

EFFECTS OF REARING DENSITY ON INDICES OF SMOLTIFICATION AND PERFORMANCE OF COHO SALMON, *ONCORHYNCHUS KISUTCH*¹

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ABSTRACT

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Effects of raceway rearing density on coho salmon, *Oncorhynchus kisutch*, were evaluated at Eagle Creek National Fish Hatchery at the time production fish were released from the facility. The production rearing density, compared with lower densities (one-third and two-thirds), appeared to delay or impair smoltification as judged by lower plasma thyroxine levels, gill (Na+K)-ATPase activity, and blood sodium regulatory ability in fish at the higher density. High rearing density also lowered the capacity of the fish to resist the pathogen *Vibrio anguillarum*. Crowding seemed to elevate corticosteroid clearance rate, but appeared to have no measurable effect on plasma cortisol concentration, diameter of nuclei of interrenal cells, or hemoglobin components. Leucocrit, but not hematocrit, tended to covary with density. These data suggest that rearing densities can affect development of the fish and that the tests used have heuristic potential for evaluating crowding levels at hatcheries.

INTRODUCTION

It is well established that pond loading levels of hatchery stocks of salmonids can affect production (Piper, 1970, 1972; Westers, 1970; Brauhn et al., 1976; Banks and Fowler, 1982) and performance (Sandercock and Stone, 1982). For the last several years, coho salmon (*Oncorhynchus kisutch*) at Eagle Creek National Fish Hatchery near Estacada, Oregon, were reared at different densities to help define optimum pond loading levels. Our

specific objective was to develop criteria to define smolt quality relative to some performance characteristics affected by pond loading density.

The basic premise on which our tests were founded rests on the observations that hatchery practices can affect the parr-smolt transformation process and the performance of anadromous salmonids subsequent to release (Schreck, 1981, 1982). Elevated rearing densities may thus represent a form of stress, or less than healthful situation, for the fish. In support of this contention, Fagerlund et al. (1981) showed that coho salmon living in crowded raceways developed clinical signs of stress (hypertrophied interrenal cells). The environmental factors affecting smoltification were reviewed by Wedemeyer et al. (1980).

We thus ran a set of tests evaluating the clinical signs of stress and performance capacities of fish reared at the hatchery under various densities, to establish their potential fitness once released. Because timing of release is critical to performance of the salmon, we also ascertained the "state of smoltification" of the fish at release by clinical means, recognizing that interpretation of candidate smolt-indicators is highly subjective. Possibly, fish of different sizes at a particular density respond differently (Fagerlund et al., 1981). We therefore evaluated our data to determine not only if density affected mean values of various parameters for a particular population of fish, but also if crowding affected variability among individuals.

METHODS AND MATERIALS

Tests at Eagle Creek National Fish Hatchery

Coho salmon at Eagle Creek were raised in duplicate raceways at density factors calculated to yield 0.15, 0.30, and 0.45 which represent total pounds of fish divided by the product of cubic foot of raceway and length of fish at release (6 May 1982) in inches. English units are employed because the density factor was developed using these units (Piper, 1970, 1972), and conversion to metric would be confounding. However, appropriate values for rearing conditions, allowing calculation of density factors in metric terms, were for the average of the two raceways at each density: low density, 654 kg of fish at 15.1 cm length; medium density, 1281 kg of fish at 14.6 cm length; and high density, 1867 kg of fish at 14.2 cm length at the time of release. The flowthrough raceways were 45.4 m³. The latter density factor of 0.45 defines the typical production load used at this hatchery. The fish were fed Oregon Moist Pellets (OMP) at a rate calculated to result in fish of equal sizes in the three density groups at the time of release. Blood and tissue samples were collected from about 15 fish per raceway on 29 April 1982. Fish were killed by a blow to the head, weighed, measured, sexed, and bled into heparinized tubes. Plasma was separated by centrifugation and stored frozen at -20°C until analyzed. Other blood samples were collected for determination of hematocrit and

leucocrit. Head kidneys were also taken from the fish. On 5 May 1982, whole blood was collected from fish for hemoglobin typing.

Saltwater challenge tests were conducted at Eagle Creek by placing 10 fish from each of the six raceways, representing the three densities, into individual 19-l buckets filled with solutions of 31.0–32.3 g Instant Ocean[®]/l of fresh water. Blood was collected from the fish 24 h later for the determination of total plasma sodium.

The influence of rearing density on the ability of the fish to pay-back an oxygen debt was determined the day before release. Groups of fish from each of six raceways were suspended in a net in the air for 15 s, after which they were placed in buckets of aerated fresh water to recover. A group of fish was bled 10 min and another group 5.5 h thereafter to determine their plasma lactic acid content.

Test at the Marine Science Center

To determine the growth capacity of fish from the various densities after entry into sea water, we trucked fish from each of the six raceways to Oregon State University's Marine Science Center at Newport in individual tanks on 29 April 1982. Upon arrival, fish from duplicate densities were pooled and stocked at the rate of about 50 individuals each into three tanks containing fresh water (one for each original density treatment) and fed to satiation with OMP. The fish were weighed and measured 1 week later, after which the fresh water was turned off and salt water (about 29 ‰ salinity) was turned on. After 1 month (8 June) the fish were again weighed and measured to determine their growth in sea water.

The effect of density on the ability of the fish to resist the bacterial pathogen *Vibrio anguillarum* was evaluated by transporting fish from each raceway to the Marine Science Center and placing them in 85-l troughs of flowing fresh water (14°C), maintaining their respective original densities with duplicate troughs per raceway (i.e., four troughs per density). The fish were challenged on 18 May with *Vibrio anguillarum* (type I in TSB) at a rate of about 6.34×10^6 cells per ml of water. The water flow was turned off for 30 min after the bacterial broth was added. One trough representing each raceway was then switched to flowing sea water to determine if the disease resistance differed between fish held in fresh water and those held in sea water.

Tests at Smith Farm

To conduct tests that could not be run at the production hatchery, to replicate the production situation, and to evaluate effects of an extremely high density in addition to those tested at Eagle Creek, we brought fish from Eagle Creek to Oregon State University's Smith Farm facility, Corvallis, on 12 January 1982. We recognize that densities established at one

facility cannot be duplicated in a strict sense at any other facility because each location is unique. The fish were placed in individual flowthrough circular tanks (diameter 0.9 m) at Smith Farm. The volume of water in the tanks was approximately 0.31 m³, and the water flow was set at 15 l/min. The density factors [lbs/(ft³·in)] at the end of the experiment were 0.11, 0.29, and 0.43 for the low (L), medium (M), and high (H) density, roughly corresponding to loading levels at Eagle Creek at that time, and 0.55 for a very high (VH) density. Fish were fed OMP twice daily to satiation. The water flow in the tanks was increased for a brief period during the spring to avoid gas supersaturation. The flow was restored to 15 l/min on 21 April.

All experiments were conducted during the same week in which the fish at Eagle Creek were released (4–8 May) to migrate to the ocean. Ten fish from each of the four densities were sampled to determine plasma levels of cortisol and thyroxine, gill (Na+K)-ATPase activity, and diameters of interrenal cell nuclei. Clearance rate of cortisol (total radioactivity after injection of ³H-cortisol) and sodium regulatory ability following seawater challenge were also estimated. Mean length, weight, and condition factor for fish from each treatment were determined.

Assays

Plasma cortisol was determined by a modification of a radioimmunoassay developed by Foster and Dunn (1974) in 10 μ l plasma (Redding et al., 1984b). Thyroxine was assayed in 10 μ l plasma as outlined by Dickhoff et al. (1978) and modified by Specker and Schreck (1982). Lactic acid was measured in 10 μ l plasma according to the methods of Passonneau (1974).

To determine the kinetics of cortisol and its metabolites, we injected 3.6 μ Ci (about 12.2 ng) of 1, 2, 6, 7 ³H-cortisol (Research Products International), without unlabeled cortisol, intracardially into individual fish, and sampled the blood from 7–10 fish each from the severed caudal peduncle at 1, 4, and 8 h after the injection. Plasma was solubilized in 200 μ l Protosol[®], and activity determined in a scintillation spectrophotometer. We realize that clearance characteristics of hormones are best determined by serial monitoring of individual fish, but this was not feasible because of the small size of our fish. Unfortunately, we could not ascertain the nature of the radioactivity, but we have evidence indicating the cortisol is rather rapidly converted primarily to cortisone (Patino, 1983).

The data were analyzed according to the one-compartment model of Normand and Fortier (1970). Linear regression analysis showed that the decline of radioactivity in plasma followed a straight line in a semilogarithmic plot (r^2 : 0.80–0.91). Therefore, the slopes and intercepts of the decline curves were compared by using the general linear test approach to multiple regression with indicator variables (Neter and Wasserman, 1974). The inverse

of the area ($1/A$) under the clearance curves was also calculated, as described by Normand and Fortier (1970), and the values obtained were divided by the value corresponding to the low density factor to express the results in relation to the lowest density. The final values thus obtained were referred to as the relative plasma clearance rates.

The ability of the fish to withstand a seawater challenge was tested by subjecting them to a 24-h artificial seawater challenge (Instant Ocean[®]; 30–31 g/l). Mortalities and behavior of the fish were recorded and the blood of survivors was sampled to determine plasma Na^+ levels on a Perkin-Elmer Coleman flame spectrophotometer.

ATPase activity was measured in gills from fish reared at Smith Farm by the method of Johnson et al. (1977).

For hemoglobin typing, blood was drawn from the severed caudal artery into heparinized capillary tubes. Blood samples were spun at $700 \times g$ for 5 min at 0°C in a Beckman TJ-6R centrifuge. The hemolyzate was prepared by methods adapted from Fyhn et al. (1979) and Riggs (1981). Mini-slab-gel electrophoresis was conducted on an apparatus made by Idea Scientific Company, Corvallis, Oregon.

To distinguish each hemoglobin component, we calculated relative mobilities as the ratio of the migration distance of the hemoglobin component to the migration distance of the BSA marker protein in the same sample of gel. We determined relative concentrations of each hemoglobin component stained by scanning each individual column of the polyacrylamide slab gels at 560 nm, using a Beckman spectrophotometer with an attached Gilford linear transport. The resulting scan tracings were resolved into individual peaks manually, when needed. Areas under the absorption peaks were determined by cutting and weighing individual peaks on a Metler analytical balance.

Results of the tests other than cortisol clearance rate determinations were analyzed with ANOVA, and if significant differences were found, the means were compared using Duncan's multiple range test. Chi square tests were used to compare proportionate data.

RESULTS

Clinical tests

Inasmuch as results of replicate treatments were similar, we pooled the data for each treatment. Plasma thyroxine levels were inversely related to rearing density, both in fish reared at Eagle Creek and at Smith Farm (Fig. 1); titers were significantly higher in fish held at low densities ($P < 0.05$). This relation was also evident in gill (Na+k)-ATPase activity among fish at Smith Farm; the trend was for fish held at the lower densities to have the higher enzyme activities (Fig. 1), although this trend was not significant. However, correlation analysis showed significant covariances ($P < 0.05$)

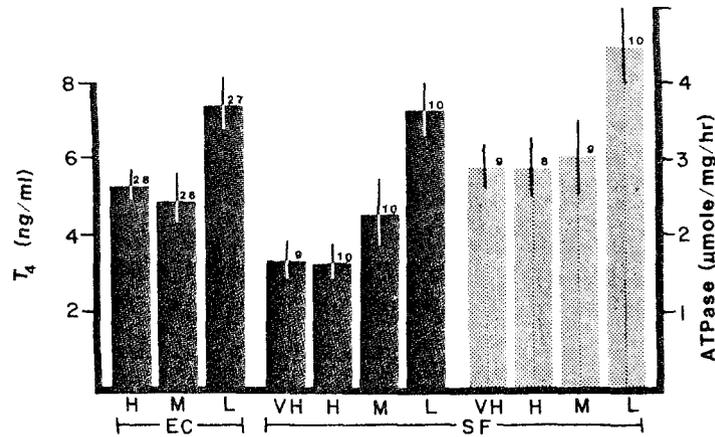


Fig. 1. Mean (\pm S.E.) plasma thyroxine level (data from replicate raceways pooled, Eagle Creek) and gill (Na+K)-ATPase activity (stippled bars) in Eagle Creek coho salmon raised at Eagle Creek (EC) at high (H), medium (M), and low (L) densities (0.45, 0.30, and 0.15 total wt in lb/[volume in ft³ mean fork length in inches], respectively) and at Smith Farm (SF) at very high (VH), high (H), medium (M), and low (L) densities (0.55, 0.43, 0.29, and 0.11, respectively) at the time of release. Sample sizes given above each bar.

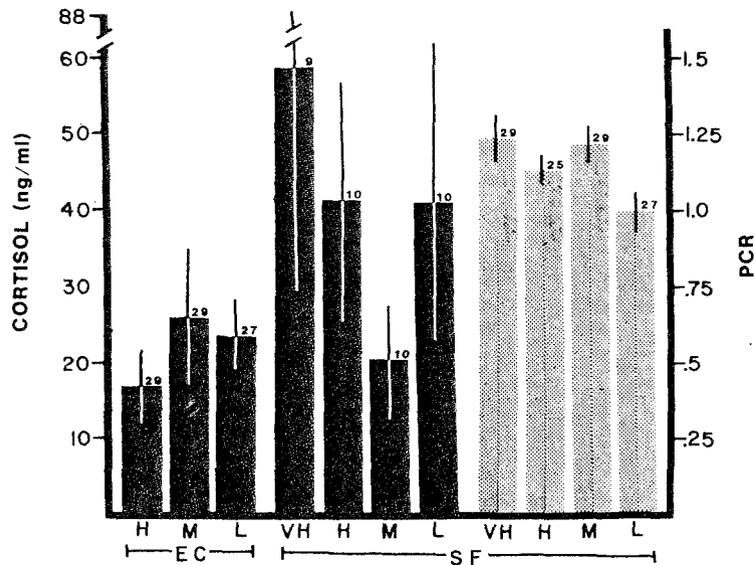


Fig. 2. Mean (\pm S.E.) plasma cortisol levels (replicates pooled, Eagle Creek) and relative plasma clearance rate for cortisol and its metabolites (PCR) (stippled bars) at the time of release. Relative PCR's were employed since clearance curves generated from only three time-points are insufficient to calculate true clearance rates. However, since the areas under the clearance curves are inversely proportional to clearance rates, we expressed these data relative to those of the fish reared at low density. That is, the inverse of the areas under the clearance curves for each treatment was divided by the area under the curve for the low density group to yield the PCR's reported here. See Fig. 1 for key. Sample sizes given above each bar.

between plasma thyroxine and gill ATPase activity when all groups were analyzed simultaneously, but not when groups were considered separately. The trend for ATPase activity to vary inversely with density was also evident in fish reared at Eagle Creek (W.S. Zaugg, personal communication, 1982).

Plasma cortisol levels did not vary significantly among the fish held at different densities either at Eagle Creek or at Smith Farm (Fig. 2). Individual cortisol levels were not correlated with fish size. Variability in cortisol was inconsistent between duplicate raceways; it appeared that density did not measurably affect the between-fish variability ($P < 0.05$).

Diameters of nuclei of interrenal cells also did not vary significantly between density groups at either Eagle Creek (averages 6.34, 6.13, and 6.21 μm for fish reared at low, medium, and high densities, respectively) or Smith Farm (average 5.75 and 5.78 μm for fish held at low and very high densities). Variability about the means was inconsistent between replicates and could not be attributed to treatment effects. Interrenal nuclear size could not be correlated with individual fish size within a density group, even when only the smallest and largest individuals were used in the analyses.

The relative plasma clearance rate of corticosteroids was lower in fish reared at the low density than in those reared at very high densities (Fig. 2, t' -test, $P < 0.05$). The slopes of the clearance curves of the ^3H -cortisol and labeled metabolites did not vary with density ($P > 0.05$). However,

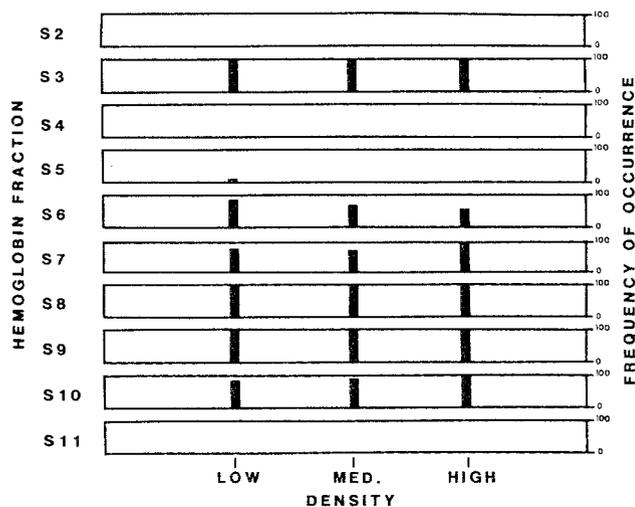


Fig. 3. The frequency expressed as a percent of occurrence of various hemoglobin fractions (referred to as S_3 – S_{11}) — i.e., the number of fish having a particular fraction divided by the total number sampled — in Eagle Creek coho salmon reared at high ($n = 10$), medium ($n = 15$), and low ($n = 11$) densities. Relative mobilities (proportionate distance between hemoglobin band and bovine serum albumin marker on the same gel) for S_3 – S_{11} are 0.44, 0.50, 0.56, 0.61, 0.65, 0.71, 0.81, 0.85, and 0.92, respectively.

the intercepts of the clearance curves for the very high and medium densities were significantly ($P < 0.05$) lower than the intercept for the low density group, indicating that the clearance or metabolism of the hormone may have been faster at the higher densities.

Hematocrit levels did not differ statistically between density treatments, although packed cell volumes appeared to be lower in fish held at the low density at Eagle Creek, where average hematocrits were 29, 36.3 and 35.4% for the low, medium, and high density groups, respectively. Leucocrit appeared to be inversely, but not significantly, related to rearing density; the levels were high for some fish from the low density group (means for low, medium, and high density groups were 0.74, 0.47, and 0.24%, respectively).

Rearing density appeared to have little effect on hemoglobin profile (Fig. 3). Although no fish from the high density raceways completely lacked a band with 0.92 relative mobility, this band was detected in only one fish among those held at each of the other densities.

Performance tests

Among coho salmon introduced into sea water, those reared at lower densities were able to osmoregulate more effectively than those held at higher densities. This difference was evident in fish held both at Eagle Creek where differences were just barely not significant ($P > 0.05$) and at Smith Farm where they were significant ($P < 0.05$) (Fig. 4). After the fish had been exposed to sea water for 24 h, the plasma sodium levels of those reared at low density had returned to a value below or near the 170

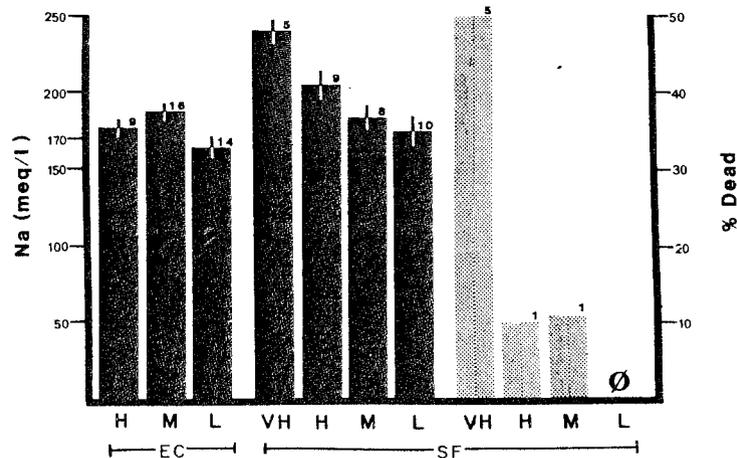


Fig. 4. Mean (\pm S.E.) plasma sodium level (replicates pooled, Eagle Creek) and % dead (stippled bars) coho salmon following 24-h seawater challenge at the time of release. See Fig. 1 for key. Sample size given above each bar.

meq/l, a level perhaps considered characteristic of smolts (Clarke and Blackburn, 1977). Fish held at other densities had proportionately higher sodium levels. Also, at Smith Farm, the mortality due to 24-h seawater challenge was substantial among fish from the high density group, but was less for fish held at lower densities and nil among fish held at low density (Fig. 4). Handling the fish and placing them in the test containers did not confound results; the plasma sodium levels in fish placed in containers of fresh water were similar after 24 h, i.e. independent of rearing density and comparable to levels in fish sampled from the raceways.

Although the fish had been fed at rates calculated to result in similar growth between densities, the fish reared at the low density at Eagle Creek were just slightly, but significantly (*f*-test), larger. The fish from the low, medium, and high densities averaged 26.4 (\pm 0.5 S.E.), 25.1 (\pm 0.4 S.E.), and 24.7 (\pm 0.4 S.E.), respectively. In fish at Eagle Creek, a test of skewness (Sokal and Rohlf, 1969) showed that fish reared at low density had weights significantly (although just barely at $P = 0.05$) skewed to the right. Weights of fish reared at medium density were not (although just barely not significant at $P = 0.05$) skewed; neither were those of fish reared at high density. These very slight differences in growth were apparent only when analyzing the total of fish sampled in this project; size of fish did not differ meaningfully between groups within any one test. At Smith Farm, fish at very high density appeared to grow significantly ($P < 0.05$) less than those at lower densities; their average weight was 28.3 g compared with 34.7, 28.9, and 32.9 g, for fish reared at low, medium, and high densities, respectively. Inexplicably, when fish from Eagle Creek were stocked into seawater tanks at the Marine Science Center, those originating from the high density raceways grew fastest, adding 16% to their weight at stocking, compared with 3% and 8% for fish held at low and medium densities, respectively.

Plasma lactic acid levels following hypoxia for 15 s did not differ between fish reared at the three densities at Eagle Creek. Resting lactate levels became substantially elevated due to the suspension of the fish in the air, as evidenced by the high titers at 10 min, averaging 55.1 (\pm 3.5 S.E.), 50.4 (\pm 2.8 S.E.), and 58.9 (\pm 3.2 S.E.) mg/100 ml in fish held at low, medium, and high densities, respectively. The fish appeared to be recovering to their resting levels within 5.5 h, when levels averaged 15.2 (\pm 3.1 S.E.), 7.8 (\pm 1.2 S.E.), and 10.6 (\pm 1.2 S.E.) for the low, medium, and high densities, respectively.

Fish reared at the low density were significantly more resistant to *Vibrio* in sea water than were those raised at higher densities (Fig. 5). Disease resistance was poorest among fish from the two high-density raceways. (Chi-square analysis showed highly significant differences between mortality of fish held at low and at high densities). The pathogen was insufficient to cause enough mortality to allow conclusions regarding the fish challenged while in fresh water.

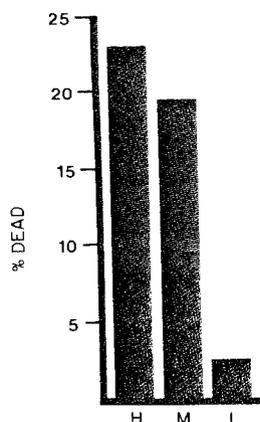


Fig. 5. Mortality (percent dead) of coho salmon raised at high (H), medium (M), and low (L) densities (0.45, 0.30, 0.15) exposed to *Vibrio anguillarum* in sea water (replicates pooled).

DISCUSSION

Loading densities evaluated at Eagle Creek, Smith Farm, and the Marine Science Center appeared to affect development of coho salmon, the lower densities yielding the more "advanced" fish at the time of release. This difference was evident from the higher plasma thyroxine titers, gill (Na+K)-ATPase activities, and sodium regulatory abilities after seawater challenge in fish reared at the lower densities, suggesting that rearing density affected development — inasmuch as parr-smolt transformation may be associated with increases in plasma thyroxine, gill (Na+K)-ATPase activity (Folmer and Dickhoff, 1980, 1981; Zaugg, 1982) and sodium regulatory ability (Clarke and Blackburn, 1977). However, the tight coupling of these "smolt markers" may vary between stocks and species (Ewing and Birks, 1982).

Plasma concentrations of cortisol may also change during smoltification of coho salmon (Specker and Schreck, 1982; Specker, 1982; Patino, 1983). We detected no influence of raceway crowding level on plasma cortisol concentration — a finding similar to those in another stock of coho salmon (Schreck, 1981) and in channel catfish, *Ictalurus punctatus* (Klinger et al., 1983). Possibly, the cortisol secretion rates by the interrenal were actually lower in fish raised at low density, since the clearance of this steroid appeared to be slower in this group. Changes in cortisol clearance from the plasma appear to be coincident also with smoltification (Patino, 1983). Crowding more severe than the levels tested here is known to stimulate interrenal activity at least temporarily (Schreck, 1981). Adrenal activity reportedly is elevated in overcrowded natural populations of mammals (Christian, 1978), as similarly suggested for fish (George, 1977). We were unable, however, to demonstrate increases in plasma cortisol at the densities tested. Redding et al. (1984a) have shown that chronic crowding

stress results in elevated plasma clearance of cortisol in coho salmon, which would perhaps explain our inability to demonstrate an elevation in plasma cortisol attributable to chronic crowding. The lack of density-effect on size of interrenal cell nuclei, however, seemed to indicate that the crowding did not affect cortisol secretion — at least not markedly. Contrary to findings of Fagerlund et al. (1981), we detected no variable interrenal development associated with fish size at high density.

Hemoglobin profiles of the salmon at the time of release were unrelated to the rearing history, although developmentally associated changes in hemoglobin patterns have been reported for salmonids by others (Giles and Vanstone, 1976; Koch, 1982; Pring, 1984). We found no significant effects of density on hematocrit levels, whereas Klinger et al. (1983) reported an increase in hematocrit of channel catfish, and Burton and Murray (1979) suggested a decrease in hematocrit of goldfish, *Carassius auratus*, with increasing stocking density. Crowding density may affect morphology of catfish erythrocytes (Murray, 1980).

Performance tests suggested that rearing density inversely affected performance capacities of the fish. Only the fish reared at low densities osmoregulated sufficiently after seawater challenge to lower their blood sodium to levels suggestive of smolts (Clarke and Blackburn, 1977). Crowding also affected the ability of fish to resist disease; fish reared at low densities were much more able to resist the marine pathogen *Vibrio* than were those reared at higher densities. It would be interesting to know if the seemingly inverse correlation between leucocrit and density is related to disease resistance. McLeay and Gordon (1977) suggested that leucocrit is depressed by various stresses, including degree of crowding. Leucocyte numbers appeared to be depressed in channel catfish by elevated rearing density, whereas leucocrit was only mildly affected (Klinger et al., 1983). Stress and resistance to disease are related, a relationship apparently modulated through the neuro-endocrine axis involving the interrenal (Ellis, 1981). It would be interesting to know if the suppressive effects of certain hatchery rearing conditions on disease resistance are carried over to influence survival of the fish following their release from the hatchery.

The faster growth of salmon raised under high rather than low densities when the fish were moved to sea water is perplexing — particularly since their growth rates were similar when in fresh water. (A similar phenomenon was noticed by Refstie (1977) in rainbow trout, *Salmo gairdneri*, in fresh water.) Perhaps a history of crowding resulted in fish that were more efficient feeders, or perhaps this result was due to a tank-effect since this performance test was unreplicated. Wedemeyer (1976) showed that transfer of coho salmon and rainbow trout to high density was stressful and inhibited feeding behavior in the salmon, activating corynebacterial kidney disease.

Our failure to find that rearing density measurably affected the variability within a treatment group should not be interpreted to mean that

subtle differences within a group did not exist. It is clear, however, that many of the tests used here were sensitive enough to be useful in demonstrating differences between salmon reared at different densities. A concurrent release experiment at Eagle Creek, in which tagged fish reared under the densities evaluated here were tested, suggested a slight inverse relation between crowding level and yield of adults returning in 1982. These findings would have been predicted by our tests. However, there was no noticeable effect of rearing density on adult return rate in the groups monitored by us (1983 return), perhaps because the total recovery of tagged fish was extremely low (about 0.05% of release) — suggested to be a consequence of El Nino.

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