arc-en-ciel (*Oncorhynchus mykiss*) femelles élevées pendant 1 an (traditionnelles, « S1 ») et 3 ans (expérimentales, « S2 ») en captivité dans le bassin de la rivière Methow (État de Washington) ont produit des nombres très semblables de rejetons dans des chenaux de frai répliqués sur 3 années consécutives. En comparaison, des mâles anadromes S1 ont eu le dessus sur des mâles S2 pour l’accès à des femelles génitrices et, par conséquent, ont produit significativement plus de rejetons. La domination des mâles montrait une association positive avec la masse corporelle, mais cette dernière n’explique pas les différences entre les mâles S1 et S2. Des mâles S2 précoces beaucoup plus petits arrivés à maturation durant l’élevage dans des bassins piscicoles ont produit des rejetons dans les six groupes de reproduction. Les contributions des mâles précoces étaient presque toujours le résultat d’évènements de frai furtifs, bien que, en de rares occasions, des mâles précoces soient arrivés à entraîner des femelles à frayer en l’absence d’un mâle anadrome. Des similitudes du succès de reproduction des femelles indiqueraient que les truites arc-en-ciel S1 et S2 d’élevage devraient présenter des productivités semblables dans des conditions naturelles, mais que les truites arc-en-ciel mâles S2 pourraient produire de plus grands taux de flux génétique de populations d’élevage vers des populations naturelles. [Traduit par la Rédaction]

Introduction

The widespread use of artificial propagation in conservation and management of anadromous salmonids includes efforts to reintroduce species to extirpated habitats (Galbreath et al. 2014; Kock et al. 2018), re-establish populations that have been altered by transfers from non-local populations, and maintain imperiled populations as part of larger population recovery efforts (NOAA Fisheries 2015). Regulating or at least accounting for gene flow between artificially propagated (hereinafter “hatchery”) and natural populations has become a prominent management process in the Pacific Northwestern United States (Paquet et al. 2011; Baskett et al. 2013; Waters et al. 2015). However, the degree of interbreeding between hatchery and natural populations may be difficult to predict and manage because of substantial intraspecific variation in reproductive success of hatchery-origin salmon (e.g., Williamson et al. 2010; Hess et al. 2012). Thus, understanding the influences of rearing practices, especially novel or experimental approaches, on reproductive phenotypes, behavior, and breeding success is critical for effectively managing hatchery systems and interactions with natural populations.

Variation in adult behavioral traits and breeding success resulting from alternative hatchery rearing strategies have not been assessed experimentally. A myriad of physiological, behavioral, and morphological changes in cultured salmonids can be influenced by feed delivery systems (Fast et al. 2008), structural enrichment (Johnsson et al. 2014), conditioning for predator recognition (Berejikian et al. 1999), water flow manipulation (Hoffnagle et al. 2006), and other factors. Hatchery programs intended to augment fisheries have long implemented feeding regimes, targeting rapid growth rates to achieve large body size and low size variation at release. Growth modulation to achieve specific size targets

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through intentionally varied feed rations has been shown to result in more natural size or age-at-release and ameliorate the unnatural precocious maturation caused by high feeding rations (Larsen et al. 2006; Harstad et al. 2014). Among the numerous adult behavioral traits that can influence reproductive success (Essington et al. 2000; Seamons et al. 2007; Anderson et al. 2010), variation in aggression and competitive ability may respond directly to altered hatchery rearing environments (Ryer and Olla 1995) and ultimately influence reproductive success in cultured populations.

Body size plays an important role in structuring mating patterns, reproductive tactics, and determining individual breeding success in salmonids (Quinn and Foote 1994; Schroder et al. 2010; Seamons and Quinn 2010). Large female body size may confer advantages in competition for nesting sites (Berejikian et al. 1997), greater fecundity (Knudsen et al. 2008), depth of egg burial (Steen and Quinn 1999), and possibly attractiveness to mates (Maekawa et al. 1994). Salmonid mating systems ubiquitously involve intense male intrasexual competition for primary (closest) access to mates, which maximizes fertilization success during spawning events involving multiple males (Neff et al. 2015). Male body mass can vary by several orders of magnitude in some species (Larsen et al. 2004; Fleming 1996; Kendall et al. 2015), and the alternative reproductive tactic of "sneak spawning" by younger and much smaller males functions to maintain important life history variation (Dodson et al. 2013).

Natural populations of *Oncorhynchus mykiss* exhibit partial anadromy with an anadromous form (i.e., steelhead) and a nonanadromous (i.e., "resident") form that is typically dominated by males that mature without migrating to sea (Kendall et al. 2015). Summer-run steelhead smolts in the Columbia River Basin east of the Cascade Range mountains predominantly migrate to sea at age-2 or age-3 (Peven et al. 1994) in the spring and return during summer months after one or two ocean winters. Pedigree studies conducted in natural steelhead trout populations fail to assign large proportions of offspring to potential anadromous male parents, inferring substantial contributions from nongenotyped resident male parents (Seamons et al. 2004; Christie et al. 2011). In coastal populations, smaller, resident males also attempt to spawn with nesting anadromous females, particularly later in the mating season (McMillan et al. 2007), and resident male contributions have also been inferred from other genetic methods (Kuligowski et al. 2005).

Accommodating conservation objectives in hatchery programs often involves collecting and spawning natural-origin adults (e.g., Araki et al. 2007; Tatara et al. 2017), which spawn later in the year than traditional hatchery brood stocks that have been selected for earlier run and spawn timing (Crawford 1979). The later spawn timing and reduced growth opportunity has necessitated the development of new rearing strategies to induce smoltification, migration, and higher survival rates (Tatara et al. 2017). Further, modulating growth for a more natural age and size at smoltification and release (age-2) may reduce size-selective mortality after release (Berejikian et al. 2017) to lessen a key driver of domestication selection (a potential cause of fitness loss in hatchery steelhead (Araki et al. 2008). However, age-2 smolt rearing appears to promote precocious male maturation (Tatara et al. 2019). Thus, gene flow from hatchery to natural populations may depend on the breeding success of anadromous males returning from the ocean at different ages and sizes and the contributions of precociously maturing males (hereinafter "precocious males"). The primary objectives of the present study were to (*i*) compare the breeding success of anadromous female and male steelhead reared and released as age-1 (hereinafter S1) or age-2 (S2) smolts, (*ii*) quantify the breeding success of S2 precocious males, and (*iii*) understand the mechanisms influencing breeding success of all phenotypes.

Table 1. Mean fork length (FL, mm) and mass (kg) of adult steelhead trout stocked into two spawning channels in each of the 3 years.

		S1					S2				
Year	Channel No.	*n*	FL (mm)	SD	Mass (kg)	SD	*n*	FL (mm)	SD	Mass (kg)	SD
Females											
2015	1	6	650	38	2.73	0.43	5	682	47	3.27	0.53
	2	5	640	76	2.50	0.81	7	669	69	2.63	0.84
2016	1	6	630	43	2.25	0.47	6	626	33	2.16	0.37
	2	6	594	56	2.02	0.50	6	613	63	2.25	0.60
2017	1	7	706	26	3.39	0.42	5	688	22	3.33	0.50
	2	**7**	707	35	3.23	0.51	5	682	34	2.99	0.37
Overall mean		36	657	61	2.73	0.72	36	661	54	2.75	0.70
Males											
2015	1	8	615	86	2.22	0.97	3	600	43	2.07	0.45
	2	8	662	106	2.69	1.21	3	506	30	1.23	0.24
2016	1	6	657	74	2.38	0.72	7	640	31	2.16	0.42
	2	5	608	24	2.08	0.34	6	618	31	2.01	0.33
2017	1	9	700	100	3.15	1.07	3	700	134	3.07	1.28
	2	10	721	104	3.28	1.05	2	725	7	3.30	0.01
Overall mean		46	667	96	2.72	1.04	24	627	76	2.20	0.76

Note: The rearing histories (i.e., released as age-1 (S1 smolts) or age-2 (S2 smolts)) of adult steelhead carrying PIT tags was known before stocking. The rearing history of non-PIT-tagged adults could not be known for certain and were presumed to have come from the S2 rearing group if their dorsal fin showed signs of bent or slightly shorter fin rays, caused by fin erosion during rearing, which was more common in the S2 group. Postmortem, coded-wire tags were decoded, and determinations based on fin morphology were either verified or corrected to determine S1 or S2 origin. Sixteen of the S1 males used in the study were from the Washington Department of Fish and Wildlife Methow Hatchery (MH) located ~2 km from the Winthrop National Fish Hatchery (WNFH). Because these fish were from the same genetic stock (see text) and reared for the same duration and under similar conditions, we did not discriminate between WNFH and MH S1 steelhead in our analyses.

Methods and materials

Spawning environment and experimental design

The experiment was conducted at the US Fish and Wildlife Services Winthrop National Fish Hatchery (WNFH; Winthrop, Washington, USA). Two spawning channels were stocked with S1 and S2 adult steelhead in each of 3 consecutive years (2015–2017), creating six independent breeding groups. Each spawning channel was an oval shape, measuring 23 m long by 5 m wide (1.9 to 2.7 m wide on either side of a center dividing wall) and providing a total of ~100 m² of available spawning habitat. Water depth ranged from 20 to 70 cm to provide a range of nesting habitat and holding areas. Each channel contained a mix of various gravel sizes ranging from 1 to 6 cm in diameter. A mix of spring water and water from the Methow River was supplied at a rate of 1135 L·min^{-1}. An airlift pump system was used and created a partially recirculating flow pattern within the spawning channels (~3300 L·min^{-1}).

The planned protocol was for each breeding group to comprise 12 females and 12 males (six from each rearing treatment) along with six S2 precocious males. However, actual composition was naturally constrained by the availability of returning adults from the different groups and our success at nonlethally determining the rearing history (S1 or S2) of individual adult steelhead upon collection. Initial determinations based on dorsal fin shape (more irregularly shaped in S2 fish) were either verified or revised after spawning was complete and the fish had died, by decoding of PIT tags and coded-wire tags (82% of determinations) or scale pattern analysis for nontagged fish (18%). Ultimately, individual breeding groups contained between 11 and 13 females, between 11 and 13 anadromous males, and always six precocious males (age-2) from the hatchery (72 females and 106 males total; Table 1). The numbers of fish stocked per channel were planned to provide ample space for females to spawn without necessitating (but not

Table 2. Mean fork length (FL, mm) and mass (kg) of precocious male (S2 rearing) steelhead trout stocked into two spawning channels in each of the 3 years of the study.

Year	Channel No.	n	FL (mm)	SD	Mass (kg)	SD
2015	1	6	220	15	0.14	0.04
	2	6	235	18	0.14	0.02
2016	1	6	191	12	0.08	0.01
	2	6	196	19	0.09	0.03
2017	1	6	188	13	0.09	0.02
	2	6	182	18	0.07	0.02
Overall		36	202	24	0.10	0.04

prohibiting) nest superimposition. The number of S2 precocious males was selected to ensure the potential to participate in all spawning events (assuming not more than six females would be active at one time), but not so many that their frequency would be unrealistically high. No S1 precocious males were introduced to the spawning channels because of their very low prevalence in WNFH release groups; 2% of precocious males were from the S1 release groups and 98% from the S2 release groups (Tatara et al. 2019). The fairly low number of adult steelhead stocked in each channel necessitated that the experiment be replicated within years and conducted over 3 years to create six independent breeding groups. All fish were removed after the last female had completed spawning; most died after spawning.

Fish collection and tagging

Adult steelhead were collected from traps at the WNFH and by hook-and-line in the upper Methow River above its confluence with the Twisp River. The adult steelhead collected for use in this study were predominantly reared at the WNFH (see exceptions in Table 1). Adult steelhead released at age-1 (S1) were spawned at the Wells Dam Hatchery below Wells Dam on the Upper Columbia River. Eggs were transported to the WNFH for rearing and release. Adult steelhead released at age-2 (S2) were incubated, reared, and released from the WNFH and collected as adults when they returned to the upper Methow River Basin. Both the S1 and S2 adult steelhead used in the study were produced from brood stock (parents) that were a mixture of hatchery and natural origins. Genetic analysis of adult steelhead in the Methow River Basin using 936 single nucleotide polymorphisms (SNPs) generated by restriction site associated DNA sequencing (RAD-Seq) revealed no differentiation between hatchery and natural-origin fish at a population level (Gavery et al. 2018).

From the time of capture until being introduced into the spawning channels, adult steelhead were held in a 25 m long × 3.3 m wide × 1.6 m deep covered raceway at the WNFH receiving 1135 $\text{L}\cdot\text{min}^{-1}$ water. All age-2 precocious males, collected directly from the raceways at the WNFH in which they had been reared, were identified by external body coloration and the expression of milt with slight pressure to the abdomen. Prior to stocking fish into the spawning channels, each was weighed (nearest 1.0 g), measured for fork length (nearest 1.0 mm), and marked with an individually numbered Petersen disc tag (Tables 1 and 2). Precocious males were marked with colour-coded 16 mm Petersen disk tags. A small piece of fin tissue was removed from each adult fish and preserved in 100% ethanol for subsequent DNA pedigree analyses.

Spawning behavior observations

Observations of spawning activity were conducted from dawn to dusk each day from behind visual blinds that minimized disturbance of the fish. We conducted focal sampling of male and female activity surrounding nest-digging females. Each focal observation continued until spawning occurred, the courtship terminated, or it became too dark to see. The goal of the focal observations was to determine which females were sexually active and to determine male dominance hierarchies, male participation in spawning events, and rank order of nest entry at the moment of spawning. For focal observations that culminated in a spawning event, we documented the order of male nest entry during spawning (i.e., which male entered first, second, third, or fourth as the female began depositing eggs). Sperm precedence is an important determinant of male fertilization success (Hoysak et al. 2004). Male order of nest entry was simply the order in which each male entered the nest pocket and aligned with the female or alongside another male already aligned with the female. Spawning events were confirmed by the presence of eggs in the center of the developed nest pocket, followed immediately by high-frequency nest-covering digs (Berejikian et al. 2007).

Scans of male and female activity were also conducted several times each day. A scan included a walk around the perimeter of a spawning channel and recording the status of every fish observed. Each female was observed until we could determine whether it was inactive, wandering (swimming around without any obvious intent or interaction with other fish), nest building, nest covering, or guarding a nest site. Encountered males were categorized as inactive, wandering, holding close to a non-nesting female, courting a female, or satellite. We used these observations to determine male dominance hierarchies and spawning participations (see below).

Male dominance hierarchies

To provide an overall measure of dominance for each S1 and S2 male, we used data collected during both focal and scan sampling observations within each spawning channel. Aggressive encounters between any two males were recorded, including the giver and receiver of attack (chasing and biting) and whether or not the receiver retreated in response to the attack. A winner and loser were determined when an attack (winner) resulted in a retreat (loser). To minimize the potential for unintentional bias caused by different observation duration (some focal observation lasted many hours, while others just a few minutes), we limited the number of wins and losses to one win–loss per pair for each type of observation for each day. That is, any given pair of males had a maximum of two outcomes per day (one from scan sampling and one from focal sampling). Wins and losses for each pair were summed over all observations days, and we only recorded wins and losses when one fish retreated (loser) in response to an attack by another (winner). We calculated “David’s scores” for each fish (David 1987):

$$\text{DS}_i = w_i^1 + w_i^2 - l_j^1 - l_j^2$$

where i is individual winning, j is individual losing, w_i^1 is the sum of i’s proportion of wins against all competitors, and w_i^2 is the sum of w_i^1 values weighted by the w_i^1 values of each competitor. The parameters $l_j^1 - l_j^2$ represent the same calculations for losses by the corresponding j individuals.

Offspring sampling and pedigree analysis

Offspring were collected from each channel after they had emerged from the gravel nests, beginning ~700 accumulated temperature units (mean daily temperature (°C) × number of days) after the final spawning event. Thus, offspring were collected between late June and mid-July. Offspring were collected by seining each channel to depletion (three occasions of equal seining effort). Fry were enumerated using batch weights by dividing the total weight of fish caught during each sampling occasion by the average of 30 individual fry weights. The total number of offspring produced in each channel was estimated with catch–effort models (Leslie and Davis 1939) using the software program Ecological Methodology. All offspring collected during the three seining events were pooled and held in two 1.5 m diameter tanks prior to subsampling offspring for pedigree analysis. All fry were removed from the holding tanks and euthanized with a lethal dose (300 ppm) of buffered tricane methanesulphonate (MS-222, Western Chemical, Ferndale, Washington, USA). Offspring for pedigree analysis (700 per spawning channel) were randomly selected with equal representation from each holding tank, and small tissue samples were stored in microcentrifuge tubes filled with 100% ethanol.

DNA-based pedigree analyses were performed to assign juvenile steelhead to single-pair matings. The approach followed the methods described in Berejikian et al. (2010). Briefly, adults were genotyped for a sufficient number of microsatellite loci to assign potential offspring to single pair matings with >99% certainty. DNA was isolated from fin tissues and subjected to polymerase chain reactions (PCRs) to amplify a sufficient number of loci to assign individuals with a high likelihood of correct assignment. A fragment analysis was conducted on PCR products using a genetic analyzer to determine the genotypes of every individual for each locus. A pedigree analysis was conducted by using an exclusionary approach, because genotypes for all parents were available (i.e., there were no missing parents). All offspring were assigned to two parents.

Female data analyses

One-way analysis of variance (ANOVA) was used to test for differences in mean body mass between S1 and S2 females within each breeding group. To estimate the ability of individual females to deposit eggs before death, we subtracted the number of ovulated eggs remaining in a female's body cavity at death from her estimated fecundity. Fecundity of the females stocked into the channels was estimated by least-squares regression of body mass versus fecundity for 86 females artificially spawned at the WNFH in 2014 and 2015 (C. Pasley, USFWS, unpublished data).

The primary measure of breeding success was the number of offspring produced per parent as determined by the pedigree assignments. Two-tailed paired *t* tests were used to compare breeding success between S1 and S2 females. Thus, each breeding group was considered the experimental unit of replication, and the difference in breeding success of S1 and S2 females within each group were paired for analyses. We also quantified the number of different mates and calculated the number of offspring per mate to better understand variation in breeding success. Mean values for each of these variables were compared by the same approach (paired *t* tests, $N = 6$).

Relationships between female body mass and the aforementioned measures of breeding success were tested by least-squares linear regression analysis within each breeding group. Fisher's combined probability tests were used to combine results from the six breeding groups where correlation coefficients within each breeding group were either all positive or all negative. The test statistic $\hat{P}$ (chi-square-distributed) was calculated as ($\hat{P} = -2\sum_i^n \ln P$), where P equaled the probability within the ith breeding group (Sokal and Rohlf 1996, Schroder et al. 2010).

Male data analyses

One-way ANOVA was used to test for differences in mean body mass between S1 and S2 males in each of the six breeding groups. Two-tailed paired *t* tests were used to compare breeding success measures (offspring per male, number of mates) in the same manner as for females. Significance of the pairwise tests for the three groups of males (S1, S2, and precocious males) were determined based on Bonferroni corrected *p* values. Likewise, pairwise comparisons of behavioral measures of male competitive ability (dominance score) and spawning success (order of nest entry at time of spawning) were also made among S1, S2, and precocious males.

Table 3. First and last documented spawning events by female S1 and S2 steelhead.

	S1			S2		
Year: channel	First spawn	Last spawn	Mean duration	First spawn	Last spawn	Mean duration
2015: 1	2 Apr.	15 Apr.	4.3 (1.2)	7 Apr.	20 Apr.	2.8 (1.3)
2015: 2	23 Apr.	8 May	2.0 (0.8)	23 Apr.	1 May	3.7 (2.4)
2016: 1	14 Apr.	25 Apr.	3.2 (1.5)	14 Apr.	25 Apr.	3.1 (1.9)
2016: 2	21 Apr.	4 May	3.0 (1.9)	21 Apr.	4 May	3.3 (2.1)
2017: 1	20 Apr.	1 May	2.7 (1.9)	20 Apr.	30 Apr.	2.3 (2.3)
2017: 2	13 Apr.	20 Apr.	2.7 (0.8)	17 Apr.	29 Apr.	2.3 (2.6)

Note: The mean (SD) spawning duration (last–first) for individual females is also shown. Note that only daytime spawning events could be observed, and some were obscured by turbulence in the spawning channels, so spawning durations and ranges are likely underestimated.

The significance of relationships between body size and several response variables, including breeding success, male competitive ability (David's score), and male participation in spawning events, were analyzed by least-squares linear regression. Analyses were conducted within each breeding group to assess the effects of body size relative to competitors. We made the simplifying assumption of no interaction between body size and rearing treatment because of the small numbers of individuals within a treatment in some breeding groups. Thus, data from S1 and S2 males were combined to test the effects of body size on the response variables. Fisher's combined probability tests were used to determine the significance of unidirectional relationships in the same manner as previously described for analyses of female breeding success. The number of offspring produced per male were transformed ($\ln(y + 1)$) to improve normality, equalize variances, and linearize relationships with the independent variables, with one exception. The relationship between spawning participations as the first male to enter the nest at the time of spawning and offspring produced per male was linear and did not require a transformation. All statistical analyses were performed in Systat version 13. Significance level was set at $\alpha = 0.05$ for all tests.

Results

Female breeding success

A combined 160 spawning events were observed during daylight hours from the six separate breeding groups. Female spawning activity of all six breeding groups ranged from the earliest spawning date of 2 April (2015, channel 1) through the last spawning date of 8 May (2015, channel 2). Females were observed to be sexually active (duration between first spawning event to last spawning event) for about 3 days (Table 3), but this is likely an underestimate because we did not observe all spawning events.

The number of offspring per spawning female did not differ between S1 and S2 females ($T = -0.95$, $p = 0.384$; Fig. 1) and neither did the mean number of different mates per female (S1 = 4.45 mates; S2 = 4.1 mates; $T = 1.14$, $p = 0.31$). Female body mass was positively correlated with breeding success (number of offspring produced) in two of the six breeding groups ($p < 0.05$), two were positive but not significant ($p > 0.05$), and the coefficient was negative (nonsignificant) in two others. Thus, there was no combined probability test, and we did not conclude there was a significant positive association between female body size and breeding success. Five of the six breeding groups had positive correlations between female body mass and the number of mates, one was negative, and none were significant, so there was no combined probability test, and we did not conclude there was a significant association. However, five of six positive associations suggest that female body size may have influenced the number of mates, but may have been obscured by a spurious association between the two variables in one of the channels.

Fig. 1. The mean number (±SD) of offspring produced per individual S1 (white bars) and S2 (shaded bars) females (panel A) and males (panel B), including S2 precocious males (striped bars) for each of the six breeding groups (700 offspring analyzed per channel).

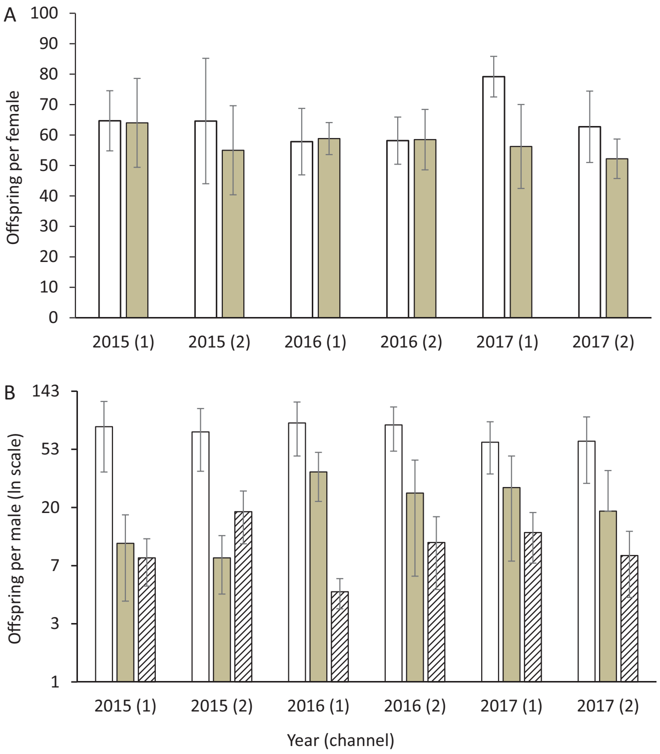


Fig. 2. The total number of offspring produced in each of the six breeding groups (year and channel number shown below each bar). Fry population abundances were estimated using a Leslie regression model with small sample confidence limits. The thick black lines above bars represent the total fecundity (see text) of all females stocked into each channel.

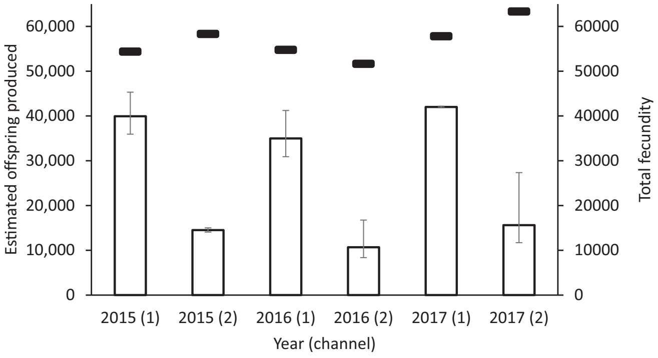


Table 4. Results of paired *T* tests comparing measures of breeding success among the three types of males (*p* values are Bonferroni-corrected) for the six independent breeding groups.

	S1–S2		S1–precocious		S2–precocious	
	T	*p*	*T*	*p*	*T*	*p*
Pedigree analyses						
Offspring per spawner	9.50	**0.001**	12.07	**0.000**	1.85	0.371
No. of mates	8.52	**0.001**	7.53	**0.002**	−0.72	1.000
Offspring per mate	3.49	0.344	8.66	**0.027**	5.17	0.504*
Behavioral measures						
Dominance score	4.54	**0.006**	—	—	—	—
First to enter	2.62	**0.006**	2.82	**0.009**	0.20	1.000
Total participations	2.96	**0.002**	0.84	0.341	−2.13	**0.009**

Note: There was no test for dominance score involving precocious males because they were always submissive to anadromous males. The asterisk (*) indicates that the significance ($\alpha = 0.05$) of the *T* test changed when excluding the 2015 (channel 2) breeding group and considering the five breeding groups in which S1 and S2 mean body size was very similar. The significance of all other tests remained unchanged. For all three comparisons, the directionality of the *T* statistic is first group minus second group.

The mean (±SD) percentage of eggs deposited over all breeding groups was similar for S1 (98% ± 0.04%) and S2 (97% ± 0.03%) females. The estimated total number of offspring produced in stream channel 1 was consistently very high, ranging from 34 979 to 42 007 over the 3 years (Fig. 2), and consistently much lower in channel 2, ranging from 10 683 to 15 610 (Fig. 2). Thus, some unknown physical characteristic of the two measurably similar spawning channels affected survival of deposited eggs in a similar manner each year.

Male breeding success and influence of opportunity (nest entry)

S1 males produced significantly more offspring per spawner than S2 males and more than precocious males, and there was no significant difference in offspring produced between S2 males and precocious males (Table 4; Fig. 1). Over all breeding groups combined, individual S1 males sired an average of 10.1% of the sampled offspring, S2 males sired an average of 3.5%, and precocious males sired an average of 1.5% (Fig. 1). Among the six breeding groups, the average number of offspring per S2 male ranged between 11.6% and 46.1% that of S1 males (29.4% overall). Precocious males produced between 5.6% and 25.5% as many offspring per male as S1 males (14.6% overall) and between 12.9% and 220.0% as many offspring as S2 males (74.6% overall).

Sixty-nine of the 160 observed spawning events (43%) involved participation by just one male. Two males participated in 45 (28%) of observed spawning events, three males in 34 (21%), and four males in 12 (7%). Observed male order of nest entry during spawning could only be determined during daytime spawning events; nevertheless, the number of times a male was observed as first to enter the nest at the time of spawning was positively correlated with the number of offspring produced in all six breeding groups and overall ($\hat{P} = 58.7$, $p < 0.001$; Fig. 3A). S1 males were first to enter the nest during spawning significantly more often than both S2 males and precocious males, and there was no significant difference between S2 males and precocious males (Table 4). The mean number of total participations (i.e., regardless of order of nest entry) by S1 males was greater than that of S2 males, but not greater than that of precocious males, and precocious males participated significantly more often than S2 males (Table 4).

Treatment effects on male dominance hierarchies

S1 males had significantly greater mean dominance (David's score) than S2 males (Table 4), and the single most dominant male in each channel was always an S1. Interestingly, the male with the lowest dominance score in five of the six channels was also from the S1 group; our observations suggested that these males were tenacious (undeterred) in competing for access to females and as a result were repeatedly chased by numerous more dominant males (Fig. 4). Precocious males frequently attacked each other, but were never observed attacking or chasing anadromous males and were always submissive; thus, dominance hierarchies (and David's scores) were only documented and calculated for anadromous males. Dominance scores were positively correlated with the number of offspring produced in each of the six breeding groups (Fig. 3B), and overall the relationship was significant ($\hat{P} = 43.2$, $p < 0.001$).

Influence of male body size

The mean body size of S1 and S2 males did not differ significantly within any of the six breeding groups, but there was a

Fig. 3. Relationships between offspring produced per individual male and (A) frequency of first-to-enter the nest at the time of spawning and (B) dominance hierarchy (David's score) for each of the six breeding groups. Symbols represent different years and channels: 2015 (squares), 2016 (triangles), and 2017 (circles). Channel 1 is shown as solid symbols and channel 2 as open symbols. Correlations were positive and significant within all six breeding groups for both variables ($p < 0.01$). Panel A includes S1 and S2 anadromous males and precocial males, and panel B includes just anadromous males.

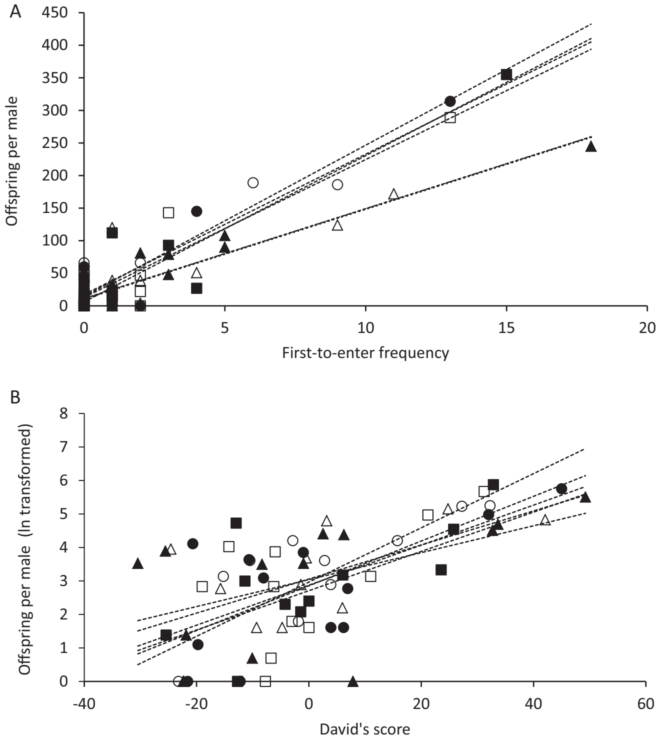


Fig. 4. The mean, minimum, and maximum David's score, which describes the relative position in the dominance hierarchy for S1 and S2 anadromous male steelhead. Each bar shade represents one of the six independent breeding groups.

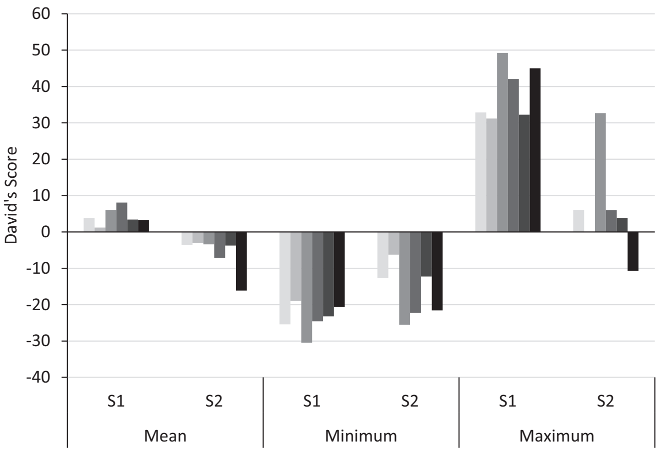
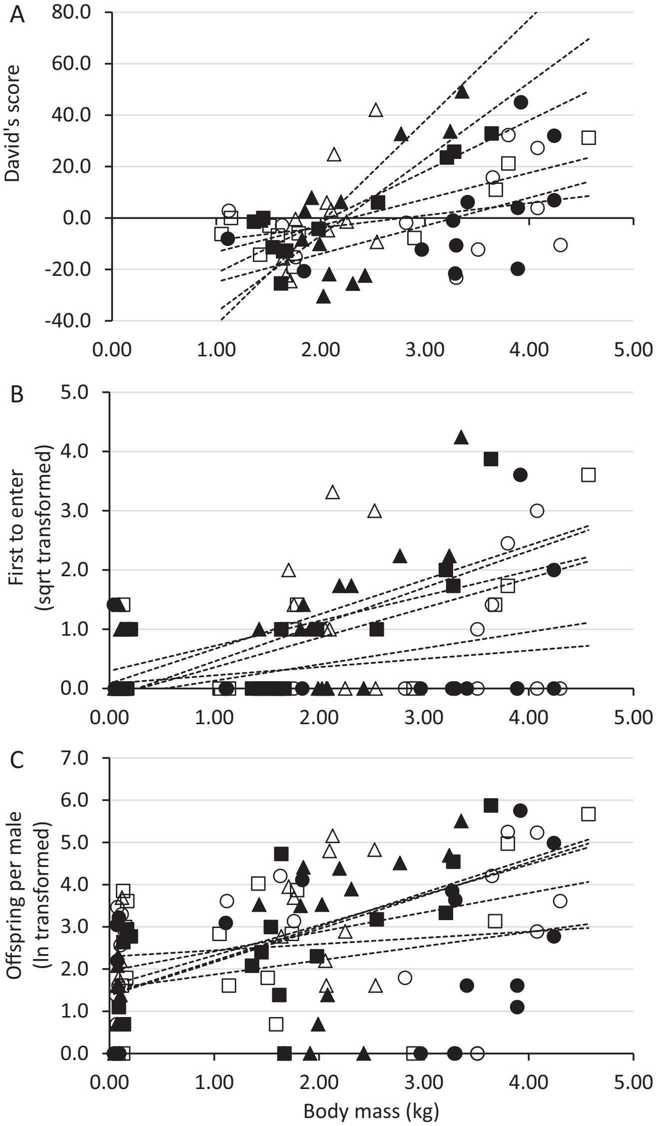


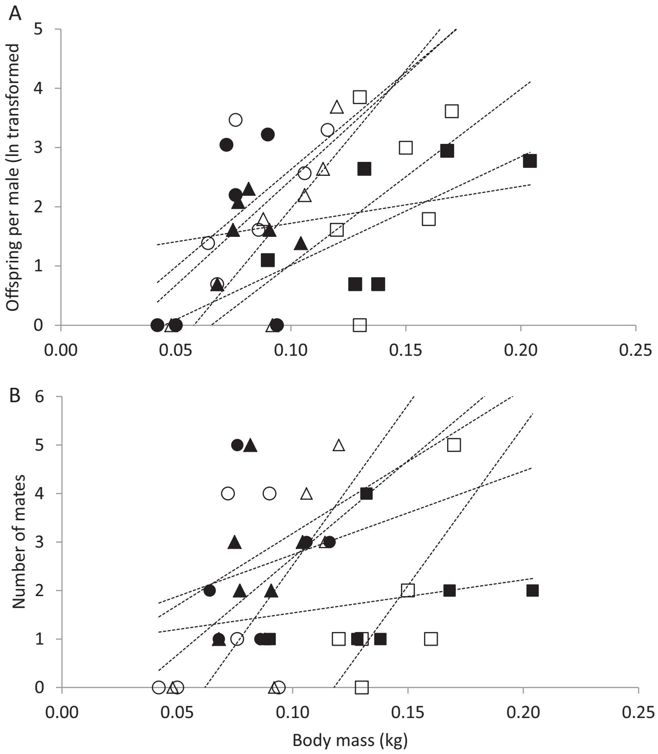
Fig. 5. Relationships between male body mass and David's score (measure of male dominance for S1 and S2 anadromous males only (A), frequency of first-to-enter the nest at the time of spawning (B), and offspring production (C). Symbols represent different years and channels: 2015 (squares), 2016 (triangles), and 2017 (circles). Channel 1 is shown as solid symbols and channel 2 as open symbols. Correlations were positive and significant within all six breeding groups and significant overall for all three response variables ($p < 0.01$).



substantial (nonsignificant) difference in one group in 2015 (larger S1 males in channel 2; $p = 0.078$; Table 1). To ensure that treatment-specific differences in male body size in that group did not confound the overall assessment of S1 and S2 success, we conducted the paired *t* tests on the remaining five breeding groups where mean body sizes were very similar (Table 1; all $p > 0.50$). The results remained unchanged for all response variables except that the comparison between anadromous S2 males and precocious males was no longer significant when group 2015 (2) was excluded from the analysis (see Table 4).

Body mass was positively correlated with position in the anadromous male dominance hierarchy in all six breeding groups, and the relationship was significant overall ($\hat{P} = 53.21$, $p < 0.001$; Fig. 5A). Body size was positively correlated with the number of times a male was first to enter the nest at the time of spawning in all six breeding groups and overall ($\hat{P} = 43.01$, $p < 0.001$; Fig. 5B). Male body size was positively correlated with the number of offspring produced. When all males were analyzed together (anadromous S1 and S2 and precocious males), the relationships were positive within each group and significant overall ($\hat{P} = 31.35$, $p < 0.001$; Fig. 5C). However, analyses of just S1 and S2 anadromous

Fig. 6. Relationship between S2 precocious male body mass and (A) the number offspring produced (natural log transformation) and (B) the number of mates in 2015 (squares), 2016 (triangles), and 2017 (circles). Within each year, channel 1 is shown as solid symbols and channel 2 as open symbols. Correlations were positive and significant within all six breeding groups and significant overall for both response variables ($p < 0.01$).



males indicated five of the six correlation coefficients were positive and one was slightly negative (no test).

S2 precocious males

Thirty-three of the 36 precocious males (92%) produced at least one offspring, and the number of matings obtained by individual precocious males ranged from zero to five. Precocious male body mass was positively correlated with the number of offspring produced within each of the six breeding groups, and the combined probability test indicated that the relationship between precocious males body mass and the number of offspring produced was significant ($\hat{P} = 17.5$, $p = 0.034$; Fig. 6). Likewise, precocious male body mass and the number of mates were also positively correlated in all six breeding groups and overall ($\hat{P} = 17.5$, $p = 0.043$; Fig. 6). The same was not true for the number of offspring per mate; only four of the six correlations were positive (hence, no test). Thus, overall, the results suggest that within the precocious male populations, body mass had a significant positive effect on breeding success, primarily through increased mating opportunities. The most productive precocious males over the 3 years of study produced 46 offspring (6.6% of the sampled offspring population) in 2015 (channel 2). Although anadromous males always dominated precocious males, we observed two instances where a female spawned with a precocious male when no anadromous males were present because they were attending other females. Females never spawn unattended, so this indicates that precocious males provided sufficient courtship stimuli to induce females to spawn.

Discussion

Hatchery rearing duration had a significant influence on the breeding success of male but not female steelhead under the conditions provided in this study. S1 and S2 females exhibited similar behavior and produced very similar numbers of offspring. S1 anadromous males were competitively superior to S2 anadromous males, tended to dominate access to nesting females, and, as a result, produced significantly more offspring. However, precocious males, which are only produced in appreciable numbers under the S2 rearing regime (Tatara et al. 2019), sired offspring in all six breeding groups, and, in some breeding groups, their contributions were similar to anadromous S2 males. Contributions from precocious males were nearly always the result of sneak spawning events, although on rare occasions, precocious males were able to stimulate females to spawn in the absence of an anadromous male. The results of this study reflect expectations when anadromous S1 and S2 adults and S2 precocious males spawn at the same times and locations, and we recognize that relative breeding success may vary depending on natural environmental conditions, which is discussed further below. However, because the differences in male breeding success were clearly linked to competitive interactions, and mating patterns and results were consistent among channels and over 3 years, we would expect similar responses in natural environments.

Competitive asymmetries among the three groups of males clearly explain the primary proximate cause of differences in breeding success. S1 males dominated S2 males, which were more frequently forced to sneak spawn from satellite positions along with precocious males. The ultimate cause of competitive differences between S1 and S2 males most likely resulted from the different early rearing environments, but the specific mechanism is difficult to identify. S1 and S2 rearing strategies differ in duration, daily feed rations, temperature regimes (see Tatara et al. 2017 and their supplement for details), and potentially other less obvious environmental conditions. Lower feed rations in S2 rearing likely increased aggression (Forest et al. 2017) and the intensity of competition, which is evidenced by fin nipping and dorsal fin erosion in steelhead (Berejikian and Tezak 2005), and has had a consistently noticeable effect on dorsal fin condition in S2 steelhead at WNFH (C. Pasley, personal communication). Increased agonistic activity may create greater separation in the behavioral development of S2 winners and losers, that is, very aggressive winners and very submissive losers. One possibility is that some S2 males become precocious because they are the most aggressive, dominate access to limited food resources, and achieve physiological thresholds for maturation (Kendall et al. 2015). If so, the most dominant S2 males would mature in the hatchery, leaving fewer dominant males in the anadromous S2 population. Because of their young age, S1 males rarely mature precociously. Consequently, the most aggressive S1 males likely grow faster (Nicieza and Metcalfe 1999; Naslund and Johnsson 2016), become successful smolts with high survival rates (Tatara et al. 2017) without triggering maturation, migrate to the ocean, return as adults, and dominate contests with remaining (and less dominant) S2 anadromous males.

This study informs two important considerations for the appropriate use of hatcheries in the management of anadromous salmonids: (*i*) the potential contribution of naturally spawning hatchery-origin fish to population productivity and (*ii*) likelihood of interbreeding between hatchery-origin and natural-origin, naturally spawning fish. Relative reproductive success is important because it can affect estimates of natural population productivity when hatchery and natural populations mix on the spawning ground and cloud productivity assessments of Distinct Population Segments listed under the US Endangered Species Act (McClure et al. 2003). The present study suggests that females from S1 and S2 smolt strategies should contribute similarly to productivity in natural systems. S1 and S2 hatchery populations may, however, exhibit different domestication pressures because of interactions among hatchery growth rates, size at release, and size-selective mortality after release (Berejikian et al. 2017; Tatara et al. 2017). The significant reduction in breeding success of S2 anadromous males is unlikely to suppress productivity of populations in natural streams (i.e., in the year they are spawning) because their reduced performance could be clearly linked to inferior competitive ability and does not likely reflect postzygotic mechanisms. The finding that females did not differ in breeding success may reflect the lesser importance of competition in determining female breeding success than for males. Females do compete for nesting territories, but in the experimental environment provided, nest quality within a channel may not have varied as much as in the natural environment. Hatchery-produced females very rarely mature precociously, so our hypotheses regarding competition, aggression, and male precocity may not pertain to females. Further, Quinn et al. (2011) did not find evidence that freshwater age of wild female steelhead influenced reproductive traits (egg size and fecundity), which is consistent with our finding of no breeding success differences related to duration of freshwater rearing. It is possible that behavioral traits related to migration behavior, nest site selection, or physical abilities to construct nests leaves open the possibility that S1 and S2 females may differ in their breeding success under natural conditions.

Gene flow from hatchery to natural populations has become a major consideration for regulating hatchery programs in the Pacific Northwestern United States over the past two decades (Mobrand et al. 2005; Paquet et al. 2011; Trushenski et al. 2018). The importance has most recently been substantiated by empirical studies indicating that reduced reproductive success of hatchery-origin steelhead is heritable (Araki et al. 2009; Ford et al. 2016). In recent years management processes have placed a strong emphasis on regulating the interbreeding between hatchery- and natural-origin salmon and steelhead (Paquet et al. 2011; Baskett et al. 2013; Waters et al. 2015). In some locations, returning anadromous hatchery fish might be removed from natural spawning areas through a fishery or by weirs or other barriers that provide access to returning adults. In most cases, the proportion of hatchery- and natural-origin adults can only be estimated based on counts at passage facilities or after the fact from observations, instream PIT detectors, or carcass and angler surveys. Even where the proportion of hatchery fish on spawning grounds can be estimated with some degree of certainty, effective management of gene flow between hatchery- and natural-origin adults requires an understanding of the relative success of hatchery and natural fish **after** they reach the spawning grounds and the proximate factors that influence it. The present study adds that gene flow from hatchery-reared S1 anadromous male steelhead to natural steelhead populations may be greater than that from S2 anadromous males because of their greater access to females and breeding success. That said, the production and release of S2 precocious males will likely increase interbreeding between hatchery and natural steelhead populations, because we found that nearly all of the precocious males in this study produced some offspring.

A number of factors may influence the breeding success of precocious males released from hatcheries. Recent pedigree reconstructions in natural systems have inferred that the missing parents of unassigned offspring reflect contributions from precociously maturing males (or resident ecotypes). Male parents of 26%–45% of spring Chinook salmon (*Oncorhynchus tshawytscha*) adults could not be assigned to captured adults returning to White River (Wenatchee River Basin, Washington) in the previous generation; however, this may overestimate precocious male contributions because 0%–23% of female parents, which do not mature precociously, also could not be identified (Ford et al. 2015). Spring Chinook salmon hatcheries typically produce very large numbers of precocious males (Larsen et al. 2019), many of which likely do not survive the 4- to 5-month period between release and spawning (Pearsons et al. 2009). Hatchery steelhead released as yearlings (similar to our S1 treatment) comprised only about 1% of missing parents in the Hood River population (Oregon; Christie et al. 2011). Precociously maturing steelhead may suffer less mortality than Chinook salmon between release and spawning because they mature in hatchery raceways or soon after release. However, Hood River steelhead (all S1) have a very low rate of precocious maturation (Larsen et al. 2017), which may explain their minimal contributions. In the present study, S2 precocious males introduced into the spawning channels exhibited lower per-capita reproductive success than anadromous males, but a very high percentage (92%) of the individual precocious males produced some offspring. Although we did not evaluate S1 precocious males because of their rarity in this hatchery population (Tatara et al. 2019), we expect their breeding success would be at least similar to S2 precocious males, and hatchery programs that release precocious males of either age should expect some gene flow into naturally spawning populations.

The abundance of hatchery-reared precocious males spawning in nature may be managed by altering rearing strategies to reduce maturation rates or identifying and removing maturing males from raceways prior to release. Trade-offs associated with precocious male contributions should be considered in determining whether, and to what extent, precocious male contributions should be limited. *Oncorhynchus mykiss* is partially anadromous throughout most of their natural range, and contribution from the resident (nonmigratory) males is nearly ubiquitous and likely important from an evolutionary perspective (Kendall et al. 2015). Frequency-dependent selection (FDS) may naturally limit gene flow from precocious males because of the stabilizing effect on contributions from alternative reproductive tactics (Hutchings and Myers 1994; Berejikian et al. 2010). For example, in a comparable Chinook salmon study, Schroder (2011) documented a consistent contribution from large anadromous males (~89%) even though there was a twofold variation in their frequencies relative to "sneaker" males (precocious and age-3 "jack" males). Recent data from natural systems suggests FDS plays an important role in maintaining alternative reproductive tactics (DeFilippo et al. 2018). Nonmigratory males have been shown to contribute substantially to naturally spawning populations (Seamons et al. 2004; Kuligowski et al. 2005), may function to stabilize the effective number of breeders under high variance in anadromous adult abundance (Araki et al. 2007), and provide an important source of adaptive genetic diversity (Dodson et al. 2013). Thus, any genetic benefits should be considered along with potential genetic diversity and fitness risks associated with artificial propagation programs (Baskett et al. 2013) on a case-by-case basis.

In summary, we found no effects of rearing duration on female breeding success in spawning channels that provided both high- and low-quality spawning and incubation environments; thus, S1 and S2 females should be equally productive when spawning naturally. Increasing the culture duration from 1 to 2 years resulted in a consistent negative effect on male competitive ability and breeding success; thus, gene flow from naturally spawning anadromous S2 males into natural populations should be less than that for S1 males. However, release of S2 precocious males would likely increase contributions from S2 programs. Whether to remove precocious males from hatchery populations prior to release, develop methods to produce fewer of them, or let natural breeding dynamics such as FDS regulate contributions will require careful consideration and will depend on management objectives.

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