Effects of Juvenile Steelhead on Juvenile Chinook Salmon Behavior and Physiology

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Abstract.—Experiments were designed to determine whether and how steelhead Oncorhynchus mykiss affect chinook salmon O. tshawytscha when the two species are confined together. In a behavioral experiment, we observed groups of juvenile chinook salmon and steelhead together and groups of chinook salmon alone to determine whether the steelhead were aggressive and their presence changed the behavior of chinook salmon. We also performed two runs of a physiological experiment to determine whether the addition of steelhead to tanks containing chinook salmon would stress the chinook salmon, as determined by a change in their plasma cortisol levels. Behavioral changes were observed in the chinook salmon when they were held with steelhead; they reduced their movements, darted less, were attacked up to 16 times as often, and were found less frequently in the shade than chinook salmon held without steelhead. Steelhead were found to establish territories and defend them with chases, charges, and nips. In their attempts to establish and defend territories, the steelhead attacked the chinook salmon as often as they attacked other steelhead, but the chinook salmon showed little aggression toward the steelhead. Cortisol concentrations were significantly higher for chinook salmon in tanks receiving steelhead than in tanks receiving additional chinook salmon or no additional fish after 2 h (in one of the two experimental runs) and after 32 h (in both experimental runs; combined data). These results suggest that confining steelhead and chinook salmon together, such as in raceways and barges in the Columbia River system and in other situations, is stressful to the chinook salmon.

The juvenile salmonid transportation program on the Columbia River collects out-migrating ju-
responses that include adverse changes in feeding (Wedemeyer 1976), metabolic rates (Barton and Schreck 1987), osmoregulatory processes (Redding and Schreck 1983), avoidance behavior (Sigismondi and Weber 1988), and immune system functions and disease resistance (Maule et al. 1989). Studies have been conducted on the physiological response of juvenile salmonids to the various aspects of collection and transportation. Few studies, however, have focused on the physiological and behavioral responses of one species of salmonid to the presence of another species, even though several species are collected and transported together.

Juvenile chinook salmon *Oncorhynchus tshawytscha*, steelhead *O. mykiss*, coho salmon *O. kisutch*, and sockeye salmon *O. nerka* are the species transported in the Columbia River system, with chinook salmon and steelhead predominating. These species are not completely separated before transport and therefore may be collected, held in raceways, and transported together. If barge holds are not filled to capacity at an upstream dam, additional fish are sometimes added to the holds at downstream dams. Transportation of mixed species could affect the ability of a species to adjust to the transportation environment. Juvenile chinook salmon subjected to a saltwater challenge experienced greater mortality when held with migrant steelhead than when held with conspecifics (Mathews et al. 1986). Juvenile sockeye salmon that were given a saltwater challenge experienced greater mortality when held with juvenile steelhead than when held alone or with juvenile chinook salmon (C. B. Schreck, Oregon Cooperative Fisheries Research Unit, personal communications). Steelhead are known for their aggressive behavior toward other species of salmonids and conspecifics (Gibson 1981; Abbott and Dill 1985; Abbott et al. 1985). We wondered whether aggressive behavior by steelhead would adversely affect chinook salmon. Our objectives in this study were (1) to determine whether steelhead show aggression toward chinook salmon in confined areas, (2) to evaluate whether the presence of steelhead causes changes in the behavior of chinook salmon and steelhead, and (3) to determine whether the presence of steelhead activates the endocrine-stress axis (Schreck 1981) in chinook salmon, thereby adding to the other stressful aspects of collection and transportation.

**Methods**

All experiments were conducted at Oregon State University’s Fish Performance and Genetics Laboratory at Smith Farm, Corvallis. The chinook salmon used in the experiments were reared from fry or eggs at this laboratory. The fry or eggs were obtained from Oregon’s Willamette Salmon Hatchery or Marion Forks Salmon Hatchery. Steelhead were obtained as yearlings and were similar in size and developmental stage to the migrating juvenile salmonids that are transported from the Snake and Columbia River dams. We also mimicked the range of loading densities and species proportions of juvenile salmonids observed in barge transport (Verhey et al. 1998; Congleton et al. 2000).

**Behavioral interactions.**—Two rectangular fiberglass tanks (1.83 × 0.66 × 0.60 m) with Plexiglas viewing windows along one side were used for observing fish behavior. Well water (12°C) flowing at 3 L/min entered at one end and exited through a standpipe at the other end. The depth of the water was 0.55 m. The tanks were located in a dark room to allow control of outside light. The upstream one-third of the tank was shaded and the remainder was exposed to light from an incandescent 100-W bulb suspended 0.30 m above the water. The tank lights were turned on at 0800 hours and off at 1700 hours over the 2 d of each trial, roughly corresponding to natural day length. We observed no prolonged effect on fish behavior when the lights were abruptly turned on. The distribution of fish after the lights were turned on was the same as it would have been when the normal photoperiod increased in intensity to the levels in our facility.

Fish to be tested were given their morning feed approximately 1 h before the start of a trial. At the start of each trial, fish were netted (a “grab” sample) from stock tanks and placed into a 20-L bucket. A bucket of fish was loaded into each of two test tanks at approximately 0800 hours. One tank served as the treatment tank (randomly selected at the first trial only), with 10 chinook salmon and 10 steelhead (the mix treatment), and the other as a control, with 20 chinook salmon (the chinook-salmon-only treatment). The chinook salmon averaged 144 mm in length (range, 84–200 mm) and 37 g in weight (range, 7–96 g) and the steelhead 187 mm in length (range, 117–226 mm) and 75 g in weight (range, 29–127 g). Using a camcorder (Sony TR81), fish activity was videotaped through the Plexiglas window for 15-min periods at 3 and 8 h and for 30-min periods at 27 and 32 h after loading. Videotaping was easily
accomplished without a blind as long as the observer moved slowly. Test subjects were tank-raised salmon, which experienced all inherent danger and response to danger from a vertical position above the tanks and not from a horizontal position. This made close-up observation through the Plexiglas extremely easy. Times were chosen to correspond to the hours of sampling in the physiological response experiments described later. Also, 32 h is roughly the length of time spent by fishes during transportation in a barge. Upon completion of a trial, the fish were weighed and measured and the tanks were drained and flushed. A new trial was then run with naive fish and the treatment and control tanks alternated. Ten trials were conducted in the above manner. Two trials (four tanks) were then run with 20 steelhead (the steelhead-only treatment) in the same manner as the previous trials as a second form of control. This entails a loading density of approximately 2.5 g/L for the whole tank and 7.5 g/L for the shaded area if all 20 fish occupied the shade (the density varied between treatments and trials due to the size difference of the two species). Although our tanks are but a fraction of the size of a barge compartment, we attempted to mimic a few of the conditions within the barge compartments (U.S. Army Corps of Engineers 1997). These include having flow-through water, exposure to light and the availability of shade, species size, species proportions, loading densities, and fasting during transportation. Trials were conducted from 3 to 30 January 1996.

For statistical analysis, only the first 15 min of videotape of each observation period were used to ensure that periods were equal in length and timing. Videotaped behavior was analyzed for aggressive interactions between and among species and for incidents of darting. Using Kalleberg’s (1958) paper as a guide, an aggressive act was defined as a charge, chase, nip, or any combination of these three initiated by one fish toward another. The following data were collected on each aggressive interaction: the time it started, the species that initiated it, the species that was the recipient (or target) of the act, and the species and number of fish that were not target fish (nonrecipients) but that appeared startled or frightened by the act. Darting was defined as a sudden, rapid swimming motion that was sustained for more than four fish body lengths and that was not the result of a recipient fish’s flight from an aggressive act. The number of darting acts was recorded for each species, and the species and number of fish responding to the darting action were noted. Notes were taken on whether any fish became dominant, whether other fish held other territories, and the relative location within the tank of individuals of each species at each sampling time. A dominant fish was defined as the holder of the largest territory (usually the shaded end of the tank) or the winner of aggressive bouts.

Nonparametric statistical tests were used because variation in individual fish behavior between trials, tanks, and time of sampling led to data with unequal variances and nonnormal distributions. The Mann–Whitney U-test in STATGRAPHICS (Manugistics, Inc., Rockville, Maryland) was used to test whether aggressive behavior, darting, and the fish affected (recipient fish plus nonrecipient fish responding) by the two types of behaviors (aggression and darting) were different among the treatments and species at each observation time. The Friedman repeated-measures one-way analysis of variance (ANOVA) in GraphPad PRISM (GraphPad Software, Inc., San Diego, California) was used to test whether behavior changed through time in each type of treatment. When significant differences were indicated by the Friedman tests, subsequent pairwise comparisons between observation times were conducted using post hoc Wilcoxon rank tests in STATGRAPHICS. The use of tank space by chinook salmon was tested with Fisher’s exact test (Siegel 1956), and the emergence of dominant steelhead was tested with Cochran’s Q-test (Siegel 1956). For all analyses, differences were considered to be statistically significant when the P-value was less than 0.05.

Physiological responses.—In a physiological experiment (conducted twice) we examined plasma cortisol levels in juvenile chinook salmon as an indicator of the endocrine-stress response to the addition (loading) of more fish (chinook salmon or steelhead) to their tanks. Runs 1 and 2 of the experiment differed as to the size and number of animals, depending on availability. Groups of chinook salmon (run 1 included 40 fish averaging 157 mm [range, 123–195 mm] and 35 g [range, 16–61 g]; run 2 included 60 fish averaging 153 mm [range, 92–210 mm] and 45 g [range, 9–107 g]) were acclimated for 3 weeks to six 1-m-diameter fiberglass tanks supplied with well water at 12°C (water flow was 7 L/min in run 1 and 4 L/min in run 2). Loading consisted of adding juvenile salmonids to the tanks of chinook salmon. Replicate experimental groups (two tanks each) were as follows: (1) no fish added (negative control); (2) chinook salmon added (positive control; the added
fish averaged 170 mm [range, 98–201 mm] and 46 g [range, 13–70 g] in run 1 and 187 mm [range, 102–241 mm] and 88 g [range, 11–213 g] in run 2); and (3) steelhead added (treatment; the added fish averaged 180 mm [range, 124–211 mm] and 53 g [range, 18–80 g] in run 1 and 201 mm [range, 173–225 mm] and 87 g [range, 55–124 g] in run 2). The chinook salmon that were added to the tanks with chinook salmon were clipped (adipose fin) to distinguish the two groups. A preliminary experiment was conducted in which the physiological response of chinook salmon to the addition of water alone was evaluated. We found that this had no effect on the physiological response; cortisol levels were low initially and remained low after the water disturbance.

Blood samples for analysis of plasma cortisol levels were taken from the original chinook salmon in groups of 8–12 (run 1) and 10 (run 2) from each tank at each sampling time. Samples were collected 2 h (run 1) or 24 h (run 2) before the addition of fish (baseline) and then 2, 8, and 32 h (run 2) or 34 h (run 1) after the addition of fish. Blood was collected via caudal severance and centrifuged. Plasma samples were then analyzed for levels of cortisol to determine the severity of stress (Schreck 1981) using a radioimmunoassay as described in Foster and Dunn (1974) and adapted by Redding et al. (1984) for use with chinook salmon. The experiment was conducted from 10 to 22 March 1994 for run 1 and from 24 January to 16 February 1996 for run 2.

Cortisol data were analyzed by repeated-measures ANOVA (RM ANOVA) with treatment (the addition of steelhead, chinook salmon, or no fish) and run (1 or 2) as the between-run factors and the sampling time interval (2, 8, or 32–34 h) as the within-run factor. The data analyzed were the mean cortisol concentrations for the different tanks (the experimental unit); data were log transformed to provide for homogeneity of variances. Calculated P-values for the effect of the within-run factor and the interactions between this factor and the other factors were corrected by the Greenhouse–Geisser factor epsilon (Kirk 1995). Because behavioral observations indicated that the interactions between steelhead and chinook salmon were highly variable between tanks and over time, the P-value required for further examination of main effects and interactions was set at 0.1 or less. If a significant sampling time × treatment interaction was observed in the RM ANOVA, additional ANOVAs were performed for each of the three sampling intervals with treatment and experimental run as factors. If the treatment × experimental run interaction was significant in these two-way ANOVAs, separate one-way ANOVAs were performed for each experimental run. If the effects of treatment were significant in ANOVA tests, the means for the three treatments were compared pairwise by Fisher’s protected least-significant-difference (PLSD) test. Finally, to test the assumption that mean cortisol concentrations did not differ between tanks prior to the experimental runs, the concentrations in tanks receiving different (future) treatments were compared by one-way ANOVA. Analyses were done with SuperANOVA software (Abacus Concepts, Berkeley, California).

Results

Behavioral Interactions

Qualitative observations.—The steelhead in this experiment behaved in a manner typical of territorial salmonids during the establishment and defense of territories (Newman 1956; Kalleberg 1958). Their aggressive acts could be vicious, sometimes resulting in collisions and producing a fight-or-flee response in the recipient fish, while nonaggressive contact produced little or no response. Chasing, especially by a dominant steelhead, would at times continue into other areas of the tank for several minutes at a time. On one occasion, a steelhead attacked several different fish continuously, for a total of 38 aggressive acts over a span of 6 min and 20 s. It was common for a steelhead to act more aggressively when an earlier display, such as a threat nip, wigwag, fin flares, or crowding, did not elicit a fleeing response from the recipient fish. The maximum number of continuous aggressive acts against an individual fish was initiated by a dominant steelhead and included 10 such acts in 22 s. Several fights were observed, always between steelhead. A typical fight involved lateral displays and aggressive acts as two steelhead circled each other nose to tail. Several blows would be exchanged over a few seconds of time until one of the fish retreated. Steelhead that lost a fight or that were the object of an aggressive act were sometimes observed to redirect that aggression against other fish immediately after the original act. Fish that were fleeing from an aggressive act or darting often prompted other steelhead to attack them. In one such episode, a steelhead was attacked 13 times by five different conspecifics as it passed by or through their territories. There was no indication that size played a role in either re-
receiving or delivering an aggressive act. However, size appeared to play a role in which steelhead become dominant, for most of the dominant steelhead were among the larger ones in the tank during the trial.

Aggressive acts by dominant steelhead accounted for approximately 25% of all aggressive acts by steelhead in the mix treatment during each observation time (Figure 1a). Approximately 60% of the aggressive acts in the steelhead-only treatment were by dominant steelhead (Figure 1b). The number of aggressive acts initiated by chinook salmon in the mix treatment was very small (Figure 1a).

As a steelhead became dominant and defended a station or territory (usually the shaded area), several other steelhead in each trial established smaller territories of their own. It appeared that the shaded area was the first preference for steelhead and that the vicinity of the standpipe was the second preference. Steelhead not holding territories dispersed to the water surface or to the corners of the tank.

In treatments with steelhead, a dominant steelhead usually emerged and several others held smaller territories. Over the 32 h of the mix treatment, an increasing number of the trials had one dominant steelhead per tank (one-tailed Cochran’s Q-test: \( P < 0.001 \); Figure 2b). The sample size was too small (four) for statistical comparisons of increasing numbers of trials with a dominant steelhead in the steelhead-only treatment. Within that treatment, however, while fish did not have territories at the first observation time, by the 32-h observation time there was a dominant steelhead that occupied the shade and others that held smaller territories in all four trials (Figure 2c).

The darting behavior of steelhead was very different from that of chinook salmon. Steelhead often darted (a quick zigzag dash around the tank) for no apparent reason. Other incidents of darting by steelhead followed shortly after a loss in an aggressive encounter. A darting steelhead sometimes collided with other fish or the walls of the tank.

Although aggression (as defined for this study) was observed in both chinook salmon and steelhead, the aggression style of these two species was very different. Chinook salmon tended to shoal or aggregate and exhibited very few aggressive displays or acts. When aggression occurred, in most cases it consisted of a slight head movement to nip at a neighboring fish (threat nip). A recipient chinook salmon was never observed returning a nip to the aggressor. A typical response of a recipient usually involved moving a short distance away and later returning to the original area or position. Chinook salmon rarely responded to a threat nip or a physical contact nip from another chinook salmon by fleeing, as they did when threatened or attacked by steelhead. A small social distance around an individual fish appeared to be more important to chinook salmon than a territory at a particular location. A threat nip appeared to serve as a communication to a neighboring fish that it was crossing the boundary of an individual’s

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**Figure 1.** Percentage of aggressive acts by (a) subordinate and dominant steelhead and chinook salmon in the mix treatment and (b) subordinate and dominant steelhead in the steelhead-only treatment at four observation times. The numbers within the bars are the mean number of total aggressive acts in the mix treatment (10 trials, 10 tanks each) and the steelhead-only treatment (2 trials, 4 tanks) during 15 min of videotaped activity at each observation time.
social distance. We saw no indication that size determined which individuals received or delivered nips or threat nips. Any movement (aggressive or passive) by one chinook salmon caused a rippling effect throughout the group, as each fish readjusted the distance to its neighbors. Chinook salmon became startled and darted away when nonaggressive body contact was made with another fish. Charges, chases, or aggressive body contacts were rarely observed among the individual chinook salmon within groups.

After loading, chinook salmon in the presence of steelhead quickly followed steelhead into the shade, while chinook salmon with only conspecifics took longer to move into the shade. Within the mix treatment, both species appeared reluctant to leave the shade even when a steelhead (dominant or not) delivered repeated blows. During the 3-h observation time, chinook salmon in the mix treatment appeared to be more agitated and to have a higher ventilation rate than those in the chinook-salmon-only treatment group. By the end of the trial, the chinook salmon in the mix treatment used tank space differently than those in the chinook-salmon-only treatment (one-tailed Fisher’s exact test: $P < 0.03$). Chinook salmon in the mix treatment usually left the shaded area as dominance was being established by a steelhead (Figure 2a). After they left the shade, they often formed an aggregate and were positioned in the middle of the tank close to the bottom. These fish did not have similar social distances or show the same shoaling behavior as their counterparts in the chinook-salmon-only treatment: The chinook salmon in the mix treatment rarely moved, instead hovering in one position. By contrast, those in a tank with only conspecifics slowly milled about in the shade and would make short excursions into the bright part of the tank as a shoal or as individuals. In neither treatment were chinook salmon observed at the water surface or in the corners of the tank, nor were they observed establishing a station or territory within the tank or taking a dominant position.

Darting behavior also differed between the two species. Darting by chinook salmon usually involved a quick dash to the water surface. Often, bubbles were released from the mouth or opercula shortly after the dart. Dashes to the surface by either steelhead or chinook salmon usually startled other chinook salmon but rarely startled steelhead.

Quantitative measurements.—At the first observation time (3 h after loading), levels of aggression were low and there was no difference in the mean number of aggressive acts between the mix treatment and either of the treatments with only one species (Figure 3a). At 8 h, aggression in the mix treatment exceeded that in the chinook-salmon-only treatment. At 27 h, aggression in the steelhead-only treatment exceeded that in the mix treatment. The differences between treatments contin-
Mean number of aggressive acts compared by (a) treatment (chinook salmon only, mix, and steelhead only), (b) species (chinook salmon and steelhead in the mix treatment), and (c) dominant steelhead in the mix and steelhead-only treatments at four observation times after the loading of fish into experimental tanks. Means and standard errors are based on the number of aggressive acts by each species during 15 min of videotaped activity at each observation time in 10 trials (10 tanks each) in the chinook-salmon-only and mix treatments and 2 trials (4 tanks) in the steelhead-only treatment. Note the differences in the y-axis scales. Asterisks between bars denote significant differences (Mann–Whitney U-test) between adjacent pairs; $P < 0.05^*$, $P < 0.01^{**}$, and $P < 0.001^{***}$.

Overall, groups with only steelhead had the greatest number of aggressive interactions, followed by mixed groups and groups with only chinook salmon.

The number of aggressive acts within treatments with steelhead increased as the number of steelhead increased. Overall, the fish in the steelhead-only treatment experienced 1.8–4.6 times the mean number of aggressive acts experienced by the fish in the mix treatment, and the fish in the mix treatment experienced 1.5–13.6 times the mean number of aggressive acts experienced by the fish in the chinook-salmon-only treatment (Figure 3a).

The increase in the number of aggressive acts within the mix treatment was attributed to aggression by steelhead (Figure 3b); the chinook salmon showed little aggressive behavior throughout the experiment, and when they did initiate an aggressive act they targeted their own species (Figure 4a). When steelhead initiated an aggressive act, there was no preference for one species over the other as the recipient (Figure 4b). At the last observation time, dominant steelhead in the steelhead-only group exhibited a greater number of aggressive acts than dominant steelhead in the mix group (Figure 3c). There was no difference in the number of aggressive acts per steelhead, which was calculated as the total number of aggressive acts per treatment divided by the number of steelhead in that treatment ($P > 0.05$ for all four observation times; in this analysis, the aggressive acts by the dominant steelhead are included in the total).

Within treatments, aggressive acts increased over the 32 h of a trial when steelhead were present. Steelhead held with only conspecifics showed a peak number of aggressive acts, both as a group and per individual, 27 h after being loaded into the experimental tanks (Friedman ANOVA: $P = 0.02$ for both group and individual; Figure 3c). Steelhead and chinook salmon in the mix treatment also showed an increase in aggressive acts per species and individual over the 32 h of a trial. While aggression by steelhead was significantly higher in all later observation periods than in the first period (Friedman ANOVA: $P < 0.001$ for group and individual), that by chinook salmon was low in numbers and similar for most of the observation periods (there was, however, a significant difference between the 3-h and 27-h observation periods [Wilcoxon rank test: $P = 0.01$ for group and individual]). There was no change in the amount of aggression for the group or per individual among
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Figure 4.—Mean number (+SE) of aggressive acts in the mix treatment by (a) chinook salmon and (b) steelhead against individuals of both species and (c) the number by species of nonrecipient fish responding to aggressive acts when steelhead attacked chinook salmon or other steelhead at four observation times after loading fish into experimental tanks. Means and standard errors are based on the number of aggressive acts by each species during 15 min of videotaped activity at each observation time in 10 trials. Note the differences in the y-axis scales. Asterisks between bars denote significant differences (Mann–Whitney U-test) between adjacent pairs: \( P < 0.05^* \) and \( P < 0.01^{**} \).

The chinook salmon with only conspecifics (Friedman ANOVA: \( P > 0.05 \) for group and individual). Aggressive acts by dominant steelhead in both the mix and the steelhead-only treatments tended to peak 27 h after loading, but the results were inconclusive due to large variation.

As might be expected, as the number of aggressive acts increased, the number of nontargeted individuals that were affected (nonrecipient fish showing a startle or flee response) also increased. The mix treatment had a greater number of affected nonrecipient fish than the chinook-salmon-only treatment at the 8-, 27-, and 32-h observation times \( (P < 0.001 \) for all three observation times), which corresponds to a greater number of aggressive acts. Similar results were obtained in comparing the steelhead-only and mix treatments. However, the number of affected nonrecipient fish per aggressive act was not different between compared treatments \( (P = 0.91 \) for the mix and chinook-salmon-only treatments, and \( P = 0.46 \) for the mix and steelhead-only treatments).

Within the mix treatment, steelhead affected more nonrecipient fish per aggressive act than chinook salmon \( (P < 0.01 \) for the 3-h and 27-h observations; \( P < 0.001 \) for the 8-h and 32-h observations). In general, steelhead in the steelhead-only treatment did not affect more nonrecipient fish per aggressive act than those in the mix treatment \( (P > 0.05 \) for all), but the dominant steelhead in the steelhead-only treatment did affect more nonrecipient fish per aggressive act than the dominant steelhead in the mix treatment at the 32-h observation time \( (P = 0.03) \). The chinook salmon in the chinook-salmon-only treatment affected more nonrecipient fish per aggressive act than those in the mix treatment \( (P < 0.04 \) for all time periods except 27 h; \( P = 0.12 \) for 27 h).

Within the mix treatment, nonrecipient chinook salmon generally responded more often to an aggressive act on a neighboring fish than did steelhead (Figure 4c). When the neighboring recipient fish was a chinook salmon attacked by a steelhead, nonrecipient chinook salmon tended to respond in greater numbers than nonrecipient steelhead. When the neighboring recipient fish was a steelhead attacked by a steelhead, nonrecipient chinook salmon and steelhead responded in equal numbers. When chinook salmon were the aggressor, they usually attacked only their conspecifics, and only nonrecipient chinook salmon responded during these aggressive acts.

There were few differences in the number of darting acts between treatments. Only the 32-h observation time showed a difference in the number of such acts between the mix and the steelhead-only treatments (Figure 5a). Within the mix treatment, chinook salmon darted less than steelhead.
after the first observation time (Figure 5b). The mean number of darts per chinook salmon was not different between the mix and chinook-salmon-only treatments ($P > 0.10$ for all). The mean number of darts per steelhead in the steelhead-only treatment was lower than that in the mix treatment at the 32-h observation time ($P = 0.03$). There was no change in the number of darts or the number of darts per fish over the 32 h of a trial in any of the treatments or for chinook salmon or steelhead within the mix treatment (Friedman ANOVA: $P > 0.05$ for all).

The number of fish responding (showing a startle response) to the darting acts of another individual was analyzed between treatments and species. Darting acts by fish in the mix and chinook-salmon-only treatments produced a similar number of responding fish (Figure 5c). Fewer fish responded to darting acts in the steelhead-only treatment than in the mix treatment. When either steelhead or chinook salmon in the mix treatment darted, fish responded in equal numbers ($P > 0.20$ for all). When chinook salmon in the mix treatment darted, fewer fish responded than when those in the chinook-salmon-only treatment darted at 27 h ($P = 0.02$). When steelhead darted in either the mix or steelhead-only treatment, there was no difference in the number of fish responding ($P > 0.10$). There was no significant change in number of fish responding to a dart over time in any of the treatments or for chinook salmon and steelhead within the mix treatment (Friedman ANOVA: $P > 0.05$ for all).

**Physiological Responses**

Chinook salmon were unstressed prior to the addition of steelhead or other chinook salmon, as indicated by relatively low baseline cortisol concentrations (Figure 6). Baseline cortisol concentrations were similar in tanks that subsequently received different treatments (ANOVA: $P = 0.53$). After initiation of the experiment (addition of fish), cortisol concentrations in chinook salmon differed between treatments (RM ANOVA: $P = 0.01$) and sampling times ($P = 0.001$). Cortisol concentrations also differed between the two experimental runs ($P = 0.0002$), with overall higher values in run 1 (the timing of this run suggests that juvenile fish were smolting). All interactions between the experimental run and other factors (experimental run × treatment, experimental run × time) were insignificant. However, the sampling time × treatment interaction was significant ($P = 0.04$), so separate ANOVAs were performed for each of the
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**Discussion**

In this study, the presence of steelhead caused adverse behavioral and physiological responses in chinook salmon. The results suggest that behavioral changes and elevated levels of plasma cortisol in chinook salmon resulted from aggressive acts by steelhead. Chinook salmon and steelhead exhibited different social behaviors: chinook salmon exhibited many of the characteristics of a shoaling species, but steelhead exhibited territory-holding characteristics. In addition, chinook salmon were largely passive, while steelhead were aggressive. Steelhead initiated aggressive acts more frequently and affected more fish per aggressive act than did chinook salmon. Aggressive acts by steelhead consisted of charges and long chases, often ending with physical contact, nipping, or collisions. Steelhead attacked chinook salmon as often as they attacked other steelhead, even though chinook salmon were never seen to provoke aggressive acts by aggressive acts or signals. Instead, chinook salmon were attacked when they approached a territorial steelhead too closely. Aggressive acts by chinook salmon were exclusively nips (most were noncontact), which appeared to be attempts to enforce a minimum distance between fish rather than to establish dominance or defend territories. Chinook salmon rarely attacked steelhead or reciprocated aggressive acts by steelhead.

In the absence of steelhead, chinook salmon moved about actively and frequented all areas of the tank, choosing the shaded area most of the time. In the presence of territorial steelhead, however, chinook salmon aggregated tightly in the well-lighted middle section of the tank, spending most of the time hovering in one position and rarely moving, either individually or as a group, into other areas. We hypothesize that chinook salmon held with steelhead reduced their normal activity level so as not to provoke aggressive acts by territory-holding steelhead. Under the experimental conditions, however, the aggregating behavior of...
Chinook salmon appeared to increase their vulnerability to aggressive acts by steelhead. Unlike some subordinate steelhead, chinook salmon seemed unwilling to spread out and move to the surface of the water or edges of the tank to avoid aggressive acts. The chinook salmon’s aggregate behavior placed them in close proximity to steelhead holding territories in the shaded end of the tank or near the tank’s standpipe and made them vulnerable to aggressive acts from several directions. At the 32-h observation period, chinook salmon held with steelhead were subject to 16 times as many aggressive acts as chinook salmon held with only conspecifics. Aggregation appeared to be a more powerful requirement for chinook salmon than the requirement for space or the need to escape from aggressive acts.

Additional changes were observed in the behavioral repertoire of chinook salmon in the presence of steelhead. When a chinook salmon nipped another (steelhead were rarely the target), fewer nonrecipient chinook salmon responded to the aggressive act if steelhead were present. A chinook salmon responding to a nip by quick movements could provoke aggressive acts by steelhead, and darting near or through a steelhead territory could also provoke an aggressive response. Chinook salmon may have perceived a nip by a conspecific as a minor threat compared with that of an aggressive act by a steelhead.

Chinook salmon that experienced the loading of steelhead into their tank were stressed, as judged from significantly elevated levels of plasma cortisol. There were some differences in cortisol dynamics between the two runs of the physiological experiment, likely reflecting the responses of chinook salmon to variable steelhead aggression. In the behavior experiment, the number of aggressive acts within the mix treatment (by both species) ranged from 3 to 126 at the 32-h observation period. In addition, we observed great variability in the time at which steelhead first established territories: In some trials, territories were established as early as the first period of observation, but in one trial, the fight for the shaded territory appeared to be just beginning at the 32-h observation period. In the physiological experiment, differences in aggressive behavior by individual steelhead and in the timing of hierarchical fighting among steelhead may have resulted in differing stress responses by the chinook salmon present. Although the addition of chinook salmon in experimental run 2 resulted in a stress response by the original chinook salmon at the 2-h sampling time, this response was smaller and more transient than the responses to the addition of steelhead.

Our experimental results support field studies that have produced indirect evidence suggesting that chinook salmon are stressed by being transported in barges with steelhead. Maule et al. (1988) observed a larger elevation of plasma cortisol in yearling chinook salmon passing through the McNary Dam collection system in May, when steelhead were present, than in physiologically similar fall chinook salmon passing through the collection system in June, July, and August, when steelhead were absent or present only in small numbers. Congleton et al. (2000) reported that stress indices were higher for yearling chinook salmon transported from Lower Granite Dam in midseason, when steelhead densities were relatively high, than for those transported earlier or later. Moreover, cortisol concentrations in chinook salmon transported to Bonneville Dam were highly correlated with steelhead loading densities but not with chinook salmon densities or smoltification indices.

Several factors are likely to contribute to stress responses in chinook salmon during barge transportation. During the peak spring migration season (late April to mid-May), juvenile steelhead make up a large percentage of the fish migrating or transported on the Snake and Columbia rivers (Gessel et al. 1985; Verhey et al. 1998; Congleton et al. 2000). In our behavioral experiment, chinook salmon and steelhead were represented in equal numbers (10 of each species) in the mix treatment. When the number of steelhead was increased from 10 in the mix treatment to 20 in the steelhead-only treatment, aggression by dominant steelhead increased. Dominant steelhead in the mix treatment delivered a quarter of the total number of aggressive acts, while dominant steelhead in the steelhead-only treatment delivered 60% of the aggressive acts. Thus, a dominant steelhead held with 19 conspecifics in the steelhead-only group had to defend its territory more often than one held with 9 conspecifics and 10 passive chinook salmon in the mix group. It is unknown whether this finding is a function of an increase in the number of aggressive steelhead or a decrease in the space available for each steelhead. Li and Brocksen (1977) found a similar increase in aggression by dominant fish as the density of conspecifics increased. Chinook salmon collected and transported with higher numbers of steelhead could experience more aggression and larger physiological responses than
those collected and transported with lower numbers of steelhead.

Size differences between the species might be another factor contributing to stress responses by chinook salmon held or transported with steelhead. Hatchery steelhead are the largest juvenile salmonids collected and transported during the spring; hatchery chinook salmon are considerably smaller, and wild chinook salmon are the smallest of the three (Congleton et al. 2000). Although predation on chinook salmon by juvenile steelhead is believed to be rare, chinook salmon could perceive large hatchery steelhead as life threatening.

Hatchery steelhead may be more aggressive than wild steelhead toward chinook salmon. Several studies have shown that aggressive interactions between hatchery salmonids occur more frequently (Mesa 1991) and are longer in duration (Swain and Riddell 1990) than aggressive interactions between wild salmonids. Noble (1991) found that the aggressive behavior of hatchery salmonids was directed at wild counterparts more often than at hatchery counterparts (hatchery and wild salmonids were present in equal numbers). McMichael et al. (1999) found that hatchery steelhead smolts released into a natural river system were highly aggressive and dominated wild steelhead. Further, they observed that the aggressive behavior of hatchery steelhead often involved physical contact but that the aggressive behavior of wild steelhead consisted largely of noncontact displays. In a study conducted in a format similar to that of our behavioral experiment, the frequency of aggressive acts by hatchery Atlantic salmon S. salar increased as loading density increased; the opposite trend was seen with wild Atlantic salmon (Fenderson and Carpenter 1971).

Although wild juvenile steelhead and chinook salmon migrate down the Columbia River at approximately the same time, they are rarely seen in the same microhabitats together within rearing streams (Edmundson et al. 1968; Leitzinger 1992; Roper et al. 1994; McMichael and Pearsons 1998). Differences in life history patterns and use of physical habitat allow the two species to coexist within stream systems with little or no interaction (Everest and Chapman 1972; Hearn 1987; Hearn et al. 1987). There are also indications that salmonid species may continue to segregate as they emigrate as smolts in major river systems (Dauble et al. 1989) and estuaries (MacDonald et al. 1987). Our experiments indicate that when juvenile steelhead and chinook salmon are collected and held together in raceways, trucks, and barges in unusually close confinement, behavioral interactions may occur that occur rarely or not at all under natural conditions.

The possibility of aggressive interactions between steelhead and chinook salmon, and especially between hatchery steelhead and wild chinook salmon, is cause for concern because fish that are under abnormal or continuous stress show adverse physiological and behavioral changes. Transported salmonids are exposed to multiple stressors (Specker and Schreck 1980; Barton et al. 1986; Maule et al. 1988; Congleton et al. 2000). Holding and transporting chinook salmon with steelhead appears to be a major stressor, potentially reducing the capacity of chinook salmon to perform vital metabolic and behavioral activities during and after transport. Acute stressors cause transitory disturbances in metabolic and immunological functions and in behavior; chronic stressors (imposed for weeks or longer) repress immune functions (Pickering 1993), retard growth and development (Schreck 1993) and can inhibit smoltification (Schreck et al. 1985). Chinook salmon are confined with steelhead for 36–60 h during collection and transportation, so that the exposure period is intermediate between acute and chronic stress (Congleton et al. 2000). Because it has long been suspected that chinook salmon are stressed by being transported with steelhead (McCabe et al. 1979), Park et al. (1983) recommended that the two species be separated prior to holding and transport. Our research supports this recommendation.

Two-stage submerged-bar separators (Gessel et al. 1985) are used at three of the four fish collection facilities on the Snake and Columbia rivers (Little Goose, Lower Monumental, and McNary dams) to partially separate smaller and larger fish prior to transport, but they do not do so very efficiently. For example, only 32% of the yearling chinook salmon at McNary Dam entered the “small fish” side of the separator in 1994; the remainder entered the “large fish” side, along with the majority of the juvenile steelhead (B. Eby, U.S. Army Corps of Engineers, personal communication). A 1997 study of the Little Goose separator (Congleton and LaVoie 2000) found that 58–69% of chinook salmon entered the “small fish” side of the separator. Larger and smaller fish are not currently separated at the fourth fish collection facility, Lower Granite Dam.

Our experiments indicate that chinook salmon that are transported with steelhead may experience frequent aggressive acts, especially since aggression by steelhead is stimulated by a large numbers
of conspecifics. Aggression by steelhead results in stress responses in chinook salmon that may compromise performance and reduce survival. Improvement of the procedures for separating smaller and larger salmonids prior to holding and transportation is desirable to reduce the possible adverse effects of the transportation system on juvenile chinook salmon.

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