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Abstract

High feeding and growth, relative to wild fish, can induce substantial proportions of hatchery reared male spring Chinook Salmon juveniles to mature precociously as age-2 minijacks. Released as smolts, these fish do not migrate to the ocean for rearing, and thus do not reach the adult age and size at which they can contribute to harvest mitigation fisheries and natural population supplementation. Initiation of maturation is thought to occur during a critical time period approximately one year prior to spawning. We performed a study with broodyear 2018 juveniles involving total feed deprivation over two different time periods (Early - August 8 through September 11, 2019; and Late - September 11 through October 24, 2019), to determine whether the rate of precocious maturation of the hatchery reared juveniles could be significantly reduced, while also allowing time post-treatment for the fish to achieve an adequate final smolt size through compensatory growth. At termination of the study in July 2020, the probabilities of precocious maturation of male smolts as minijacks in the Early and Late treatment tanks, as determined by logistic regression analyses, averaged 24.7% and 24.2%, respectively, which were significantly lower than the 38.1% rate for continuously fed fish in the Control tanks. Because the fish had to be pooled from their replicate tanks into a single subdivided concrete raceway for overwintering, fish from each tank were stocked into a particular section of the raceway after receiving an identifying adipose and/or pelvic fin clip combination. Unfortunately, there was some level of misidentification of fish to their original rearing tank, due primarily to regrowth of clipped pelvic fins. Errors in identification were such that the actual reduction in minijack rate was likely even larger, in particular for the Early treatment fish, for which the actual minijack rate was likely closer to 20%. In both May and July 2020, the treatment fish were similar in size to Control fish. Further study of the effects of timing and duration of the food deprivation period on precocious maturation rate and growth is recommended in order to develop a protocol for hatchery rearing of spring Chinook Salmon smolts for consideration by regional fisheries and hatchery managers.

Numerous studies have demonstrated that in spring of their second year, a substantial percentage of male hatchery reared spring Chinook Salmon smolts have precociously initiated sexual maturation. These fish will become fully mature “minijacks” (Beckman and Larsen 2005) by age-2, five to six months following smolt release (e.g., Gebhards 1960; Foote et al. 1991; Mullan et al. 1992; Larsen et al. 2004, 2006, 2013 and 2018; Harstad et al. 2014; Medeiros et al. 2018). Several of these studies were conducted at the Cle Elum Supplementation Research Facility (CESRF; Cle Elum, Washington), located adjacent to the Yakima River (rkm 297). CESRF is a hatchery managed by the Yakama Nation for the production of spring Chinook Salmon smolts with which to supplement the natural population in the upper Yakima River basin. At the CESRF, minijack rates among male smolts average approximately 42% (Larsen et al. 2018). Following release of the smolts, some of the precociously maturing minijacks never leave their natal river system (Larsen et al. 2004). Others among the maturing minijacks exhibit downstream migration behavior, though fish have also been observed to reverse their migration (Zimmerman et al. 2003; Beckman and Larsen 2005; Larsen et al. 2010), presumably in an effort to return to their natal rivers for the fall spawning season. Despite the relatively high number of minijacks that can be produced by hatchery programs, minijacks are only infrequently observed in spawning ground surveys and parentage studies indicate their reproductive success is low (Schroder et al. 2012; Pearsons et al. 2009; Ford et al. 2015). Importantly, maturing minijacks among hatchery reared smolts never attain the adult age and size at which they would help meet hatchery objectives to contribute to harvest mitigation fisheries and to natural population supplementation (Beckman and Larson 2005; Pearsons et al. 2009).

The physiological decision in salmonids to initiate sexual maturation or not occurs approximately a year prior to full gonadal maturation and spawning (Mangel 1994; Thorpe 1994; Berglund 1995; Silverstein et al. 1998). Subyearling male salmonids that have experienced relatively rapid growth and have accumulated high energy stores within their first year may make this decision to initiate maturation at a high rate. A greatly increased incidence of minijacks, relative to naturally spawned spring Chinook Salmon, is observed among hatchery reared fish, due to the high feeding rates and typically high fat content of commercial feeds. Wild reared smolts are considerably smaller and leaner at the same age, and demonstrate much lower minijack rates (Larsen et al. 2004 and 2006; Pearsons et al. 2009).

In 2002, a study was conducted at the CESRF to assess the extent to which manipulation of feeding rate would alter the incidence of minijacks among the spring Chinook Salmon smolts (Larsen et al. 2006). In April 2002, samples of broodyear (BY) 2001 juveniles were distributed among 16 rearing tanks and the fish were fed according to two different feeding regimes for two different time periods - 4 tanks per treatment in a 2x2 factorial design. The fish were fed at either a High or Low feeding rate (relative to standard CESRF rates), applied either in the Summer (June 1 – Aug 31) and/or the Fall (Sept 1-Nov 7). Afterwards, rearing of the fish continued following standard CESRF feeding rates until the next April when the smolts were harvested – April being concurrent with the March to May period of release of the CESRF production smolts (Fast et al. 2015). Summary results of the study (Table 1) indicate that the Low feeding rate effectively reduced the minijack rate relative to fish fed at the High rate, with the greatest effect being when applied during the Summer relative to the Fall period. However, the reduction in minijack rate, even for fish fed at the Low rate in both the Summer and Fall was only modest, and the final average smolt size of these fish was significantly reduced relative to fish fed at the High rate during both periods, as well as to the CESRF production smolts. Given that a decrease in smolt size is generally correlated with decreased outmigration survival of smolts and lower smolt to adult return rates (Beckman et al. 2017), the reduction in minijack rate was considered insufficient to merit incorporation of a period of reduced feeding to the standard CESRF rearing practices.

However, the manuscript describing this study concluded with the statement, "In future studies, more significant alterations in growth, perhaps through dietary lipid manipulation, may be needed to further reduce the level of precocious male maturation in hatchery-produced fish" (Larsen et al. 2006). Prompted by this recommendation, we conducted a feed study using juvenile CESRF Chinook Salmon with the goal of reducing body lipid stores during the critical period (A. L. Pierce et al. *in preparation*). While this study did yield a reduction in lipid stores, the minijack rate did not significantly decrease. As an alternative to manipulating lipid content, therefore, we proposed that a more drastic reduction in feeding rate during juvenile rearing, i.e., total feed deprivation during the maturation decision period, albeit for a more limited duration than used by Larsen et al. (2006). The objective of the study was to affect a greater reduction in growth and adiposity, that might lead to a more dramatic reduction in minijack rate. While total feed deprivation might sound extreme from a hatchery production perspective, salmonids in nature are regularly exposed to protracted periods of restricted food availability. Additionally, various studies conducted to characterize physiological responses of salmonids to periods of starvation, including studies investigating compensatory growth and repression of maturation rate, indicated that survival was similar to continuously fed control fish (e.g., Weatherly and Gill 1981; Thorpe et al. 1990; Quinton and Blake 1990; Reimers et al. 1993; Johansen et al. 2001).

TABLE 1. Final average smolt size and minijack rate for hatchery-reared spring Chinook Salmon reared under High or Low feeding rates during the Summer (June 1 to August 31, 2003) or Fall (September 1 to November 7, 2003), reported by Larsen et al. (2006).

Modified Feeding		Harvest - April 2004	
Summer : June 1 to Aug 31, 2003	Fall : Sept 1 to Nov 7, 2003	Average Body Weight (g)	Average Male Minijack Rate (%)
High	High	25	69%
High	Low	18	58%
Low	High	18	51%
Low	Low	22	42%
2004 CESRF Production smolts		22	53%

We designed our feed deprivation study to address two research questions: 1) will either of two approximately 5-week periods of total feed deprivation applied in either mid-August to mid-September (Early) or mid-September to mid-October (Late), significantly reduce the rate of precocious maturation relative to continuously fed Control fish, and 2) will *ad libitum* feeding and compensatory growth following feed deprivation permit the fish to achieve a final smolt size within sexes and maturation status, similar to that of continuously fed Control fish?

METHODS

Study fish

Broodfish for the CESRF spring Chinook Salmon program are collected each year from among returning adults at the Roza Adult Monitoring Facility (RAMF), located adjacent to Roza Irrigation Dam on the Yakima River (rkm 206), and transported to CESRF for holding until the spawning season (Fast et al. 2015). The primary smolt supplementation program uses strictly natural origin adults – a fully integrated program. A smaller number of smolts are produced for a segregated hatchery control (HC) line, which uses only returning hatchery origin adults each generation, and a limited number of returning first generation supplementation (SH) origin adults are also collected for use as broodfish. At the time of spawning (September-October), in addition to the primary CESRF production using the natural origin broodfish, a series of test crosses are made among adults of each of the three broodlines. Subsamples of eggs and milt are collected from the fish, and the gametes are matrix-mated within origins to produce multiple single-pair matings involving approximately 200 eggs per cross. The crosses are incubated separately, and the fry are reared to the swim-up stage. Percent hatch, weight of the unfertilized eggs, length and weight of the swim-up fry, and survival to swim-up is estimated for each mating, and these data are compared among crosses for each of the three brood lines, to monitor possible divergence in these reproductive traits following a generation or more of artificial propagation (Knudsen et al. 2008).

Following collection of data at the swim-up stage, fry produced with the natural origin and the HC broodfish are mixed among the production fry for their respective brood lines. Fry from the SH origin crosses are normally sacrificed. Exceptionally, approximately 5,000 of the swim-up fry from the BY 2018 SH crosses, involving 37 individual matings among 17 female and 13 male SH broodfish, were retained for use in the feed deprivation study.

Feed Deprivation Study

In February 2019, the BY 2018 SH swim-up fry were distributed for initial rearing among four circular fiberglass tanks - 390 L water volume receiving approximately 15 L per minute flow through water. The fish were fed at normal CESRF rates. On August 6, 2019, 159 juveniles (approximately 40 per tank) were sacrificed in an overdose of buffered anaesthetic (Tricaine Methanesulfonate; MS-222; Western Chemical; www.wchemical.com), and measured for fork length (mm) and body weight (0.01 g). From among the remaining juveniles, approximately 250 fish were randomly distributed into each of 16 fiberglass tanks. Each tank was assigned to one of three treatments: Control (n=6 tanks; continuous feeding), Early feed deprivation (n=5 tanks; feed withheld from Aug 8 to Sept 11), and Late feed deprivation (n=5 tanks; feed withheld from Sept 11 to Oct 24). Following their respective deprivation treatments (as of September 12 for the Early fish, and as of October 25 for the Late fish), the fish were fed *ad libitum* - feed was offered by hand four to five times per day to satiation. Feeding of the Control fish continued at normal CESRF rates. On November 20-21, 2019, the study fish were removed from the tanks and pooled into a single raceway for continued rearing, and all fish were fed *ad libitum* until the study was terminated in July 2020.

On Sept. 11 and October 24, 2019, 20 juveniles per tank were randomly collected, sacrificed, and measured for fork length and body weight. On November 20-21, 2019, 30 fish per tank were non-lethally anaesthetized, measured for fork length and body weight and replaced in their tanks. Because of the risk of freezing of the water supply pipes to the fiberglass tanks, after the November 20-21 sampling the fish were transferred to an adjacent concrete raceway for overwintering and rearing until termination of the study. To be able to identify individuals to their respective rearing tank, the fish from each tank were anaesthsized and received an identifying fin clip combination (with or without an adipose fin clip, and with or without a right pelvic fin clip). The fish were then stocked into one of four equal length sections (A, B, C and D) of the raceway (30.5 m long × 3.5 m wide × 3.1 m deep), each section receiving four groups of fish with each group having a contrasting fin clip combination. On May 12, 2020, a total of 60 fish from the raceway (15-16 per section; 3-4 fish per tank), were sacrificed, identified to treatment tank, and dissected to confirm the ease with which sex and maturation status could be visually identified. On July 8-9 and 15, 2020, all remaining fish in the raceway were sacrificed in an overdose of anaesthetic, individually identified to raceway section and fin clip, measured for fork length and body weight, and dissected to visually identify sex and maturation status. Testes were removed and weighed from a subset of the non-maturing (n=161) and precociously maturing (n=81) male smolts, randomly selected across the treatment tanks. Ovaries were removed and weighed from a smaller number (n=43) of the female smolts.

Data Analyses

Fork length and body weight data were used to calculate condition factor for each individual, and these values were compared between treatments within sample dates by ANOVA (α=0.05). Specific growth rate (% change in body weight per day) based on average individual body weight per tank was calculated between successive 2019 sampling dates (8/6, 9/11, 10/24 and 11/20), and the data compared between treatments by ANOVA. Testes weights sampled at termination of the study in July 2020 were compared by ANOVA between male smolts identified as non-maturing or maturing, to further confirm visual identification of maturation status. Data for the numbers of non-maturing and maturing minijacks among male smolts were analyzed by logistic regression (glm function in lme4 r package) to estimate the probability of male smolt maturation per treatment, with Tukey post-hoc analysis for comparison between treatments (emmeans r package).

RESULTS

Summary treatment values for average fork length, body weight and condition factor at each of the four 2019 sample dates are provided in Table 2 (with values for each tank within treatments in Appendix I), and illustrated in Figure 1. During their period of feed deprivation fish in both the Early and Late treatments showed statistically significant reductions in these measures ($p<0.05$). After resumption of feeding, the fish recovered much of the difference in size relative to Control fish, though as of the 11/20/2019 sampling the feed deprived fish were still significantly smaller on average than Controls. Summary treatment values for specific growth rate between 2019 sample dates per treatment are presented in Table 3, with values for each tank within treatments presented in Appendix II. Specific growth rates of both the Early and Late feed deprived fish compared within period(s) subsequent to deprivation were significantly greater than for Controls, indicative of a compensatory growth response. The CESRF facility uses pumped river water during the spring through early winter, and growth of the fish responded to change in temperature during the study, which averaged 18.6 ^{o}C from 8/6 to 9/11, 12.0 ^{o}C from 9/11 to 10/24, and 6.9 ^{o}C from 10/24 to 11/20 (Figure 2). Optimum temperature for growth of juvenile Chinook salmon is reported to be 15 ^{o}C to 16 ^{o}C (ODEQ 1995; USEPA 2001; WDOE 2002).

Dissection of fish sampled on May 12, 2020 (n=60) indicated that visual examination of the gonads was sufficient to readily identify males versus females, as well as the maturation status of the males. Testes for the males fell into two significantly different size groups ($p<0.001$), indicative of non-maturing (small - average 15 mg) versus maturing minijacks (large - average > 575 mg). These differences were even more significant when the study fish were all harvested in July 2020 – testes of the maturing minijacks were large and white and averaged 8,844 mg in weight, while the pink and threadlike testes of the non-maturing males averaged only 18 mg. Ovaries of the females in July 2020 were thin and yellow-orange in color with a distinct triangular thickening at the anterior end, and averaged 71 mg (Table 4).

Average fork length, body weight, and condition factor for fish sampled on May 12, 2020, categorized by sex and maturation status within treatments are presented in Table 5. No significant differences in these measures were observed. Summary treatment values of these same measures for fish at termination of the study in July 2020, categorized by sex and maturation status within treatments are presented in Table 6 (an expanded table with values for each tank is provided in Appendix III). When comparing these measures between treatments within sex and maturation status, the values were not significantly different, with the exception of a smaller average fork length for Late treatment non-maturing males ($p=0.008$; Appendix III). When comparing size measures of the fish between sex and maturation status (tank averages pooled across treatments) neither fork length, body weight, nor condition factor differed significantly between females and non-maturing males, but these measures were both significantly smaller than for maturing minijacks ($p<0.001$; Table 7).

TABLE 2. Summary treatment values, mean ± standard error (SEM), for fork length, body weight and condition factor, averaged across mean values per replicate tank at each of the sample dates in 2019. Sample size was 19-21 fish per tank on 9/11 and 10/24/2019, and 30 fish per tank on 11/20-21/2019. Food was withheld from fish in the Early treatment tanks from 8/6 to 9/11/2019, and for the Late treatment fish from 9/11 to 10/24/2019. Statistical comparisons of tank averages were performed between treatments within sample dates. Differences are indicated by the letters a, b and/or c; columns not sharing a letter differ significantly. An expanded table including values for these measures per tank is provided in Appendix I.

		Sept. 11, 2019			Oct. 24, 2019			Nov. 20-21, 2019		
Treatment		Fork Length (mm)	Body Weight (g)	Condition Factor (K)	Fork Length (mm)	Body Weight (g)	Condition Factor (K)	Fork Length (mm)	Body Weight (g)	Condition Factor (K)
Control	Avg	89.0a	8.3a	1.160a	102.8a	12.9a	1.172a	106.0a	13.9a	1.147a
	SEM	1.1	0.3	0.013	2.0	0.9	0.013	1.9	0.8	0.014
Early	Avg	77.5b	4.2b	0.884b	94.1b	9.8b	1.149b	100.1b	11.6b	1.136ab
	SEM	0.9	0.2	0.022	3.0	1.0	0.018	1.6	0.5	0.014
Late	Avg	89.4a	8.5a	1.168a	87.2c	6.7c	0.998c	91.6c	8.8c	1.119b
	SEM	2.0	0.6	0.011	0.7	0.2	0.008	2.2	0.6	0.013

TABLE 3. Summary treatment values, mean ± standard error (SEM), for specific growth rate for fish (sexes combined) between 2019 sample dates, averaged across mean values per replicate tank. Sample size was 19-21 fish per tank on 9/11 and 10/24/2019, and 30 fish per tank on 11/20-21/2019. On 8/6/2019, prior to distribution of fish from the pooled group among the 16 study tanks, a sample of 159 fish indicated an average fork length of 80.9 mm, an average body weight of 5.9 g, and an average condition factor of 1.090. These values were assumed for each tank at the start of the study period. Food was withheld from fish in the Early treatment tanks from 8/6 to 9/11/2019, and for the Late treatment fish from 9/11 to 10/24/2019. Statistical comparisons performed between treatments within sample dates, with differences indicated by the letters a, b and/or c; columns not sharing a letter differ significantly.

		Specific Growth Rate (% change in body weight per day)		
Treatment		Aug. 6 to Sept. 11, 2019 (36 days)	Sept. 11 to Oct. 24, 2019 (41 days)	Oct. 25 to Nov. 20, 2019 (27 days)
Control	Avg	0.95% a	1.01% b	0.27% c
	SEM	0.11%	0.09%	0.14%
Early	Avg	-0.94% b	1.95% a	0.64% b
	SEM	0.12%	0.21%	0.26%
Late	Avg	1.00% a	-0.53% c	0.98% a
	SEM	0.20%	0.13%	0.19%

FIGURE 1. Graphs for average fork length, body weight and condition factor ± standard error of the mean for fish at each of the four sample dates in 2019. On 8/6/2019, the study fish had an average fork length = 80.9 mm, body weight = 5.9 g, and condition factor = 1.090; these values were assumed for each tank. Food was withheld from fish in the Early treatment tanks from 8/6 to 9/11/2019, and from the Late treatment fish from 9/11 to 10/24/2019.

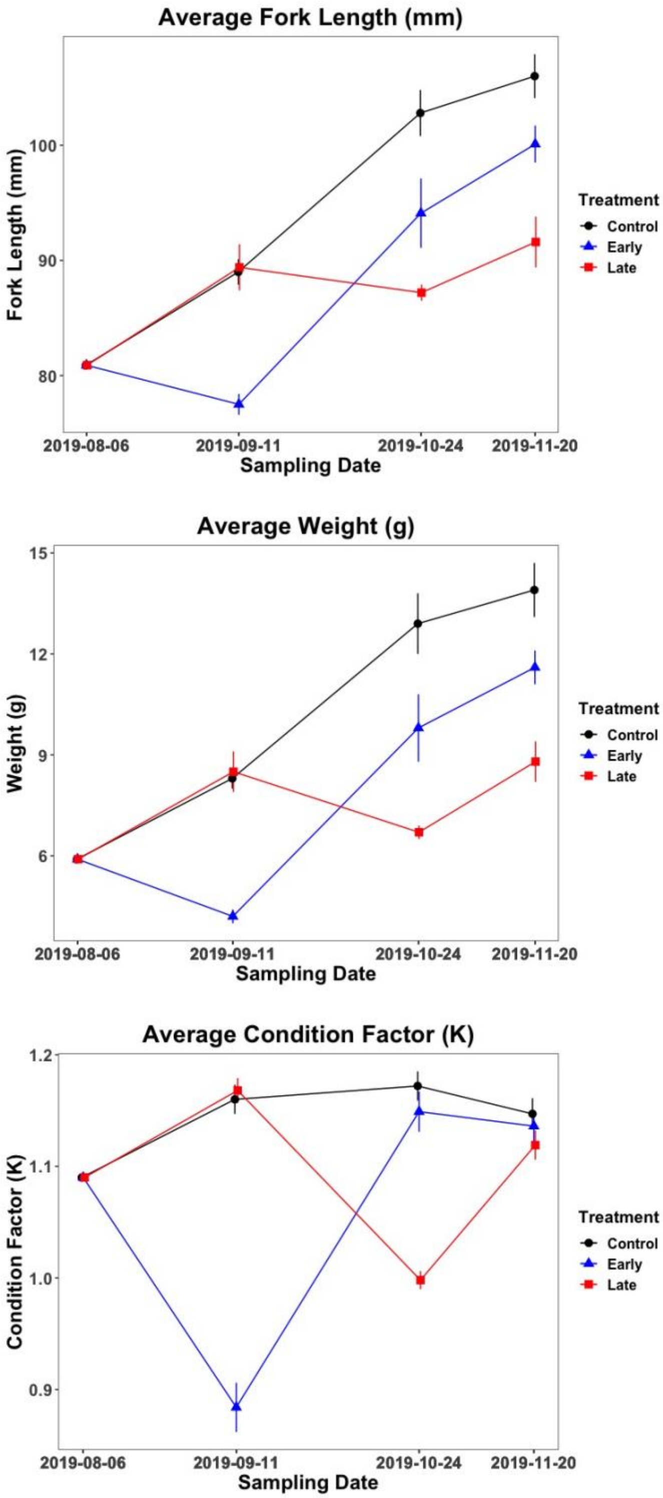

TABLE 4. Gonad weight and gonadosomatic index for females, non-maturing males, and maturing minijack males among BY 2018 juveniles at termination of the study in July 2020. Statistical comparisons performed between sex and maturation status, with differences indicated by the letters a, b and/or c; columns not sharing a letter differ significantly.

Sex and Maturation	Sample Number	Gonad Weight (g)		Gonadosomatic Index	
		Average	Range	Average	Range
Female	43	0.071 b	0.02 to 0.10	0.12 b	0.02 to 0.20
Non-Maturing	161	0.018 c	<0.01 to 0.12	0.29 c	<0.01 to 0.16
Minijacks	81	8.8 a	2.5 to 13.0	10.4 a	4.5 to 13.6

TABLE 5. Mean treatment values observed on May 12, 2020 for fork length, body weight and condition factor for female, non-maturing male and maturing minijack male smolts (data for fish from different tanks pooled within treatment). Statistical comparisons performed for measures between sex and maturation status; no significant differences (p>0.05) were observed.

Treatment	Sex and Maturation Status	Sample Number	Average Fork Length (mm)	Average Body Weight (g)	Average Condition Factor (K)
Control	Females	12	156.3	44.3	1.1522
Early	Females	8	157.3	45.5	1.1549
Late	Females	12	155.1	43.0	1.1452
Control	Non-Maturing	6	158.7	49.6	1.2252
Early	Non-Maturing	9	156.3	45.6	1.1911
Late	Non-Maturing	5	153.4	41.9	1.1606
Control	Minijacks	4	169.0	64.9	1.3307
Early	Minijacks	2	159.5	57.8	1.3885
Late	Minijacks	2	169.5	65.1	1.3372

Logistic regression analyses yielded a probability for precocious maturation of male smolts in the Control tanks of 38.1%, significantly higher (p<0.0001) than the 24.2% and 24.7% probabilities for the Early and Late treatments, respectively (Table 8). Differences between the Early and Late treatments were not significantly different (p=0.987).

TABLE 6. Summary treatment values, mean ± standard error (SEM), for fork length, body weight and condition factor for female, non-maturing male and maturing male smolts, averaged across mean values per replicate tank at termination of the study in July 2020. Statistical comparisons performed between sex and maturation status, with differences indicated by the letters a or b; columns not sharing a letter differ significantly. Values for these measures per tank within treatment groups is available in Appendix III.

Treatment	Sex and Maturation Status		Fork Length (mm)		Body Weight (g)		Condition Factor (K)	
Control	Females	Average	175.24	a	59.82	a	1.1274	a
		SEM	0.63		0.74		0.0278	
Early	Females	Average	175.93	a	61.39	a	1.1171	a
		SEM	0.66		1.11		0.0173	
Late	Females	Average	173.53	a	59.12	a	1.1482	a
		SEM	0.72		1.08		0.0342	
Control	Non-Maturing	Average	177.62	a	62.44	a	1.1034	a
		SEM	1.15		1.43		0.0087	
Early	Non-Maturing	Average	178.37	a	64.79	a	1.1306	a
		SEM	0.99		1.77		0.0136	
Late	Non-Maturing	Average	173.38	b	58.50	a	1.1092	a
		SEM	0.60		1.20		0.0158	
Control	Minijacks	Average	183.29	a	83.53	a	1.3637	a
		SEM	1.52		2.26		0.0225	
Early	Minijacks	Average	182.68	a	83.29	a	1.3593	a
		SEM	1.67		3.13		0.0166	
Late	Minijacks	Average	178.22	a	80.13	a	1.4049	a
		SEM	0.86		1.04		0.0115	

TABLE 7. Summary treatment values, mean ± standard error (SEM), for fork length, body weight and condition factor at termination of the study in July 2020 for female, non-maturing male and maturing male smolts; tank means pooled across treatments within sex and maturation status. Statistical comparisons performed between sex and maturation status, with differences indicated by the letters a or b; columns not sharing a letter differ significantly.

Treatment	Sex and Maturation Status		Fork Length (mm)		Body Weight (g)		Condition Factor (K)	
Control	Females	Average	174.92	a	60.09	a	1.1307	a
		SEM	1.76		2.27		0.0606	
Early	Non-Maturing	Average	176.53	a	61.94	a	1.1137	a
		SEM	3.06		4.11		0.0296	
Late	Minijacks	Average	181.51	b	82.39	b	1.13752	b
		SEM	3.83		5.22		0.0446	

Table 8. Estimated probability of precocious maturation of male Chinook salmon smolts as minijacks from logistic regression analyses, including standard errors of the means (SEM) and lower (LCI) and upper (UCI) 95% asymptotic confidence intervals. Multiple comparisons were performed between treatments, with differences indicated by the letters a or b.

Treatment	Probability of Maturing	SEM	LCI	UCI
Control	0.381 a	0.021	0.341	0.423
Early	0.242 b	0.022	0.202	0.288
Late	0.247 b	0.022	0.207	0.292

DISCUSSION

Results of this study provided affirmative answers to the two primary research questions: 1) both the Early and the Late feed deprivation treatments significantly reduced the rate of precocious maturation of male smolts relative to Controls, and 2) *ad libitum* feeding and compensatory growth following feed deprivation permitted the fish from both treatments to achieve a final smolt size (fork length, body weight and condition factor) within sexes and maturation status, that was similar to that of continuously fed Control fish.

Interpretation of these results, however, must take into consideration various circumstances that arose over the course of the study. The first of these involves a columnaris (*Flavobacterium columnare*) infection experienced by juveniles being reared at the CESRF in the summer of 2019. The infection led to mortalities among the study fish beginning in early August, concurrent with the beginning of the Early deprivation treatment. Because of their increasingly diminished metabolic state, the Early treatment fish experienced relatively high levels of mortality, averaging 79 fish (32%) per tank over the period 8/6 through 10/24/2019. To combat the infection, four successive salt treatments were applied every two to three days beginning August 28. Treatments involved dissolution of 1.8 kg NaCl per tank (0.47% NaCl), with the flow through water diminishing the concentration by approximately 50% every 20 min. By the time the Late deprivation treatment began on 9/11/2019, salt treatments were finished and mortality had diminished. In contrast to the Early treatment, mortality in the Late treatment tanks averaged only 17 fish (7%) over the 08/06 through 10/24/2019 period, with mortality during this period among the Control fish also being 17 fish per tank. Additionally , mortality due to the columnaris infection likely resulted in disproportionate loss among smaller fish, which are also less likely to become minijacks. Thus, the columnaris infection may have biased the observed minijack rates upwards, in particular for the Early treatment tanks.

Uncertainty in the data was also caused by possible migration of some fish from one raceway section to another. The total numbers of fish per tank counted in November 2019, and in July 2020 (plus fish sampled in May 2020) are provided in Table 9, with the difference in the adjacent column representing the loss of fish between dates. In Sections C and D the number of fish diminished by only 20 and 6, respectively. This seems reasonable as mortalities were observed only occasionally over the course of the seven months of rearing in the raceway. In contrast, Section B, lost 79 fish, and instead of losing fish the number in Section A actually increased by 19. We strongly suspect that some of the 79 fish lost from Section B were not mortalities but were instead fish that had moved past the screen (in an upstream direction) from Section B to Section A, resulting in the excess of fish counted in Section A in July. Presuming that movement of fish from Section B was random with respect to sex and maturation status, the estimates for minijack proportion for the four tanks in that section would not be affected. However, the addition of fish to Section A could have biased the resulting estimates of minijack proportion for the tanks in Section A. Specifically, given the observation that Control fish yielded a significantly higher proportion of minijacks, inclusion of Late treatment adipose clipped (Ad) fish from Section B (tank 10) to Control Ad clipped fish in Section A (tank 14) would tend to bias downwards the minijack estimate for Control tank 14. Conversely, addition of Control pelvic fin clipped (P) fish from Section B (tank 11) to Early P clipped fish (tank 15) in Section A would bias upwards the resulting minijack proportion estimate for Early tank 15 (Table 8).

An even more significant bias in some of the data likely resulted from misidentification of fish that had received a pelvic fin clip. At termination of the study of the fish in July 2020, it was remarked that while some fish wholly lacked a left pelvic fin, the fins of other previously pelvic fin clipped fish had regrown to varying lengths. The result was that for fish with substantial regrowth of the pelvic fin, we were unable to identify with assurance which fish had never received a pelvic fin clip, versus those which had been fin clipped followed by regrowth over the interim 7 months. If P clipped fish were misidentified as not having been clipped, the result would inflate the number of fish identified as unclipped (X) fish at the expense of the number of P fish within each section. Similarly, the number of Ad fish in the section would be inflated at the expense of fish that had previously received both an adipose and a pelvic fin clip (AdP). It appears certain that misidentification of some number of fish occurred, given that the counts of P and AdP fish within each section diminished over the period November 2019 through July 2020, while the counts for X and Ad fish increased (Table 9). When misidentification involved fish from two tanks of Control fish, or two treatment tanks (give the similarity of the average minijack rate between feed deprivation treatments), there would be little or no bias in the resulting estimates of minijack proportions. However, given the much higher minijack rate for Control tanks relative to treatment tanks, when the misidentification was between a Control and a treatment tank, then the group of fish receiving the misidentified fish incurred a bias upwards or downwards in the resulting minijack estimate (Table 9).

These issues, and most particularly those associated with misidentification of pelvic fin clipped fish lend uncertainty to the final estimates of minijack rate. Of the six Control tanks, four may have had their minijack rates biased low, and while none of the Late tanks are suspected to have been biased, three of the five Early tanks are possibly biased high (Table 9). The result is that the observed reduction in average minijack rate from 38.1% for Control male smolts to 24.2% for Early treatment male smolts represents a minimal difference.

Particularly suspect is the observed minijack proportion for Early Tank #9 (45.5%), which was one of the three Early tanks suspected of bias. The observed minijack rate for Tank #9 is greater than all six observed estimates for minijack rate in the Control tanks, and exceeds by greater than five SEMs the estimates for the other four Early tanks (Appendix III). If the value for Tank #9 were eliminated from the logistic regression analysis, results indicate that probability of maturation for the Early tanks diminishes from 24.2% to 19.9%. A subsequent pairwise comparison with the probability of maturation for the Late treatment tanks (24.7%) indicates a difference that remains non-significant, though the resultant p value is substantially decreased, from p=0.987 to p=0.279.

TABLE 9. Number of fish counted in November 2019 and at termination of the study in July 2020, and observed percent of minijacks among males per tank. Fish were identified to tank vis raceway section (A, B, C or D) and fin clip combination (X = no clip; P = left pelvic fin clipped, Ad = adipose fin clipped, and AdP both left pelvic and adipose fins clipped).

Raceway Section	Treat-ment	Tank No.	Fin Clip	Number Nov. 2019	Number July 2020	Change in No. of Fish	Observed % Minijacks	Possible Bias to % Minijacks
A	Late	13	X	200	230	+30	29.5	
	Early	15	P	85	91	+6	27.3	a
	Control	14	Ad	202	226	+24	42.7	b,y
	Late	16	AdP	197	156	-41	27.6	
	Total			684	703	+19		
B	Early	9	X	131	154	+23	44.8	x
	Control	11	P	200	136	-64	43.9	
	Late	10	Ad	197	206	+9	26.7	
	Early	12	ADP	183	136	-47	17.7	
	Total			711	632	-79		
C	Control	5	X	193	212	+19	30.3	y
	Late	7	P	174	161	-13	23.5	
	Early	6	Ad	104	131	+27	18.2	x
	Control	8	AdP	180	127	-53	32.8	
	Total			651	631	-20		
D	Control	1	X	183	200	+17	39.1	y
	Early	3	P	174	144	-30	18.1	
	Control	2	Ad	182	206	+24	37.0	y
	Late	4	AdP	175	158	-17	16.9	
	Total			714	708	-6		

a - % minijacks biased high due to escape from B to A
b - % minijacks biased low due to escape from B to A
x - % minijacks biased high due to misidentifying P fish as X, or AdP fish as Ad
y - % minijacks biased low due to misidentifying P fish as X, or AdP fish as Ad

Average size for both the fish sampled in May 2020 (Table 5), as well as for the remaining fish sampled at termination of the study in July 2020 (Table 6), was statistically similar among treatments, within sex and maturation status. The data collected in May was at the end of the mid-March through mid-May time period when raceways at the acclimation sites are open for volitional release of the CESRF production smolts (Fast et al. 2015). During this period, smolts leaving the acclimation sites average approximately 108 mm in fork length (B. Bosch, personal communication), or approximately 14 g/fish. Average size for the study fish sampled in May was considerably larger, generally exceeding 155 mm and 45 g/fish. In the original study design, termination of the study was scheduled for early April 2020, near the beginning of the release period, in order to compare final smolt sizes against the CESRF production smolts, but restrictions associated with the Covid-19 pandemic required postponement until a later date.

However, even if we had been able to sample in April 2020, the size of the study fish would not have been directly comparable with that of the production smolts. For most of their rearing, the CESRF production fish are reared with flow-through water from the adjacent Yakima River, the temperature of which fluctuates with the season (Figure 2). In February, prior to release, the fish are transferred to one of three acclimation sites, which are also fed with flow-through river water. At the CESRF, following transfer of the smolts, the raceways are cleaned, refilled with flow-through well water (approximately 10 ^{o}C), and the next brood year's swim-up fry are transferred to the raceways for initial 3-4 months in the relatively warm well water, before the water source is switched back to Yakima River water. Our study fish which remained at the CESRF through the duration of their rearing, and therefore experienced this change in water source, which is reflected in the sudden increase in the raceway water temperature from 3.3 to 9.3 ^{o}C in February 2020 (Figure 2). Growth of the study fish was therefore accelerated relative to that of the production smolts at the acclimation sites, resulting in the much larger size of the study fish. It would have been informative to have sampled the study fish in mid-February, the last date at which a direct comparison of size data for the study fish could have been made with those for the production smolts. Nonetheless, growth compensation of fish in the feed deprivation treatments did permit them to catch up in size with the continuously fed control fish, and it would appear that growth of the treatment fish during late winter and early spring, had they continued to be reared with river water, would likely have made up most, if not all, of the size deficit relative to the CESRF production smolts.

In summary, totally withholding feed from subyearling spring Chinook for approximately five weeks early or late in the August to October period (approximately one year post-egg fertilization) produced a significant reduction from at least 37% to 25% (and possibly as low as 20%) in rate of precocious maturation of male juveniles as age-2 minijacks. Also, *ad libitum* feeding subsequent to feed deprivation permitted the treated fish to achieve a final smolt size comparable to that of continuously fed Control fish, and likely similar to that of CESRF production smolts. Results for the Early versus the Late deprivation treatments did not exhibit a significant difference in minijack rate. However, reexamination of timing for the deprivation treatments in a follow-up study(s) would be merited, as would testing for modest increases or decreases in duration of the treatment. The objective of these studies would be to determine a protocol that could be proposed for consideration by regional fisheries and hatchery managers, for hatchery rearing of spring Chinook smolts that would minimize minijack production while also permitting production of smolts of the desired size prior to release.

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APPENDICES

APPENDIX I. Average and standard error of the mean (SEM) for length, body weight and condition factor per tank at each of the sample dates in 2019. On 8/6/2019, prior to distribution of fish from the pooled group among the 16 study tanks, a sample of 159 fish indicated an average length of 80.9 mm, an average weight of 5.9 g, and an average condition factor of 1.090. These measures were assumed for each tank at the start off the study period. Food was withheld from fish in the Early treatment tanks from 8/6 to 9/11/2019, and for the Late treatment fish from 9/11 to 10/24/2019. Statistical comparisons were performed between treatments within sample dates, with differences indicated by the letters a, b and/or c; columns not sharing a letter differ significantly.

		Sept. 11, 2019				Oct. 24, 2019				Nov. 20-21, 2019			
Treat-ment	Tank No.	Sample Number	Fork Length (mm)	Body Weight (g)	Cond. Factor (K)	Sample Number	Fork Length (mm)	Body Weight (g)	Cond. Factor (K)	Sample Number	Fork Length (mm)	Body Weight (g)	Cond. Factor (K)
Control	1	19	89.5	8.4	1.160	21	103.7	13.1	1.169	30	106.8	14.1	1.144
	2	20	87.7	8.0	1.166	20	100.0	11.8	1.168	30	102.8	12.6	1.150
	5	20	89.3	8.3	1.149	20	104.4	13.6	1.177	30	108.1	15.0	1.167
	8	20	88.5	8.1	1.144	20	102.1	12.5	1.163	30	106.1	14.0	1.147
	11	20	88.1	8.0	1.163	20	101.3	12.1	1.157	30	105.2	13.3	1.124
	14	20	90.8	8.9	1.180	20	105.4	14.1	1.196	30	107.0	14.2	1.153
	Avg		89.0a	8.3a	1.160a		102.8a	12.9a	1.172a		106.0a	13.9a	1.147a
	SEM		1.1	0.3	0.013		2.0	0.9	0.013		1.9	0.8	0.014
Early	3	21	78.6	4.4	0.875	19	91.0	8.9	1.150	30	97.8	11.0	1.146
	6	20	77.6	4.1	0.858	20	93.8	9.5	1.133	30	100.3	11.7	1.154
	9	20	76.3	4.0	0.880	20	91.9	9.0	1.130	30	100.1	11.4	1.119
	12	20	77.1	4.1	0.886	20	94.9	10.3	1.174	30	99.8	11.4	1.129
	15	20	78.1	4.4	0.919	20	98.8	11.3	1.159	30	102.4	12.4	1.131
	Avg		77.5b	4.2b	0.884b		94.1b	9.8b	1.149b		100.1b	11.6b	1.136ab
	SEM		0.9	0.2	0.022		3.0	1.0	0.018		1.6	0.5	0.014
Late	4	20	89.5	8.4	1.153	20	86.7	6.6	1.007	30	88.9	8.0	1.122
	7	19	88.8	8.3	1.166	20	87.2	6.7	0.984	30	92.1	9.0	1.139
	10	19	86.4	7.6	1.163	20	86.4	6.6	0.998	30	89.9	8.2	1.107
	13	20	91.9	9.2	1.176	20	87.9	6.9	0.998	30	94.1	9.4	1.109
	16	20	90.5	8.8	1.181	20	88.0	6.9	1.001	30	93.1	9.1	1.119
	Avg		89.4a	8.5a	1.168a		87.2c	6.7c	0.998c		91.6c	8.8c	1.119b
	SEM		2.0	0.6	0.011		0.7	0.2	0.008		2.2	0.6	0.013

APPENDIX II. Average specific growth rate in weight for fish between 2019 sample dates per replicate tank within treatments, and per treatment. Sample size was 19-21 fish per tank on 9/11 and 10/24/2019, and 30 fish per tank on 11/20-21/2019. On 8/6/2019, prior to distribution of fish from the pooled group among the 16 study tanks, a sample of 159 fish indicated an average length of 80.9 mm, an average weight of 5.9 g, and an average condition factor of 1.090. These values were assumed for each tank at the start of the study period. Food was withheld from fish in the Early treatment tanks from 8/6 to 9/11/2019, and for the Late treatment fish from 9/11 to 10/24/2019. Statistical comparisons performed between treatments within sample dates, with differences indicated by the letters a, b and/or c; columns not sharing a letter differ significantly.

	Specific Growth Rate (% change in body weight per day)		
Treatment-Tank No.	Aug 6 to Sept 11, 2019 (36 days)	Sept 11 to Oct 24, 2019 (41 days)	Oct 25 to Nov 20, 2019 (27 days)
Control-01	0.99	1.03	0.28
Control-02	0.86	0.89	0.23
Control-05	0.95	1.14	0.37
Control-08	0.89	1.00	0.42
Control-11	0.85	0.96	0.34
Control-14	1.15	1.07	0.02
Average	0.95 a	1.01 b	0.27 c
SEM	0.11	0.09	0.14
Early-03	-0.84	1.65	0.78
Early-06	-1.00	1.93	0.80
Early-09	-0.09	1.88	0.88
Early-12	-1.00	2.13	0.38
Early-15	-0.79	2.17	0.34
Average	-0.94 b	1.95 a	0.64 b
SEM	0.12	0.21	0.26
Late-04	0.97	-0.56	0.72
Late-07	0.94	-0.50	1.13
Late-10	0.71	-0.34	0.84
Late-13	1.25	-0.69	1.17
Late-16	1.12	-0.57	1.03
Average	1.00 a	-0.53 c	0.98 a
SEM	0.20	0.13	0.19

APPENDIX III. Average values observed for fork length, weight and condition factor for female, non-maturing male and maturing male smolts per replicate tank with overall average and standard error of the mean (SEM) for tank values within treatments, and observed percent minijacks per tank at termination of the study in July 2020 (including fish sampled on May 12, 2020). Statistical comparisons performed between sex and maturation status, with differences indicated by the letters a or b; columns not sharing a letter differ significantly.

Treatment-Tank No.	Sex and Maturation Status	Number	Average Length (mm)	Average Weight (g)	Average Condition Factor (K)	Observed Percent Minijacks
Control-01	Females	105	175.04	58.27	1.0816	
Control-02	Females	95	174.08	58.00	1.0957	
ontrol-05	Females	102	176.38	60.46	1.0905	
Control-08	Females	65	176.89	61.03	1.0956	
Control-11	Females	66	176.15	62.55	1.1405	
Control-14	Females	120	172.88	58.61	1.2601	
	Average		175.24 a	59.82 a	1.1274 a	
	SEM		0.63	0.74	0.0278	
Early-03	Females	69	174.00	57.27	1.0796	
Early-06	Females	53	178.11	61.87	1.0860	
Early-09	Females	85	176.13	63.13	1.1473	
Early-12	Females	72	175.50	63.42	1.1675	
Early-15	Females	44	175.93	61.24	1.1052	
	Average		175.93 a	61.39 a	1.1171 a	
	SEM		0.66	1.11	0.0173	
Late-04	Females	77	174.09	58.40	1.0993	
Late-07	Females	74	174.68	59.68	1.1143	
Late-10	Females	100	175.14	62.95	1.1626	
Late-13	Females	115	171.37	57.98	1.2756	
Late-16	Females	76	172.37	56.60	1.0894	
	Average		173.53 a	59.12 a	1.1482 a	
	SEM		0.72	1.08	0.0342	
Control-01	Non-maturing	56	179.48	63.47	1.0902	
Control-02	Non-maturing	67	175.63	59.88	1.0961	
Control-05	Non-maturing	74	177.81	62.88	1.1065	
Control-08	Non-maturing	39	180.56	65.62	1.1062	
Control-11	Non-maturing	36	179.22	65.94	1.1416	
Control-14	Non-maturing	58	173.02	56.87	1.0799	
	Average		177.62 a	62.44 a	1.1034 a	
	SEM		1.15	1.43	0.0087	
Early-03	Non-maturing	58	177.19	61.34	1.0951	
Early-06	Non-maturing	61	180.41	68.66	1.1527	
Early-09	Non-maturing	36	179.06	66.52	1.1553	
Early-12	Non-maturing	49	180.08	67.61	1.1504	
Early-15	Non-maturing	31	175.10	59.82	1.0995	
	Average		178.37 a	64.79 a	1.1306 a	
	SEM		0.99	1.77	0.0136	
Late-04	Non-maturing	63	173.41	58.01	1.1039	
Late-07	Non-maturing	64	174.88	59.76	1.1110	
Late-10	Non-maturing	76	173.78	61.76	1.1656	
Late-13	Non-maturing	79	173.61	58.48	1.0968	
Late-16	Non-maturing	54	171.20	54.47	1.0687	
	Average		173.38 b	58.50 a	1.1092 a	

	SEM		0.60	1.20	0.0158	
Control-01	Minijacks	36	180.86	79.49	1.3385	39.1%
Control-02	Minijacks	40	180.20	77.23	1.3137	37.4%
Control-05	Minijacks	32	189.56	92.83	1.3593	30.2%
Control-08	Minijacks	19	186.00	86.08	1.3305	32.8%
Control-11	Minijacks	28	182.32	84.03	1.3725	43.8%
Control-14	Minijacks	44	180.77	81.51	1.4679	43.1%
	Average		183.29 a	83.53 a	1.3637 a	
	SEM		1.52	2.26	0.0225	
Early-03	Minijacks	13	177.92	73.23	1.2958	18.3%
Early-06	Minijacks	13	187.62	91.78	1.3870	17.6%
Early-09	Minijacks	30	182.83	83.41	1.3595	45.5%
Early-12	Minijacks	11	180.45	80.77	1.3712	18.3%
Early-15	Minijacks	12	184.58	87.27	1.3831	27.9%
	Average		182.68 a	83.29 a	1.3593 a	
	SEM		1.67	3.13	0.0166	
Late-04	Minijacks	13	177.54	77.39	1.3663	17.1%
Late-07	Minijacks	19	180.95	82.82	1.3919	22.9%
Late-10	Minijacks	27	175.74	78.16	1.4288	26.2%
Late-13	Minijacks	33	177.88	80.48	1.4183	29.5%
Late-16	Minijacks	21	179.00	81.81	1.4193	28.0%
	Average		178.22 a	80.13a	1.4049 a	
	SEM		0.86	1.04	0.0115	