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Title: RESISTANCE OF COHO SALMON (ONCORHYNCHUS KISUTCH) AND STEELHEAD TROUT (SALMO GAIRDNERI) STOCKS AND TRANSFERRIN GENOTYPES TO BACTERIAL KIDNEY DISEASE AND VIBRIOSIS

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Juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*) of different stocks and three transferrin genotypes (AA, AC, and CC), all reared in identical or similar environments, were experimentally infected with the causative agent of bacterial kidney disease (BKD) or *Vibrio anguillarum*. Mortality due to the pathogens was compared among stocks within a species and among transferrin genotypes within a stock to determine if there was a genetic basis for resistance to disease among stocks and transferrin genotypes. Differences in resistance to BKD among coho salmon stocks have a genetic basis. Stock susceptibility to vibriosis is 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 BKD is stock-specific; in those stocks which showed differential resistance of genotypes the AA was the most susceptible. No differences in resistance to vibriosis among transferrin genotypes

were observed.

To further verify that stock differences, both genetic and environmental, did exist, vertebral and lateral series scale numbers were compared among four steelhead trout stocks (Alsea, Siletz, Rogue, and North Santiam) reared in a common environment and at the hatchery of their origin. The four stocks are genetically different from each other with respect to vertebral and scale number in almost all cases. Differences between steelhead trout of the same stock reared at two localities were significant for vertebral but not scale number. Not all meristic differences between hatchery-reared stocks were significant.

Resistance of Coho Salmon (*Oncorhynchus kisutch*) and Steelhead Trout (*Salmo gairdneri*) Stocks and Transferrin Genotypes to Bacterial Kidney Disease and Vibriosis

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Gary Wayne Winter

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APPROVED:

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Assistant Professor of Fisheries

in charge of major

Redacted for Privacy

Head of Department of Fisheries and Wildlife

Redacted for Privacy

Dean of Graduate School

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RESISTANCE OF COHO SALMON (ONCORHYNCHUS KISUTCH) AND STEELHEAD TROUT (SALMO GAIRDNERI) STOCKS AND TRANSFERRIN GENOTYPES TO BACTERIAL KIDNEY DISEASE AND VIBRIOSIS

I. INTRODUCTION

Bacterial kidney disease (BKD) is a major cause of serious losses among salmon reared in freshwater hatcheries of the Pacific Northwest (Leitritz and Lewis, 1976). In the marine environment epizootics caused by *Vibrio anguillarum* are particularly devastating to salmonids maintained within saltwater impoundments (Fryer, Nelson, and Garrison, 1972). Externally applied antibiotics are relatively ineffective in the treatment of these diseases. Recently, immunization with bacterins for the control of vibriosis has been shown to be feasible (Fryer, Rohovec, Tebbit, McMichael, and Pilcher, 1976). Attempts to produce a bacterin for BKD, however, have been unsuccessful (Evelyn, 1977). The use of disease resistant populations of fish may be conceivable as a means of reducing the incidence and severity of these diseases. Fish that inherit natural resistance to a disease will normally maintain that resistance throughout their lifetimes (Snieszko, Dunbar, and Bullock, 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. Among strains of brook trout (*Salvelinus fontinalis*) differences in susceptibility to ulcer disease and furunculosis have been observed (Wales and Berrian, 1937; Wolf, 1954; Snieszko, 1957; Snieszko et al., 1959). Gjedrem and Aulstad (1974) noted significant differences in resistance to vibriosis between strains of Atlantic salmon parr (Salmo salar) in Norway and calculated that these differences were slightly heritable. Unfortunately, in many of the previous studies concerning disease resistance there has been no attempt to rear the stocks in a common environment. Phenotypic expression is a combination of genotype, environment, and genotype-environment interactions. Only by rearing different stocks under identical conditions can one be certain that differences in resistance to disease are genetic in origin and not due to previous exposure of a particular stock to the disease in question. One objective of this study was to determine if there are differences in resistance to bacterial kidney disease and vibriosis among stocks of coho salmon (Oncorhynchus kisutch) and steelhead trout (Salmo gairdneri) and whether or not these differences have a genetic basis.

There are differences in resistance to bacterial kidney disease among three genotypes of transferrin, an iron binding plasma protein, in coho salmon (Suzumoto, Schreck, and McIntyre, 1977). In mammals, iron is known to enhance the growth and virulence of some pathogens. Transferrin may act to 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, owing to the fastidious nature of the organism. 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 bacterial kidney disease and vibriosis among transferrin genotypes in order to confirm earlier results with BKD and determine whether transferrin provides increased tolerance of bacterial diseases of salmonids in general. I also sought to determine if differences in resistance of transferrin genotypes exist among different stocks of coho salmon and steelhead trout.

II. MATERIALS AND METHODS

Juvenile coho salmon and steelhead trout were used as test fish. Coho salmon were obtained as eyed eggs from the Fall Creek (Alsea) and Big Creek salmon hatcheries, Oregon. Two crosses, Big Creek X Sol Duc (B x S) and Big Creek X Umpqua (B x U), were also obtained from the Big Creek hatchery. The Big Creek stock was reared at Oregon State University's Smith Farm facility in Corvallis, Oregon, while the Alsea stock and the two crosses were reared at the Oregon Department of Fish and Wildlife's Research Section and Oregon State University's Fish Disease Laboratory, respectively, in Corvallis. These rearing facilities presented similar though not identical environments for the fish. Due to insufficient numbers of fish the two crosses were only included 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 the Smith Farm facility.

Transferrin genotype was determined for experimental fish. Approximately 0.1 ml of blood was withdrawn from the caudal vein with a 1-ml tuberculin syringe and ejected into heparinized hematocrit tubes which were then centrifuged. The plasma obtained from the salmon was frozen until time of analysis. Steelhead samples were placed on ice and processed within several hours after collection because frozen storage reduces the stability of transferrin in steelhead trout. Fish were individually indentifiable by dangler tags applied just posterior of the dorsal fin. Starch-gel electrophoresis using a discontinuous buffer system as described by Ridgeway, Sherburne, and Lewis (1970) was employed to determine transferrin genotypes. Only the AA, AC, and CC genotypes were considered in this study, and in some stocks only two of these were used. The transferrins of Siletz and North Santiam steelhead stocks are not included due to poor resolution on the electrophoretic gels. Following bleeding, fish were given a recovery period of at least two weeks after which they were transferred to experimental tanks.

Bacterial Kidney Disease Study

All experimental fish were placed in 70-L fiberglass tanks with flowing, aerated, chilled (12 ± 2^oC) declorinated water located in Oregon State University's Nash Hall and allowed to acclimate to these conditions for two weeks. Each stock of coho salmon and steelhead trout consisted of 125 fish divided into two test replicates of 50 each and a control of 25 fish. Included in the steelhead trout experiment was one group of hatchery-reared (Cole Rivers) Rogue River stock (N = 34) without a replicate. The respective transferrin genotypes were distributed randomly among all tanks.

The BKD (*Corynebacterium sp.*) strain (RB-1-73) used in this study was isolated on cysteine serum agar from a spring chinook salmon at the Round Butte state hatchery, Oregon, by J. E. Sanders, fish pathologist, Oregon Department of Fish and Wildlife. A stock culture was maintained on Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) enriched with cysteine (0.1%) and calf serum (20%). Prior to each experiment cells were passed once in the species being tested to produce a fresh isolate which was further cultured until sufficient cells were available for an inoculum.

All test fish received an ip injection of 0.1 ml of a suspension of kidney disease bacteria in phosphate-buffered saline (PBS) while all control fish received an ip injection of only 0.1 ml PBS. The approximate inocula for the coho salmon ($\bar{x}$ weight = 23 g) and steelhead trout ($\bar{x}$ weight = 36 g) were 9×10^7 cells and 3×10^8 cells, respectively. The coho salmon were injected on March 17, 1977, while the steelhead trout were treated on September 12, 1977. All mortalities were necropsied and identification of BKD as the causative agent was based on presumptive diagnosis using gram stains of kidney smears. In addition, kidney smears from 10% of the mortalities were cultured on Mueller-Hinton media. Experiments were terminated at the end of 4 months or earlier depending on the progress of infection.

One week following injections into coho salmon all experimental fish were accidentally exposed to chlorine which resulted in mortalities as high as 50% within some stocks. This study was still continued but a second abbreviated one was begun August 24, 1977. Only Alsea and Big Creek stocks ($\bar{x}$ weight = 33.2 g) were utilized, the latter having been obtained directly from the hatchery. The inoculum for this second experiment was increased to 3×10^8 cells.

Vibriosis Study

The *Vibrio anguillarum* strain (LS-174) used in these experiments was isolated on brain heart infusion agar from a coho salmon at Lint Slough, Waldport, Oregon, by J. S. Rohovec. The inocula were either prepared from lyophilized cells or recent passage isolates.

Experimental fish were exposed to the pathogen in stainless steel tanks located at Oregon State University's Fish Disease Laboratory. Two experiments were undertaken with the coho salmon. In the first (initiated October 8, 1976), 225 fish ($\bar{x}$ weights for Big Creek and Alsea 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 particular experiment, having not been bled and tagged for transferrin genotype identification, were freeze branded to differentiate the stocks within each tank. In the second coho salmon experiment (June 10, 1977) the sample size for each tank was reduced to approximately 25 fish ($\bar{x}$ weight = 36.6 g) due to the unavailability of sufficient numbers, but transferrin genotypes had been determined. In the steelhead trout phase of the study (October 21, 1977) 75 fish from each stock with a mean weight of 36 g were divided equally among three test replicates while 15 from each stock were placed in a fourth tank that acted as a control. A hatchery-reared Rogue stock was also used in this steelhead trout experiment. A second experiment (December 27, 1977) utilizing steelhead trout from the Cole Rivers (Rogue), Alsea, and Marion Forks (North Santiam) salmon hatcheries was also done. In this latter experiment 50 fish with a mean weight of 42.2 g were divided equally among two replicates. Transferrin genotypes were distributed randomly among the tanks.

The initial temperature in all experimental tanks was 12.2^{o}C to which all fish had been acclimated. The temperature was then raised to 17.7^{o}C over a period of 1.5 h. Water flow was then discontinued in all tanks for 15 min. Test tanks then received the bacteria suspended in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) while the controls remained uninfected. The inocula for the first and second coho salmon exposures were 5 x 10^6 cells/ml and 8.6 x 10^6 cells/ml, respectively, while the steelhead trout received concentrations of 8.8 x 10^6 cells/ml in the first experiment and 7.2 x 10^6 cells/ml in the second. All mortalities 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 week.

Statistical comparisons of 3 or more stocks involved a one-way analysis of variance based on arc-sin transformations of percentages and least significant difference while comparisons of transferrin genotypes or 2 stocks were based on a χ^2 test employing a 2 x k contingency table (Snedecor and Cochran, 1967).

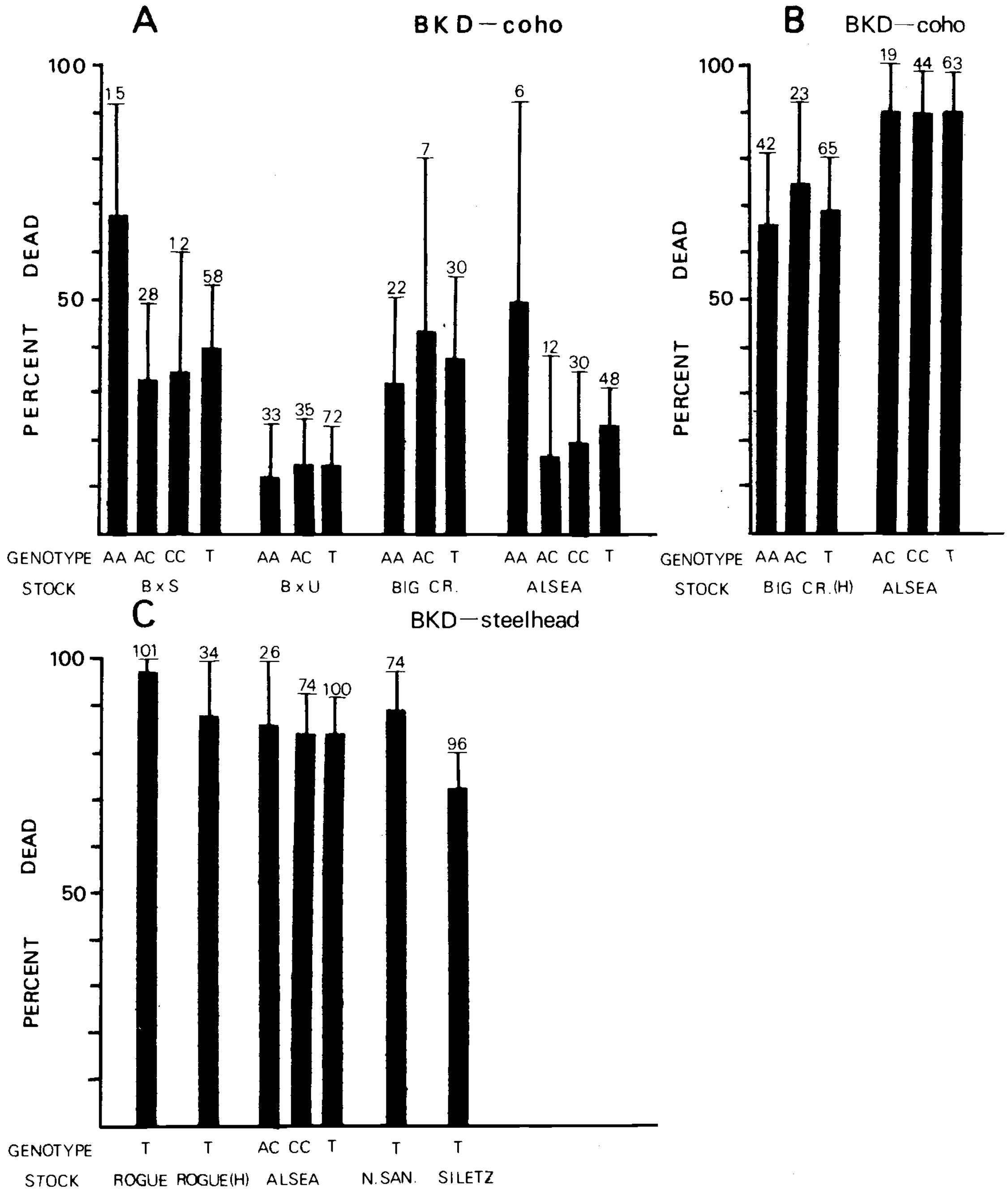
III. RESULTS AND DISCUSSION

In the first experiment in which coho salmon were infected with BKD, the Alsea stock and Big Creek x Umpqua cross were approximately twice as resistant to the disease as fish of the Big Creek stock and Big Creek x Sol Duc cross (Fig. 1A). This difference in mortality between the Big Creek x Umpqua and the two more susceptible groups was significant ($P<0.05$) while the Alsea mortality was only significantly lower than that of the Big Creek x Sol Duc ($P<0.06$). A comparison of mean times to death as shown below revealed a similar pattern:

	Stock or Cross			
	Big Cr. x Sol Duc	Big Cr. x Umpqua	Big Cr.	Alsea
Mean Time to Death (in days)	79.5	99.9	88.4	95.4

The mean times to death for the Big Creek x Umpqua and Alsea coho salmon were significantly greater than the Big Creek x Sol Duc ($P<0.05$). Concerning transferrin, only the Big Creek x Sol Duc cross and Alsea stock showed any differences in resistance to BKD among genotypes (Fig. 1A). In both groups the AA genotype was the most susceptible while the AC and CC both showed similar mortalities. The difference in resistance was significant ($P<0.07$) between the AA and AC genotypes within the Big Creek x Sol Duc cross. The Alsea transferrin results though not significant are substantiated by a previous study using Alsea coho salmon in which the AA genotype was also the most susceptible to BKD (Suzumoto et al., 1977). Because of similar transferrin results in the Big Creek x Sol Duc cross and Alsea stock the data were combined. The AC (28% mortality) and CC (24% mortality)

Figure 1. Percentages of different stocks and transferrin genotypes that died of bacterial kidney disease (BKD). A and B, coho salmon (*Oncorhynchus kisutch*) experiments 1 and 2, respectively; C, steelhead trout (*Salmo gairdneri*). T indicates total mortality for the stock; AA, AC, and CC indicate mortality for individual genotypes within a stock; B x S = Big Creek x Sol Duc cross and B x U = Big Creek x Umpqua cross; (H) indicates hatchery-reared fish. Sample number is indicated above bars; the line above each bar represents the upper limit of the 95% C.I.


A
BKD—coho
100
50
PERCENT DEAD
15 28 12 58 33 35 72 22 7 30 6 12 30 48
GENOTYPE AA AC CC T AA AC T AA AC T AA AC CC T
STOCK B x S B x U BIG CR. ALSEA
B BKD—coho
100
50
PERCENT DEAD
42 23 65 19 44 63
GENOTYPE AA AC T AC CC T
STOCK BIG CR.(H) ALSEA
C
BKD—steelhead
100
50
PERCENT DEAD
101 34 26 74 100 74 96
GENOTYPE T T AC CC T T T
STOCK ROGUE ROGUE(H) ALSEA N.SAN. SILETZ

genotypes were significantly (P<0.01) more resistant to BKD than 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 produced transferrin results similar to those of the first (Fig. 1B). Unfortunately, the AA genotype was not included in the Alsea comparison due to lack of sufficient numbers. No stock comparison was made because the Big Creek stock came directly from the hatchery. At that time 91.5% of mortalities in production fish at Big Creek were due to BKD (Conrad, pers. comm.). The probability of previous exposure to BKD in the Big Creek coho used in the experiment was very high.

The third BKD study involved the four steelhead stocks and a second Rogue stock reared at the hatchery (Fig. 1C). Three weeks into the study mortalities in the test groups began to increase at a high rate due to a secondary infection caused by _Aeromonas hydrophila_. This trend continued for another 4 weeks at which time mortalities leveled off and the study was then terminated. A comparison of stocks with respect to resistance is not really valid since the test tanks were obviously not challenged equally with the secondary infection of _A. hydrophila_. However, there were no significant differences (P<0.10) between replicates and the Siletz steelhead trout showed the lowest mortality (72%) which was significantly different (P<0.05) from all other stocks except the Alsea. As the mortality within 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_.

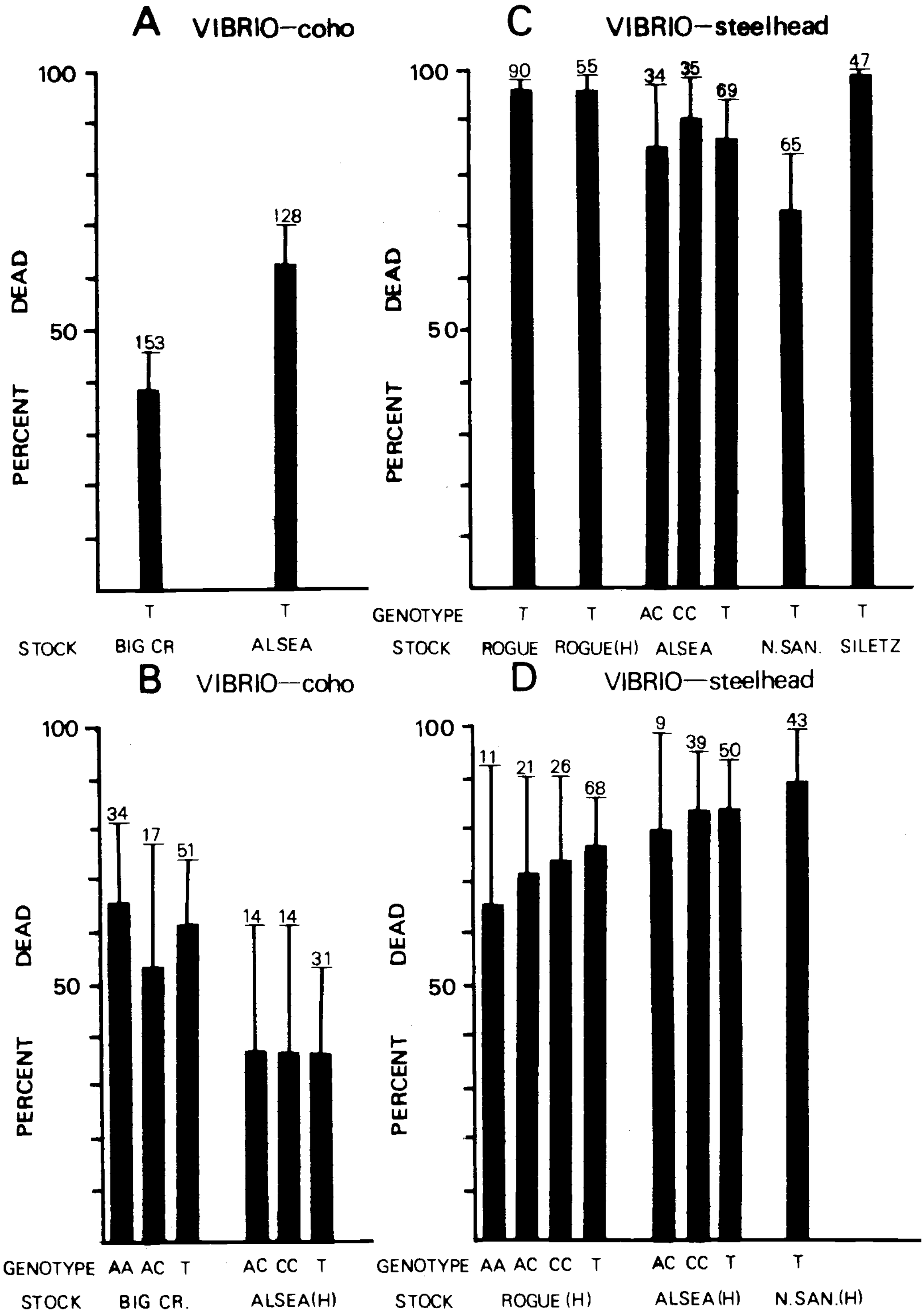
Although percent mortality is a better measure of an organism's ability to tolerate disease, mean time to death is also an indication of resistance to diseases, especially chronic ones such as BKD. There were no differences in mean time to death among either the Rogue or Alsea steelhead transferrin genotypes as is evident in the table below:

	Stock	Transferrin Genotype		
		AA	AC	CC
Mean Time to Death (in days)	Rogue	28.5 (N=30)	30.0 (N=41)	29.7 (N=19)
	Alsea		30.4 (N=21)	30.0 (N=62)

The importance of transferrin was probably reduced by the double infection.

The second disease considered in this study was vibriosis. In the first experiment in which coho salmon were exposed to V. anguillarum (Fig. 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 particular comparison. I repeated this experiment with coho salmon to observe if the results would be similar. In this second test (Fig. 2B) the resistance trend was reversed though at a much lower level of significance than the previous experiment ($P<0.07$). However, the Alsea coho used in this second test came directly from the hatchery. It is unlikely that any of these fish would have been previously exposed to V. anguillarum in freshwater, but a difference in susceptibility to vibriosis still existed. This, then, demonstrates the importance of eliminating environmental factors in studies of genetic resistance to disease by rearing fish under identical conditions. Within both the Alsea

Figure 2. Percentages of different stocks and transferrin genotypes that died of vibriosis. A and B, coho salmon experiments 1 and 2, respectively; C and D, steelhead trout experiments 1 and 2, respectively. T indicates total mortality for the stock; AA, AC, and CC indicate mortality for individual genotypes within a stock; (H) indicates hatchery-reared fish. Sample number is indicated above bars; the line above each bar represents the upper limit of the 95% C.I.


A VIBRIO–coho
100
50
PERCENT DEAD
153
128
T
T
STOCK BIG CR ALSEA
C VIBRIO–steelhead
100
50
PERCENT DEAD
90 55 34 35 69 65 47
GENOTYPE T T AC CC T T T
STOCK ROGUE ROGUE(H) ALSEA N.SAN. SILETZ
B VIBRIO–coho
100
50
PERCENT DEAD
34 17 51 14 14 31
GENOTYPE AA AC T AC CC T
STOCK BIG CR. ALSEA(H)
D VIBRIO–steelhead
100
50
PERCENT DEAD
11 21 26 68 9 39 50 43
GENOTYPE AA AC CC T AC CC T T
STOCK ROGUE (H) ALSEA (H) N.SAN.(H)

and Big Creek stocks there was no differential resistance shown by the transferrin genotypes although the AA genotype was not included in the Alsea transferrins (Fig. 2B).

The final two vibriosis experiments involved steelhead trout. In the first test (Fig. 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 were still significantly more resistant than the remaining stocks ($P<0.05$). Because mortality was high in the Rogue stock (96%) transferrin genotype differences were not considered. However, no differences in resistance were observed among genotypes within the Alsea stock. These results are similar to those observed with the coho salmon. The second experiment (Fig. 2D) utilizing hatchery-reared steelhead trout from the Rogue, Alsea, and North Santiam revealed the same transferrin results as the previous steelhead trout experiment. No differential resistance was shown among genotypes including the AA's within both the Alsea and 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 need for eliminating environmental differences in order to make a genetic comparison. The Rogue replicates in this experiment were significantly different ($P<0.025$) with respect to stocks which made a genetic comparison even less valid. Except for the hatchery-reared Rogue replicates in the last experiment there were no significant differences between replicates for both stocks and genotypes in all four vibriosis tests for which reason replicates were combined in the data analysis.

Differential resistance of coho salmon stocks to BKD has been demonstrated and has a genetic basis since the stocks were reared in similar environments. The Alsea and Big Creek x Umpqua coho salmon were more tolerant of BKD as shown by the lower percent dead and greater mean times to death. It is difficult to draw any valid conclusions in terms of stock resistance from the steelhead exposures to BKD owing to the secondary infection of *A. hydrophila*. The vibriosis results are not very convincing as shown by the reverse trends in some of the experiments. In the first vibriosis experiment with coho salmon there was a significant difference ($P<0.005$) in mean weight (t'-test, Snedecor and Cochran, 1967, p. 114) between the Alsea and Big Creek fish. However, there were no significant differences ($P<0.10$) in resistance to vibriosis among 4 size classes (5.1-10.0 g; 10.1-15.0 g; 15.1-20.0 g; and 20.1-25.0 g) within either stock. The difference in resistance appears to be genetic. The reverse trend of the second coho salmon experiment demonstrated a strong effect of environment in determining the degree of resistance to vibriosis. The difference in resistance to vibriosis of the North Santiam stock between the two steelhead trout exposures also indicates the importance of environmental factors. There was a significant difference in vertebral number between North Santiam steelhead reared at the hatchery and at Smith Farm, indicating an environmental difference (see appendix). No differences in resistance to either BKD or vibriosis were shown between Rogue steelhead trout reared at Smith Farm and at the hatchery, but the difference in vertebral number is significant (see appendix). The environmental difference indicated by the difference in vertebral number between the Smith Farm- and hatchery-reared Rogue steelhead trout perhaps was masked by the high mortality (96%) which makes a comparison difficult. Perhaps stock resistance to acute diseases such as vibriosis may depend more on which stock has an edge at the time of infection rather than genetic make-up. Genetic factors are probably more important in chronic diseases such as BKD. Apparent genetic resistance to infection by Ceratomyxa shasta, normally not an acute condition, has been observed among hatchery strains of chinook salmon (O. tshawytscha) (Zinn, Johnson, Sanders, and Fryer, 1977).

It is also evident that a stock may be resistant to one disease and not to another. While the Siletz steelhead trout were most resistant to the double infection of BKD and A. hydrophila, they showed the greatest susceptibility upon exposure to V. anguillarum. This statement is not totally valid since all the steelhead trout stocks may not have had equal exposure to A. hydrophila. Ehlinger (1977) observed that certain selected brook trout (S. fontinalis) strains though quite resistant to furunculosis were more susceptible to gill disease than the native stock. Consequently selection of stocks for resistance to several diseases would be very difficult (McIntyre, 1977) except possibly in the case of closely related pathogens (Hutt, 1970).

In reference to the transferrin results it appears as though the importance of transferrin genotype in resistance to disease is stock-specific. The AA genotype was the most susceptible to BKD infection in the Alsea and Big Creek x Sol Duc coho salmon whereas no differences were observed among genotypes within the Big Creek and Big Creek x Umpqua groups. 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. Otherwise, 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 is perhaps 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, in which case transferrin would only serve as a marker. McIntyre and Johnson (1977) observed higher growth rates and better survival in AA's than AC's within Big Creek coho salmon. Alsea coho salmon have been used to supplement the broodstock at the Big Creek hatchery. While the frequency of the C allele is high in the Alsea stock, that frequency is depressed in the mixed population at Big Creek (McIntyre, unpublished data). Although BKD selects for the C allele in the Alsea coho the advantage given to individuals with 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 might be favored by 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, Cole, and McGhee, 1977). Perhaps the iron chelators of *V. anguillarum* are more efficient at removing iron from transferrin than those of BKD bacteria. This would explain to some extent the lack of differential resistance to vibriosis among genotypes within both coho salmon and steelhead trout stocks. It is also possible that differences among transferrin genotypes would be more significant in the case of a chronic disease such as BKD and less so in acute diseases such as vibriosis, or perhaps the rapid death rate following exposure to *V. anguillarum* just squeezed the results together too much to allow any differences to be observed. Because of the short time span involved in vibriosis infections the benefit of such differences to individual fish would be negligible. In addition, it is also difficult to draw valid conclusions concerning either stock or transferrin differences when mortalities approach 100%. Any immunity that was present may have been surpassed.

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. Selection for one particular genotype might provide resistance to BKD along with lower growth rates or even greater susceptibility to other diseases. McIntyre (1977) cautiously recommends selective breeding for disease resistance only in the case of propagated fish being held under carefully controlled conditions or when one particular pathogen is a recurrent problem. It would be best to maintain variability in a stock to meet the demands of a variable environment.

IV. LITERATURE CITED

Arnold, R. R., M. F. Cole, and J. R. McGhee. 1977. A bacterial effect for human lactoferrin. Science 197: 263-265.

Balakhnin, I. A., and N. P. Galagan. 1972. Distribution and survival rate of individuals with different transferrin types among carp offspring from combinations of parents. Hydrobiol. J. (USSR) 8: 41-45.

Ehlinger, N. F. 1977. Selective breeding of trout for resistance to furunculosis. N. Y. Fish and Game J. 24: 26-36.

Evelyn, T. P. T. 1977. Immunization of salmonids. Pages 161-176 in Proceedings from the International Symposium on Diseases of Cultured Salmonids. April 4-6, 1977. Tavolek, Inc., Seattle, Wash.

Fryer, J. L., J. S. Rohovec, G. L. Tebbit, J. S. McMichael, and K. S. Pilcher. 1976. Vaccination for control of infectious diseases in Pacific salmon. Fish Pathology 10: 155-164.

Fryer, J. L., J. S. Nelson, and R. L. Garrison. 1972. Vibriosis in fish. Pages 129-133 in R. W. Moore, eds. Prog. in fishery and food science. Vol. 5. Univ. of Wash. Publications in Fisheries New Series, Seattle.

Gjedrem, T., and D. Aulstad. 1974. Selection experiments with salmon. I. Differences in resistance to Vibrio disease of salmon parr (Salmo salar). Aquaculture 3: 51-59.

Hershberger, W. K. 1970. Some physiochemical properties of transferrins in brook trout. Trans. Am. Fish. Soc. 99: 207-218.

Hutt, F. B. 1970. Genetic resistance to infection. Pages 1-11 in R. H. Dunlop and H. W. Moon eds. Resistance to infectious disease. Saskatoon Modern Press, Saskatchewan.

Leitritz, E., and R. C. Lewis. 1976. Trout and salmon culture. Cal. Fish Game. Fish Bull. 164: 197 pages.

McIntyre, J. D. 1977. Heritable tolerance of disease in salmonids. Pages 87-90 in Proceedings from the International Symposium on Disease of Cultured Salmonids. April 4-6, 1977. Tavolek, Inc., Seattle, Wash.

McIntyre, J. D., and A. K. Johnson. 1977. Relative yield of two transferrin phenotypes in coho salmon. Prog. Fish-Cult. 39: 175-177.

Reinitz, G. L. 1977. Tests for association of transferrin and lactate dehydrogenase phenotypes with weight gain in rainbow trout (Salmo gairdneri). J. Fish. Res. Board Can. 34: 2333-2337.

Ridgeway, G. J., S. W. Sherburne, and R. D. Lewis. 1970. Polymorphism in the esterases of Atlantic herring. Trans. Am. Fish. Soc. 99: 147-151.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods. 6th ed., Iowa State Univ. Press, Ames. 593 pages.

Snieszko, S. F. 1957. Disease resistant and susceptible populations of brook trout (Salvelinus fontinalis). U.S. Fish and Wildl. Serv. Spec. Sci. Rep.-Fish. 208: 126-128.

Snieszko, S. F., C. E. Dunbar, and G. L. Bullock. 1959. Resistance to ulcer disease and furunculosis in eastern brook trout, Salvelinus fontinalis. Prog. Fish-Cult. 21: 111-116.

Suzumoto, B. K., C. B. Schreck, and J. D. McIntyre. 1977. Relative resistances of three transferrin genotypes of coho salmon (Oncorhynchus kisutch) and their hematological responses to bacterial kidney disease. J. Fish. Res. Board Can. 34: 1-8.

Wales, J. H., and W. Berrian. 1937. The relative susceptibility of various strains of trout to furunculosis. Cal. Fish Game 23: 147-148.

Weinberg, E. D. 1974. Iron and susceptibility to infectious disease. Science 184: 952-956.

Wolf, L. E. 1954. Development of disease-resistant strains of fish. Trans. Am. Fish. Soc. 93: 342-349.

Zinn, J. L., K. A. Johnson, J. E. Sanders, and J. L. Fryer. 1977. Susceptibility of salmonid species and hatchery strains of chinook salmon (Oncorhynchus tshawytscha) to infections by Ceratomyxa shasta. J. Fish. Res. Board Can. 34: 933-936.

APPENDIX

V. APPENDIX

MERISTIC COMPARISON OF FOUR STOCKS OF STEELHEAD TROUT (SALMO GAIRDNERI). - I compared numbers of vertebrae and lateral series scales among four stocks of steelhead trout reared in a common environment to determine if stocks could be separated by these characters. Environmental factors were also considered by comparing these meristic characters between the same stocks reared in two different locations. Salmonid populations from different drainage systems or areas within large lakes have been separated by comparisons of meristic characters (Seymour, 1959; Vernon, 1957). Differences in characters such as vertebral number may be influenced by genetic or environmental factors, or both (Bailey and Gosline, 1955). Seymour (1959) attributed differences in vertebral counts among four stocks of chinook salmon (*Oncorhynchus tshawytscha*) to genetic factors while Vernon (1957) observed both environmental and genetic influences in the determination of vertebral number within three races of Kootenay kokanee salmon (*O. nerka*) in British Columbia. Meristic characters are influenced by environmental factors such as light, temperature, and dissolved oxygen during the period from fertilization to hatching (Mottley, 1937; Wallace, 1973; Kwain, 1975).

Materials and methods. - Steelhead trout were obtained 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). The eggs from each stock were taken within a week of each other in all cases except the Alsea eggs which were fertilized two months earlier. Although the exact number of parents for each stock is uncertain, the parental matings were sufficiently random to insure that any variability observed would be due to stock characteristics and not heritable individual variability. These four stocks were then reared at Oregon State University's Smith Farm facility in Corvallis, Oregon, until they attained a length of approximately 15 cm. During egg incubation all stocks were exposed to a constant water temperature of 10.6°C, dissolved oxygen near saturation, and equal light intensities (incubator trays covered by black polyethylene sheets). Steelhead trout (length $\geq$ 15 cm) from each stock that had been reared at the respective hatcheries were obtained later. Since vertebral number is determined by the eyed stage (Taning, 1946), mean water temperatures during the period of fertilization to hatching were obtained from each hatchery as shown below:

	Alsea	Siletz	Rogue	North Santiam
Mean Temp. (°C)	6.9	6.5	10.8	6.0

Thirty fish from each stock, both Smith Farm and hatchery, were X-rayed and vertebral counts were determined from the negatives. Lateral series scale counts were done for 15 fish from each of the respective stocks. Data were analyzed using a one-way analysis of variance and least significant difference (Snedecor and Cochran, 1967).

Results and discussion. - All four stocks reared at Smith Farm were significantly different from each other with respect to both vertebral and lateral series scale number except the Siletz-North Santiam scale comparison (Table 1). Since the stocks were reared in a common environment these differences are genetic. Seymour (1959) showed

Table 1. Mean vertebral and lateral series scale numbers of four stocks of steelhead trout (<u>Salmo gairdneri</u>) reared in identical environments (Smitn Farm) and their respective hatcheries of origin (Hatchery).

	STEELHEAD TROUT STOCKS							
	ALSEA		SILETZ		ROGUE		NORTH SANTIAM	
	Smith Farm	Hatchery	Smith Farm	Hatchery	Smith Farm	Hatchery	Smith Farm	Hatchery
Vertebral Number (N=30) ± SE [a]	62.2±0.16	64.4±0.13	62.8±0.15	63.3±0.14	61.5±0.11	63.0±0.13	63.4±0.13	64.1±0.14
Range	61 - 65	63 - 66	61 - 64	62 - 65	60 - 62	61 - 64	62 - 64	63 - 66
Lateral Series Scale Number (N=15) ± SE [b]	124.5±0.64	126.3±0.61	132.2±0.86	131.9±0.98	136.6±0.93	136.9±1.35	130.1±0.75	131.5±0.98
Range	121-128	122-130	127-138	127-137	130-142	130-147	126-135	125-138

[a] At P<0.01 a difference of 0.5 vertebrae is significant.

[b] At P<0.01 a difference of 3.4 lateral series scales is significant.

genetic differences in vertebral number among chinook salmon stocks, but some of his parental matings involved only a single male and female.

Differences between the same stocks reared at Smith Farm and their respective hatcheries are significant for vertebral number but not lateral series scale number (Table 1). This indicates that vertebral number may be influenced more by environmental factors while the similarity of scale counts between identical stocks reared at two different locations points strongly to genetic influence. Genetic factors were responsible for differences in number of scales on and above the lateral line between Cowichan, British Columbia, steelhead and rainbow trout that spawned at the same time and place (Neave, 1944). Smith (1969) found genetic influence on lateral line scale number to be neglible in a comparison of winter and summer races of Capilano, British Columbia, steelhead trout. In all comparisons of Smith Farm and hatcheries lower water temperatures at the hatcheries (6.0 - 6.9°C) were associated with greater vertebral number, except in the case of the Rogue stock (Table 1; Materials and methods). In general, vertebral number is inversely related to temperature, but this relationship does not necessarily hold (Mottley, 1937; Bailey and Gosline, 1955).

Differences in vertebral number of the hatchery-reared stocks could be due to selection in favor of individuals with a certain vertebral number, but this seems unlikely. Mottley (1937) found no evidence of selection for vertebral number under hatchery conditions. Although Vernon (1957) considered an adaptive significance of differences in vertebral number also unlikely, he mentioned the possibility of their association with anatomical or physiological variations that could have survival value.

Not all differences in meristic counts, both vertebrae and lateral series scales, between stocks reared at their respective hatcheries were significant (Table 1). Neither the Siletz-Rogue and Alsea-North Santiam vertebrae comparisons nor the Siletz-North Santiam scale comparison were significant. This observation and the overlap in ranges of the meristic counts would make the use of single meristic characters in the identification of ocean-caught salmonids according to stocks unfeasible. However, a multivariate analysis based on a large number of meristic characters is conceivable.

LITERATURE CITED

Bailey R. M., and W. Gosline. 1955. Variation and systematic significance of vertebral counts in the American fishes of the family Percidae. Misc. Publ. Mus. Zool. Univ. of Mich. 93: 44 pages.

Kwain, W. 1975. Embryonic development, early growth, and meristic variation in rainbow trout (Salmo gairdneri) exposed to combinations of light intensity and temperature. J. Fish. Res. Board Can. 32: 397-402.

Mottley, C. McC. 1937. The number of vertebrae in trout (Salmo). J. Biol. Board Can. 3: 169-176.

Neave, F. 1944. Racial characteristics and migratory habits in Salmo gairdneri. J. Fish. Res. Board Can. 6: 245-251.

Seymour, A. 1959. Effects of temperature upon the formation of vertebrae and fin rays in young chinook salmon. Trans. Am. Fish. Soc. 88: 58-69.

Smith, S.B. 1969. Reproductive isolation in summer and winter races of steelhead trout. Pages 21-38 in Symposium on Salmon and Trout in Streams, H. R. MacMillan Lectures in Fisheries, Univ. of British Columbia.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods. 6th ed., Iowa State Univ. Press, Ames. 593 pages.

Taning, A. V. 1946. State of determination of vertebrae in teleostean fishes. Nature 157: 594.

Vernon, E. H. 1957. Morphometric comparison of three races of kokanee (*Oncorhynchus nerka*) within a large British Columbia lake. J. Fish. Res. Board Can. 14: 573-598.

Wallace, C. R. 1973. Effects of temperature on developing meristic structures of smallmouth bass, *Micropterus dolomieui* Lacepede. Trans. Am. Fish. Soc. 102: 142-145.