RESISTANCE OF DIFFERENT STOCKS AND TRANSFERRIN GENOTYPES OF COHO SALMON, *ONCORHYNCHUS KISUTCH*, AND STEELHEAD TROUT, *SALMO GAIRDNERI*, TO BACTERIAL KIDNEY DISEASE AND VIBRIOSIS[1]

GARY W. WINTER,[2] CARL B. SCHRECK,[2] AND JOHN D. MCINTYRE[3]

ABSTRACT

Juvenile coho salmon and steelhead trout of different stocks and three transferrin genotypes (AA, AC, and CC), all reared in identical or similar environments, were experimentally infected with *Corynebacterium* sp., the causative agent of bacterial kidney disease, or with *Vibrio anguillarum*, the causative agent of vibriosis. Mortality due to the pathogens was compared among stocks within a species and among transferrin genotypes within a stock to determine whether there was a genetic basis for resistance to disease. Differences in resistance to bacterial kidney disease among coho salmon stocks had a genetic basis. Stock susceptibility to vibriosis was strongly influenced by environmental factors. Coho salmon or steelhead trout of one stock may be resistant to one disease but susceptible to another. The importance of transferrin genotype of coho salmon in resistance to bacterial kidney disease was stock specific; in stocks that showed differential resistance of genotypes, the AA was the most susceptible. No differences in resistance to vibriosis were observed among transferrin genotypes.

Bacterial kidney disease (BKD) caused by *Corynebacterium* sp. is a major cause of serious losses among salmon reared in freshwater hatcheries of the Pacific Northwest (Leitritz and Lewis 1976), and epizootics caused by *Vibrio anguillarum* in the marine environment are particularly devastating to salmonids maintained in saltwater impoundments (Fryer et al. 1972). Externally applied antibiotics are relatively ineffective in the treatment of these diseases. Immunization with bacterins for the control of vibriosis has been shown to be feasible (Fryer et al. 1976), but attempts to produce a bacterin for BKD have been unsuccessful (Evelyn 1977). The use of disease resistant populations of fish may conceivably reduce the incidence and severity of these diseases. Fish that inherit natural resistance to a disease normally maintain that resistance throughout their lives (Snieszko et al. 1959). In addition, information on the resistance of donor stocks, for use in transplants to infected waters, would be valuable.

The existence of disease resistant strains within a species has been demonstrated. Stock or strain refers to a population of fish of one species which shares both a common environment (a particular stream) and common gene pool (discrete breeding group) and, as such, can be considered as a self-perpetuating system (Larkin 1972). Differences in susceptibility to ulcer disease and furunculosis have been observed among different strains of brook trout, *Salvelinus fontinalis* (Wales and Berrian 1937; Wolf 1954; Snieszko 1957; Snieszko et al. 1959), and Gjedrem and Aulstad (1974) noted significant differences in resistance to vibriosis, which they showed to be slightly heritable, between different strains of Atlantic salmon, *Salmo salar*, parr in Norway. Unfortunately, in most previous studies of disease resistance, fish of the different stocks were not reared in a common environment. Since phenotypic expression is a combination of genotype, environment, and interactions between these two variables, different stocks must be reared under identical conditions if one is to be certain that differences in resistance to disease are genetic in origin and not due, for example, to previous exposure of a particular stock to the disease in question or some other factor such as nutritional history. One objective of the present study was to determine whether there are differences in resistance to BKD and vibriosis

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[2]Oregon Cooperative Fishery Research Unit, Oregon State University, Corvallis, OR 97331. Cooperators are Oregon State University, Oregon Department of Fish and Wildlife, and U.S. Fish and Wildlife Service.

[3]Oregon Cooperative Fishery Research Unit; present address: U.S. Fish and Wildlife Service, Box 1050, Tyler Road, Red Bluff, CA 96080.

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among stocks of coho salmon, *Oncorhynchus kisutch*, and steelhead trout, *Salmo gairdneri*, and whether these differences have a genetic basis.

Suzumoto et al. (1977) reported differences in resistance to BKD among three genotypes of transferrin (an iron-binding plasma protein) in coho salmon. In mammals, iron is known to increase the growth and virulence of some pathogens. Transferrin may reduce infection by binding the metal, thereby reducing its availability to invading bacteria, a process known as nutritional immunity (Weinberg 1974). No iron requirement has been demonstrated for BKD bacteria, although it is likely that one exists, judging by the fastidiousness of the organisms. Hershberger (1970) observed differences in iron binding capacity among transferrin genotypes in brook trout and suggested that individuals more efficient in the uptake and release of iron might fare better under "adverse conditions" such as disease. A second objective of this study was to compare resistance to BKD and vibriosis among transferrin genotypes, to evaluate earlier results with BKD, and to determine whether transferrin increases the tolerance of bacterial diseases of salmonids in general. We also sought to determine whether differences in resistance of transferrin genotypes exist among different stocks of coho salmon and steelhead trout.

MATERIALS AND METHODS

Juvenile coho salmon were obtained as eyed eggs from the Fall Creek (Alsea) and Big Creek salmon hatcheries, Oreg. The Big Creek hatchery was also the source of two crosses, Big Creek × Sol Duc (B × S) and Big Creek × Umpqua (B × U). All stocks were reared at Corvallis, Oreg.—the Big Creek stock at Oregon State University's Smith Farm; the Alsea stock at the Oregon Department of Fish and Wildlife's Research Section; and the two crosses at Oregon State University's Fish Disease Laboratory. These rearing facilities presented similar, though not identical, environments for the fish. Because we lacked sufficient fish of the two crosses to include them in all studies, we used them only in the BKD study.

Steelhead trout were obtained as green eggs from the following Oregon State hatcheries: Alsea (winter run), Roaring River (Siletz summer run), Cole Rivers (Rogue summer run), and Marion Forks (North Santiam winter run). All four stocks were reared under identical conditions at Smith Farm.

For determination of the transferrin genotypes of the experimental fish, we withdrew about 0.1 ml of blood from the caudal vein of anesthetized fish with a 1 ml tuberculin syringe and ejected it into heparinized hematocrit tubes, which were then centrifuged. The plasma from the salmon was frozen until the time of analysis. Blood samples from steelhead trout were placed on ice and processed within 4 h after collection because we found that frozen storage reduces the stability of transferrin in this species. Fish were individually identified by dangler tags applied immediately behind the dorsal fin. We used starch-gel electrophoresis, adapting the discontinuous buffer system described by Ridgeway et al. (1970), to determine transferrin genotypes. Only the AA, AC, and CC genotypes were considered, and in some stocks only two of these were used. The transferrins of Siletz and North Santiam steelhead trout stocks were not included in this study because resolution on the electrophoretic gels was poor. After the fish were bled, they were given a recovery period of at least 2 wk before they were transferred to experimental tanks.

Bacterial Kidney Disease

All experimental fish were held indoors in 70 l fiber glass tanks supplied with flowing, aerated, chilled (12°±2° C), dechlorinated water. The fish were allowed to acclimate in these tanks for 2 wk. Fish were fed once daily with Oregon Moist Pellet. Each stock of coho salmon and steelhead trout consisted of 125 fish divided into two test replicates of 50 each plus 25 control fish. Included in the steelhead trout experiment was one group of 34 fish of hatchery-reared (Cole Rivers) Rogue River stock, without a replicate. The respective transferrin genotypes were distributed randomly among all tanks.

The BKD (*Corynebacterium* sp.) strain (RB-1-73) used was isolated on cysteine serum agar from a spring chinook salmon, *O. tshawytscha*, at the Round Butte Oregon State Hatchery by J. E. Sanders, fish pathologist, Oregon Department of Fish and Wildlife. A stock culture was maintained on Mueller-Hinton agar (Difco Laboratories,[4] Detroit, Mich.) enriched

[4]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

with cysteine (0.1%) and calf serum (20%). Before each experiment, cells were passed once in the species being tested to produce a fresh isolate, and this isolate was further cultured until sufficient cells were available for an inoculum.

All test fish received an intraperitoneal injection of 0.1 ml of a suspension of kidney disease bacteria in phosphate-buffered saline (PBS), and all control fish received a 0.1 ml intraperitoneal injection of only PBS. The approximate inocula were 9×10^7 cells for the coho salmon (mean weight, 23 g), and 3×10^8 cells for the steelhead trout (mean weight, 36 g). The coho salmon were injected on 17 March 1977 and the steelhead trout on 12 September 1977. We examined all fish that died and identified BKD as the causative agent on the basis of presumptive diagnosis, using gram stains of kidney smears. In addition, kidney smears from 10% of the fish that died were cultured on Mueller-Hinton media. Experiments were terminated at the end of 4 mo or earlier, depending on the progress of infection.

One week after the coho salmon had been injected, an accidental exposure of the fish, including the controls, to chlorine resulted in mortalities as high as 50% in some stocks. The study was nevertheless continued, but a second, abbreviated test was begun on 24 August 1977. Only Alsea and Big Creek stocks (mean weight, 33.2 g) were used; the Big Creek fish were obtained directly from the hatchery. The inoculum for this second experiment was increased to 3×10^8 cells.

Vibriosis

The *V. anguillarum* strain (LS-174) used in these experiments was isolated on brain heart infusion agar from a coho salmon at Lint Slough, Waldport, Oreg., by J. S. Rohovec. The inocula were either prepared from lyophilized cells or recent passage isolates. Experimental fish were exposed to the pathogen in 93 l stainless steel tanks at Oregon State University's Fish Disease Laboratory.

Two experiments were undertaken with the coho salmon. In the first (8 October 1976), 225 fish (mean weights for Big Creek and Alsea stocks were 10.4 g and 14.5 g, respectively) from each stock were divided equally among two test replicates and an untreated control. The three tanks contained fish from each stock to insure identical treatment. The fish in this experiment, having not been bled and tagged for transferrin genotype identification, were freeze branded to differentiate the stocks in each tank. In the second experiment (10 June 1977) the number of fish per tank was reduced to about 25 (mean weight, 36.6 g) because larger numbers were not available, but transferrin genotypes had been determined.

In the steelhead trout phase of the study (21 October 1977), 75 fish from each stock (mean weight, 36 g) were divided equally among three test replicates and 15 from each stock were placed in a fourth tank for controls. A hatchery-reared Rogue stock was also used in this steelhead trout experiment. In a second experiment (27 December 1977) in which we used steelhead trout from the Cole Rivers (Rogue), Alsea, and Marion Forks (North Santiam) hatcheries, 50 fish (mean weight, 42.2 g) were divided equally between two replicates. Transferrin genotypes were distributed randomly among the tanks.

The initial temperature in all experimental tanks was 12.2° C, to which all fish had been acclimated. The temperature was then raised to 17.7° C over a period of 1.5 h, and at this temperature water flow was discontinued in all tanks for 15 min. The bacteria suspended in brain heart infusion broth (Difco Laboratories) were then introduced into the test tanks (other than those of the controls). The inocula were 5×10^6 cells/ml for the first coho salmon exposure and 8.6×10^6 cells/ml for the second; the steelhead trout received concentrations of 8.8×10^6 cells/ml in the first experiment and 7.2×10^6 cells/ml in the second. All fish that died were necropsied and kidney smears were cultured on brain heart infusion agar. Positive diagnosis of *V. anguillarum* was confirmed by slide agglutination with specific antiserum. The experiments were terminated at the end of 1 wk.

Statistical comparison of three or more stocks involved a one-way analysis of variance based on arcsin transformations of percentages and least significant difference, and comparisons of transferrin genotypes of two stocks were based on χ^2 test employing a $2 \times k$ contingency table (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

Bacterial Kidney Disease

In the first experiment in which coho salmon were infected with BKD, the Alsea stock and B × U cross were about twice as resistant to the disease

as were fish of the Big Creek stock and B × S cross (see totals, Figure 1A). The difference in mortality between the B × U and each of the two more susceptible groups (Big Creek and B × S), was significant ($P<0.05$), but the Alsea mortality was significantly lower than that of only the B × S, cross ($P<0.06$). A comparison of mean times to death (days) revealed a similar pattern: B × S, 79.5; B × U, 99.9; Big Creek, 88.4; and Alsea, 95.4. The mean times to death for the B × U and Alsea coho salmon were significantly greater than the B × S ($P<0.05$). The differential resistance of coho salmon stocks to BKD probably has a genetic basis because the stocks were reared in similar environments.

Among transferrin genotypes, only the B × S cross and Alsea stock showed any important differences in resistance to BKD (Figure 1A). In both groups the AA genotype was the most susceptible, and the AC and CC both showed lower, similar mortalities. The difference in resistance was significant ($P<0.07$) between the AA and AC genotypes within the B × S cross. The Alsea transferrin results, though not significant due to small sample size, are substantiated by a previous study in which Suzumoto et al. (1977)

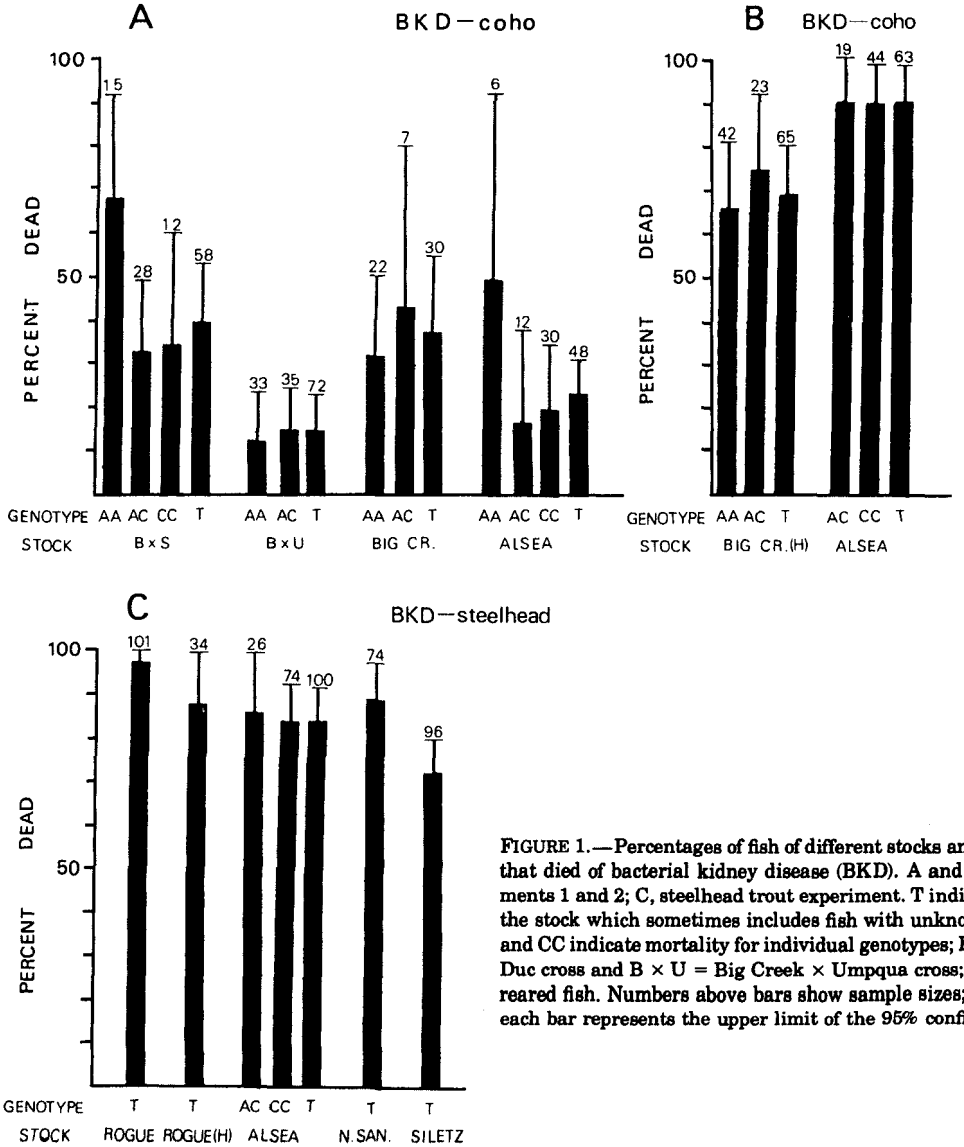

FIGURE 1.—Percentages of fish of different stocks and transferrin genotypes that died of bacterial kidney disease (BKD). A and B, coho salmon experiments 1 and 2; C, steelhead trout experiment. T indicates total mortality for the stock which sometimes includes fish with unknown genotypes; AA, AC, and CC indicate mortality for individual genotypes; B × S = Big Creek × Sol Duc cross and B × U = Big Creek × Umpqua cross; (H) indicates hatchery-reared fish. Numbers above bars show sample sizes; the vertical line above each bar represents the upper limit of the 95% confidence interval.

used Alsea coho salmon in which the AA genotype was also the most susceptible to BKD. Because of similar transferrin results in the B × S cross and Alsea stock, the data were combined. For the combined data, the AC (28% mortality) and CC (24% mortality) genotypes were significantly ($P<0.01$) more resistant to BKD than was the AA genotype (62% mortality). Within both the stocks and transferrin genotypes, differences between replicates were not significant.

The second BKD experiment with coho salmon gave results similar to those of the first on the basis of transferrin genotypes (Figure 1B). Unfortunately, the AA genotype was not included in the Alsea comparison because we lacked sufficient fish. No stock comparison was made because the Big Creek stock came directly from the hatchery, at a time when 91.5% of the mortalities in production fish at Big Creek were due to BKD (J. Conrad[5]). The probability that the Big Creek coho salmon used in the experiment had previously been exposed to BKD was therefore very high.

In the third BKD study, which involved the four steelhead stocks and a second Rogue stock reared at the hatchery (Figure 1C), mortalities in all the test groups began to increase at a high rate 3 wk after the study began because of a secondary infection with *Aeromonas hydrophila*. This trend continued for another 4 wk, at which time mortalities leveled off, and the study was terminated. A comparison of the resistance of the different stocks is not fully valid because the fish in the different test tanks were obviously not challenged equally with a secondary infection of *A. hydrophila*. However, there were no significant differences ($P>0.10$) between replicates, and the mortality of the Siletz steelhead trout (72%) was significantly lower ($P<0.05$) than that of all other stocks except the Alsea. Because mortality in the Rogue stock was extremely high (96%), a transferrin genotype comparison was not considered. The AC and CC genotypes within the Alsea stock were equally susceptible to the double infection of BKD and *A. hydrophila*. Although percentage mortality is a better measure of an organism's ability to tolerate disease, mean time to death is also an indication of resistance to diseases, especially chronic ones such as BKD. There were no differences in mean time to death (days) among either the Rogue or Alsea steelhead transferrin genotypes (numbers of fish in parentheses): Rogue—AA, 28.5 (30); AC, 30.0 (41); and CC, 29.7 (19); Alsea—AC, 30.4 (21); and CC, 30.0 (62). The importance of transferrin was probably reduced by the double infection.

[5]J. Conrad, Oregon Department of Fish and Wildlife, Clatskanie, OR 97015, pers. commun. February 1978.

Vibriosis

In the first experiment in which coho salmon were exposed to *V. anguillarum* (Figure 2A), the Big Creek stock (38% mortality) was significantly more resistant ($P<0.005$) than the Alsea stock (62% mortality) (transferrin was not considered in this comparison). There was a significant difference ($P<0.005$) in mean weight (t'-test, Snedecor and Cochran 1967:114) between the Alsea and Big Creek fish. However, there were no significant differences ($P>0.10$) in resistance to vibriosis among four weight classes (5.1-10.0, 10.1-15.0, 15.1-20.0, and 20.1-25.0 g) within either stock. The difference in resistance between the two stocks appears to be genetic. In a second test, the resistance trend between the Alsea and Big Creek stocks was reversed (Figure 2B), though at a lower level of significance ($P<0.07$) than the previous experiment. However, the Alsea coho salmon used in this second test came directly from the hatchery. Though it is unlikely that any of these fish would have been previously exposed to *V. anguillarum* in freshwater, a difference in susceptibility to vibriosis still existed. These conflicting results thus demonstrate that the environment has a strong effect in determining resistance to vibriosis. In both the Alsea and Big Creek stocks, no differential resistance was shown by the transferrin genotypes, although the AA genotype was not included in the Alsea transferrins (Figure 2B).

In the first of the two vibriosis experiments with steelhead trout (Figure 2C), the North Santiam steelhead trout were the least susceptible to vibriosis of all the stocks ($P<0.05$). The Alsea steelhead trout, though exhibiting a higher mortality (87%) than the North Santiam fish, were still significantly more resistant than the remaining two stocks ($P<0.05$). Because mortality was high in the Smith Farm- and hatchery-reared Rogue stocks (96%), transferrin genotype differences and the effects of rearing environment on resistance were not considered. However, no differences in resistance were observed among genotypes within the Alsea stock. These results using steelhead trout are similar to those observed in the coho salmon exposed to vibriosis.

The second vibriosis experiment (Figure 2D),

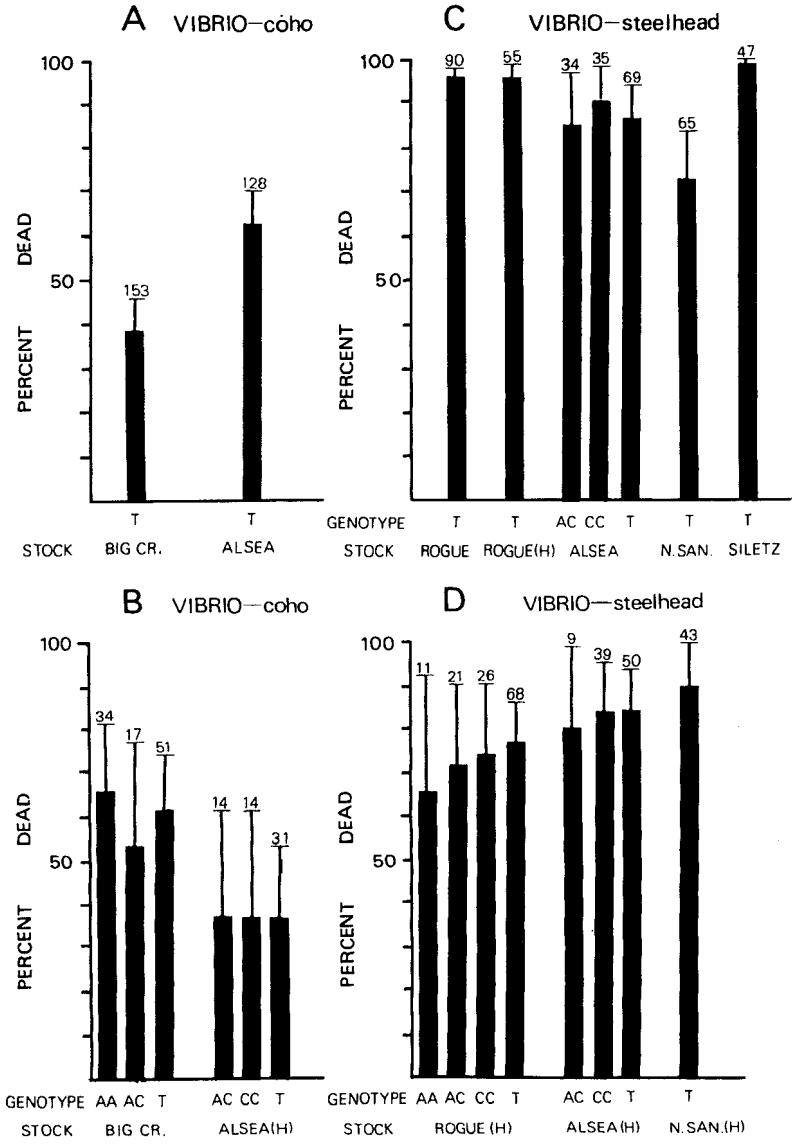

FIGURE 2.—Percentages of fish of different stocks and transferrin genotypes that died of vibriosis. A and B, coho salmon experiments 1 and 2; C and D, steelhead trout experiments 1 and 2. For interpretation of other features see Figure 1.

involving hatchery-reared steelhead trout from the Rogue, Alsea, and North Santiam, revealed the same results as did the first, with respect to transferrin genotypes. No differential resistance was shown among genotypes, including the AA's, within either the Alsea or Rogue stocks. Although resistance to vibriosis among the three stocks was similar, the North Santiam stock showed the highest mortalities this time—which again emphasizes the importance of environmental factors in the determination of resistance and the need for eliminating environmental differences in making genetic comparisons. There was a significant difference in vertebral number between North Santiam steelhead trout reared at the hatchery and at Smith Farm, indicating an environmental difference (our unpubl. data). The Rogue replicates in this experiment were significantly different ($P<0.025$) with respect to stock mortality; consequently a genetic comparison was invalid. Except for the hatchery-reared Rogue replicates in the last vibriosis experiment using steelhead trout, there were no significant differences between replicates for stocks or genotypes in all four vibriosis tests; consequently we combined replicates in the data analysis.

Perhaps stock resistance to acute diseases such as vibriosis depends more on which stock has an environmental advantage at the time of infection, rather than on genetic make-up. Also, when mortalities in experiments are high, resistance comparisons are difficult to make because any immunity that was present may have been overwhelmed. Genetic factors are probably more important in chronic diseases such as BKD. For example, Zinn et al. (1977) observed apparent genetic resistance to infection by *Ceratomyxa shasta,* normally not an acute condition, among hatchery strains of chinook salmon.

It is also evident that a stock may be resistant to one disease and not to another. Although the Siletz steelhead trout were most resistant to the double infection of BKD and *A. hydrophila*, they showed the greatest susceptibility to *V. anguillarum.* Ehlinger (1977) observed that certain selected brook trout strains, though resistant to furunculosis, were more susceptible to gill disease than was the native stock. Consequently selection of stocks for resistance to several diseases would be difficult (McIntyre 1977), except possibly when the pathogens are closely related (Hutt 1970).

Judging by the present results, it appears that the importance of transferrin genotypes in resistance to disease is stock specific. Differences among genotypes were only observed in the Alsea and B × S coho salmon infected with BKD. Weinberg (1974) noted that different host species may vary in the extent to which they rely on iron-specific nutritional immunity. Although only the most common genotypes were compared within each stock, it is unlikely that other genotypes would have shown greater resistance to BKD; their frequencies within the stocks would have been increased by natural selection if the disease plays an important role as a selective agent. However, it is apparent that factors other than disease may select for different transferrin genotypes. In Ukranian carp, *Cyprinus carpio*, general survival rates were highest among individuals with the AC genotype (Balakhnin and Galagan 1972). There is also an association of transferrin phenotype with weight gain in juvenile rainbow trout that may be due to the linkage of the transferrin locus with a gene or gene complex affecting growth (Reinitz 1977). The association of resistance to BKD with transferrin genotype may also be due to a gene linkage; if so, transferrin serves only as a marker. McIntyre and Johnson (1977) observed higher growth rates and better survival in AA than in AC transferrin genotypes of Big Creek coho salmon. While the frequency of the C allele is high in the Alsea stock, that frequency is depressed in a mixed population at Big Creek where Alsea coho salmon have been used to supplement the broodstock (J. D. McIntyre unpubl. data). Although BKD selects for the C allele in the Alsea coho salmon, the advantage of this allele is offset by some other more important selective factor, such as growth rate, within the Big Creek stock.

It is also conceivable that transferrin genotypes provide resistance to different diseases, or not at all—as with vibriosis. The ability to synthesize iron chelators—compounds necessary to remove iron from transferrin—is considered a virulence factor for certain pathogens (Arnold et al. 1977). Perhaps the iron chelators of *V. anguillarum* remove iron from transferrin more efficiently than do those of BKD bacteria. This more efficient removal would explain to some extent the lack of differential resistance to vibriosis among genotypes within both coho salmon and steelhead trout stocks. Pratschner (1978) observed differential resistance among transferrin phenotypes to vibriosis and several other diseases in coho salmon from the Skagit River, Wash. The AA phenotype exhibited greater susceptibility to vibriosis and cytophagosis but greater resistance to furunculosis while the CC phenotype was most resistant to vibriosis and very susceptible to furunculosis and cytophagosis. The disparity between Pratschner's and our results with respect to vibriosis may be due to the stock-specific nature of transferrin. Possibly differences among transferrin genotypes are more significant in a chronic disease such as BKD, and less so in an acute disease such as vibriosis—or perhaps the rapid death rate following exposure to *V. anguillarum* compressed the results too much to allow differences to be observed. Because of the short time span involved to vibriosis infections, the benefit of such differences to individual fish would be negligible.

Keeping in mind such considerations as selection for transferrin genotypes by different factors such as growth or disease, it becomes clear (as with stocks) that selectively breeding for certain transferrin genotypes would not be advisable. Though selection for one particular genotype might provide resistance to BKD, it might also entail lower growth rates or even greater susceptibility to other diseases. McIntyre (1977) cautiously recommended selective breeding for disease resistance only in propagated fish being held under

carefully controlled conditions or when one particular pathogen is a recurrent problem. Otherwise, it seems advisable to maintain variability in a stock to meet the demands of a variable environment.

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