The relative effects of *Ceratomyxa shasta* on crosses of resistant and susceptible stocks of summer steelhead
The relative effects of Ceratomyza shasta on crosses of resistant and susceptible stocks of summer steelhead

Mark Wade

Oregon Department of Fish and Wildlife
Research and Development Section

October 1986
ABSTRACT

Crosses were made between a stock of summer steelhead (*Salmo gairdneri*) known to be resistant to infection by *Ceratomyxa shasta* and stocks of summer steelhead known to be susceptible. Ceratomyxosis, the disease caused by *C. shasta* was initiated by exposure to Willamette River water. I found that the crosses were intermediate in susceptibility to ceratomyxosis relative to the parental stocks. There was no difference in susceptibility to ceratomyxosis between reciprocal crosses of the same stocks. Resistance of moderate susceptibility in the F2 generation of experimental stock crosses and examples from both wild and hatchery stocks of mixed ancestry indicate long term disease problems may result from introductions of less adapted, foreign stocks.
INTRODUCTION

Returns of Skamania summer steelhead to the McKenzie River, have fluctuated widely. A possible reason may be that Skamania smolts migrate through the lower Willamette River so late that in many years the water temperature exceeds 17°C. Skamania summer steelhead smolts are highly susceptible to bacterial diseases such as *Aeromonas salmonicida* and *Aeromonas liquefaciens* at temperatures greater than 17°C (Buchanan et al. 1982; Fryer and Pilcher 1974) and warm water may physiologically terminate smolting (Zaugg and Wagner 1973; Wagner 1974). A stock that migrates earlier in the spring would avoid the warm water and possibly increase the percentage of smolts surviving to return as adults.

The Oregon Department of Fish and Wildlife (ODFW) has considered replacing the Skamania stock with other summer steelhead stocks. A suitable stock would be resistant to ceratomyxosis and would migrate before high water temperatures occur in the lower Willamette River. Testing of other available stocks revealed that the Umpqua stock is susceptible to ceratomyxosis, a disease caused by myxosporidian *Ceratomyxa shasta,* (Buchanan 1977), and that the Deschutes and Clearwater (Idaho) stocks are resistant (Buchanan 1974). Unfortunately, the Deschutes and the Clearwater stocks are carriers of infectious hematopoietic necrosis (IHN) and cannot be transferred into the Willamette basin.

Resistance to ceratomyxosis is thought to be genetically transferred (Johnson 1975, Zinn et al. 1977). Zinn (1975) tested reciprocal crosses of Big Big Creek and Trask River fall chinook salmon along with the parental stocks for resistance to ceratomyxosis. He found resistance to be inherited as a dominant sex-linked character. None of the Big Creek chinook salmon died
whereas 92.5% of the Trask River fish succumbed to ceratomyxosis. Only 4% of
the progeny of Trask males x Big Creek females died and 33% of the progeny of
Big Creek males x Trask females died.

By crossing a resistant stock (Skamania) with an early migrating but
ceratomyxosis susceptible stock (Siletz or Umpqua) I hoped to produce
steelhead that would be resistant to ceratomyxosis and that would retain the
early smolt migration timing. A ceratomyxosis resistant, early migrating
stock of summer steelhead would provide managers with another stock for use in
the Willamette River tributaries where adult returns of Skamania stock have
been low. The objectives of this study were (1) to determine the
susceptibility of crosses of Siletz x Skamania and Umpqua x Skamania summer
steelhead stocks to ceratomyxosis and (2) to explore how resistance to
ceratomyxosis is inherited, especially if resistance is a sex-linked trait.

METHODS

Equal volumes of eggs were collected from 19 Siletz stock summer
steelhead at Cedar Creek Hatchery, 14 Skamania stock fish at South Santiam
Hatchery, and 12 Umpqua stock fish at Rock Creek Hatchery in February 1981.
Approximately 240 milliliters of eggs were placed in 1 liter plastic
containers. Sperm was collected (10 Umpqua males, 11 Siletz males, and 11
Skamania males) by stripping milt from one male into a zip-loc bag, inflating
the bag with air, and sealing the bag. All gametes were transported the same
day in ice chests at 2-4°C to the ODFW's Research Laboratory at Corvallis.

From these gametes I propagated seven genetic groups of fish; Skamania,
Siletz, Umpqua, and reciprocal crosses (male x female and female x male) of
Skamania x Siletz and Skamania x Umpqua stocks (males are listed first in all
specific stock crosses). All eggs of each stock were pooled and mixed before
being divided into groups for fertilization, and 5 milliliters of sperm were
pipetted from each bag into a beaker and mixed, one beaker for each stock.
This was done to minimize differences within each stock in genetic
contribution to the respective groups. The fertilized eggs were placed in a
Heath incubator and supplied with pathogen free, 12°C well water. When the
yolk sac was absorbed, each genetic group was placed in a 356 liter circular
tank supplied with 12°C well water and fed to repletion several times each
day. The fish were fin-clipped for group identification and each group was
divided into two equal test lots on 25 September 1981 when they had grown to
approximately 5-8 cm fork length.

Exposure to water containing the infectious stage of _C. shasta_, such as
the Willamette River near Corvallis, is the only practical way to initiate
infection (Johnson 1975). I exposed one lot to Willamette River water in a
1.5 cu m livebox in the Willamette River near Corvallis, Oregon (river km
212), from 30 September to 7 October 1981. Water temperature in the live box
ranged from 15°C to 16°C during this exposure. A second lot was exposed at
the same location from 7 October to 15 October 1981 at 12°C to 14°C. After
exposure to _C. shasta_, the fish were transferred to pathogen free well water
at 12°C and fed CPM with 3% Terramycin added to prevent bacterial infections.
Terramycin does not inhibit ceratomyxosis (Sanders et al. 1972). Dead fish
were collected daily until 12 January 1986 and frozen or examined fresh. Fish
were considered infected by _C. shasta_ if a microscopic examination (400x) of a
smear of intestinal fluid contained the characteristic spores.

Fish that survived exposure to _C. shasta_ in the fall of 1981 were
combined into one group and reexposed to _C. shasta_ the following spring for 16
days beginning 24 May 1982 at a temperature of 12° to 15°C. Dead fish were
collected and examined as before.
Fish that survived reexposure were reared to age 3+. In the spring of 1984, four males and four females were sexually mature and were bred to produce the F2 generation (Figure 1). Since not all the F1 fish matured at the same time I could not make all possible crosses. In addition, two groups of F2 progeny were lost because of a failure in the water supply. The F2 progeny were reared in pathogen free, 12°C well water at the ODFW Research Laboratory in Corvallis and exposed to C. ahaeta in the Willamette River at Corvallis for 3 weeks beginning 21 September 1984. Alsea stock steelhead, a stock that is susceptible to ceratomyxosis (Weber and Knispel 1976), were included to verify the presence of the infectious stage of C. ahaeta. After exposure the fish were transferred to pathogen free 12°C well water and fed OMP with 3% Terramycin for 86 days. Dead fish were collected and examined as before.

STATISTICAL ANALYSIS

Overall differences in mortality between reciprocal cross groups in the fall of 1981 exposures were tested using analysis of variance with each exposure as a block (Snedecor and Cochran 1967). The proportion of each group dying was transformed using the square root of the arcsine (Bartlett 1947). Differences between groups were tested using least significant difference.

A two-factor analysis of variance with no interactions (Netter and Wasserman 1974) was used to test for differences in the proportions of the F2 progeny of F1 reciprocal cross x F1 reciprocal cross matings that died from ceratomyxosis. These proportions were transformed using the square root of the arcsine.
Figure 1. Crosses made to produce the F₂ generation. The dashed lines represent groups that failed. Sk = Skamania, U = Umpqua, SI = Siletz.
RESULTS

Nearly all of the pure Umpqua and pure Siletz fish were dead and infected with spores of C. shaesta by 105 days after the first day of exposure of the first test lot and by 98 days after the first day of exposure of the second lot (Table 1). Only 3% of the Skamania fish in the first lot died and none of the Skamania fish in the second lot died. All four of the reciprocal cross groups in the first lot experienced intermediate levels of mortality relative to the pure stock groups. The reciprocal cross groups in the second lot incurred less mortality but were still intermediate to the pure stock groups. The differences between exposure lots in percent mortality of the reciprocal crosses were significant ($P = 288$, df = 1,3, $P = 0.0004$) as was the difference in mortality between the Umpqua x Skamania and Siletz x Skamania crosses ($P = 26.30$, df = 3,3, $P = 0.01$). I found no significant differences between reciprocal crosses of the same stocks and exposure lot.

There were no additional effects on the Skamania steelhead from the reexposure to the infectious stage of C. shaesta. One percent of the Skamania fish died from ceratomyxosis after being exposed to C. shaesta a second time. The reciprocal crosses once again experienced a moderate level of mortality.

Mortality from ceratomyxosis on the F2 generation is listed in Table 2. The F2 progeny of crosses of susceptible and resistant stocks exhibited a wide range of resistance to ceratomyxosis. Analysis of variance showed significant differences between F1 females of reciprocal crosses in the resistance of their F2 progeny to ceratomyxosis ($P = 11.37$, df = 2,3, $P = 0.02$). Large differences in resistance to ceratomyxosis of the F2 progeny were noted between females from the same F1 cross (Skamania x Siletz) even when they were mated to the same males. I found no difference ($P > 0.05$) between F1 males mated to the same females.
Table 1. Mortality from ceratomyxosis on three stocks of summer steelhead and their reciprocal crosses after exposure to _C. shaeta_ in the Willamette River near Corvallis, Oregon, 1981 and 1982.

<table>
<thead>
<tr>
<th>Stock cross (exposure lot)</th>
<th>Number of fish exposed</th>
<th>Number of dead fish</th>
<th>Number of fish dead and infected with <em>C. shaeta</em></th>
<th>Percentage of fish dead and infected with <em>C. shaeta</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Skamania x Skamania:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First lot</td>
<td>73</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Second lot</td>
<td>101</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reexposure</td>
<td>72</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Umpqua x Umpqua:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First lot</td>
<td>88</td>
<td>87</td>
<td>87</td>
<td>99</td>
</tr>
<tr>
<td>Second lot</td>
<td>97</td>
<td>95</td>
<td>95</td>
<td>98</td>
</tr>
<tr>
<td>Siletz x Siletz:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First lot</td>
<td>96</td>
<td>93</td>
<td>93</td>
<td>97</td>
</tr>
<tr>
<td>Second lot</td>
<td>88</td>
<td>85</td>
<td>85</td>
<td>97</td>
</tr>
<tr>
<td>Skamania x Umpqua:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First lot</td>
<td>101</td>
<td>43</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>Second lot</td>
<td>94</td>
<td>12</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Reexposure</td>
<td>54</td>
<td>11</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>Umpqua x Skamania:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First lot</td>
<td>77</td>
<td>43</td>
<td>38</td>
<td>49</td>
</tr>
<tr>
<td>Second lot</td>
<td>85</td>
<td>12</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Reexposure</td>
<td>21</td>
<td>8</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td>Skamania x Siletz:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First lot</td>
<td>95</td>
<td>67</td>
<td>65</td>
<td>68</td>
</tr>
<tr>
<td>Second lot</td>
<td>95</td>
<td>31</td>
<td>29</td>
<td>30</td>
</tr>
<tr>
<td>Reexposure</td>
<td>25</td>
<td>6</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>Siletz x Skamania:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First lot</td>
<td>94</td>
<td>64</td>
<td>63</td>
<td>67</td>
</tr>
<tr>
<td>Second lot</td>
<td>101</td>
<td>22</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Reexposure</td>
<td>40</td>
<td>16</td>
<td>13</td>
<td>32</td>
</tr>
</tbody>
</table>

*a* (male x female).

*b* The first lot was exposed from 30 September to 7 October 1981. The second lot was exposed from 7 October to 15 October 1981. The first and second lots were combined and reexposed 24 May to 19 June 1982.

*c* Too few survivors to form a lot for reexposure.
Table 2. The effects of *Ceratomyza shasta* on the F₂ generation of reciprocal crosses of summer steelhead stocks. SK = Skamania, SI = Siletz, UM = Umpqua, and AL = Alsea.

<table>
<thead>
<tr>
<th>Cross F₁ Male x F₁ Female</th>
<th>Number of fish exposed</th>
<th>Number of dead fish</th>
<th>Number of dead fish infected with <em>C. shasta</em></th>
<th>Percentage of fish dead and infected with <em>C. shasta</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>SKxSK X SKxSK</td>
<td>40</td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>SKxSI X SKxSK</td>
<td>53</td>
<td>5</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>SKxUM X SkxSK</td>
<td>47</td>
<td>6</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>SKxSI X SKxSI #1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23</td>
<td>4</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>SixSK X SKxSI #1</td>
<td>93</td>
<td>34</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>SKxUM X SKxSI #1</td>
<td>27</td>
<td>17</td>
<td>10</td>
<td>37</td>
</tr>
<tr>
<td>SKxSK X SKxUM</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>SKxUM X SKxUM</td>
<td>36</td>
<td>17</td>
<td>17</td>
<td>47</td>
</tr>
<tr>
<td>SKxSI X SKxUM</td>
<td>60</td>
<td>29</td>
<td>29</td>
<td>48</td>
</tr>
<tr>
<td>SiXSK X SKxSI #2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88</td>
<td>58</td>
<td>58</td>
<td>66</td>
</tr>
<tr>
<td>SKxUM X SKxSI #2</td>
<td>33</td>
<td>24</td>
<td>24</td>
<td>73</td>
</tr>
<tr>
<td>SKxSI X SKxSI #2</td>
<td>51</td>
<td>43</td>
<td>43</td>
<td>84</td>
</tr>
<tr>
<td>AL X AL&lt;sup&gt;b&lt;/sup&gt;</td>
<td>59</td>
<td>55</td>
<td>55</td>
<td>93</td>
</tr>
</tbody>
</table>

<sup>a</sup> SKxSI #1 and SKxSI #2 are different female fish from the same F₁ cross.

<sup>b</sup> *C. shasta* positive control.
DISCUSSION

By obtaining differences in resistance between stocks after rearing them in a common, pathogen free environment I demonstrated that resistance to ceratomyxosis is an inherited trait in summer steelhead. Hoffmaster (1985) crossed male coho salmon resistant to ceratomyxosis with susceptible female rainbow trout and tested the hybrids and pure species for resistance to ceratomyxosis. She concluded that susceptibility to ceratomyxosis is inherited as a dominant genetic trait. The intermediate susceptibility of the resistant steelhead x susceptible steelhead crosses in my work and that of Hemmingsen et al. (in press) with coho does not support this conclusion. Our results suggest an additive genetic model in which each allele substitution produces an incremental increase or decrease in a particular character.

The patterns of inheritance of resistance in the $F_2$ generation of reciprocal crosses suggests a strong maternal influence. I do not know if this was also true in the $F_1$ generation because I did not test individual families. However I did not observe resistance to be a dominant sex-linked trait in steelhead as was seen by Zinn (1975) in chinook salmon.

The specific physiological mechanism that allows Skamania summer steelhead to prevent ceratomyxosis, was partially broken down by crossbreeding with the Umpqua stock and with the Siletz stock. Differences in resistance to ceratomyxosis between the Umpqua x Skamania and Siletz x Skamania crosses demonstrate that genetic differences exist between the Umpqua and Siletz stocks although the pure stocks are equally susceptible at the exposure levels I tested. Differences in resistance to ceratomyxosis of the $F_2$ progeny of $F_1$ females of the same stock cross (Skamania x Siletz) when mated to the same males indicates genetic differences within stocks.
Differences between lots exposed in the fall of 1981 in the incidence of ceratomyxosis in the reciprocal cross groups demonstrated the difficulties extend to comparisons of exposures at the same location and period of the year but at different times. Although the length of exposure was the same, incidence of ceratomyxosis in the reciprocal cross groups averaged 56% for lot 1 and 18% for lot 2. Differences between exposures in resistance to ceratomyxosis can probably be attributed to dilution of the infective stage of *C. shasta*. Flow in the Willamette River at Albany, the nearest gauging station, increased from an average of 8,077 cfs during exposure of the first lot to 11,284 cfs during exposure of the second lot. The incidence of infection by *C. shasta* gradually tapers off in the fall as flows increase (Johnson 1975).

Natural selection for ceratomyxosis resistant individuals may be the mechanism by which resistant stocks evolve (Johnson 1975, Zinn et al. 1977). Threshold characteristics, such as disease resistance, can be selected for (Dempster and Lerner 1950). Differences in the F₂ progeny of F₁ Skamania x Siletz females indicate genetic differences in resistance to ceratomyxosis within stocks. A resistant stock may possibly be developed from susceptible x resistant crosses by selecting resistant families. Attempts to produce ceratomyxosis resistant hybrid steelhead should start with as many F₁ families as possible to minimize random genetic drift and reduce them to the number needed through high levels of exposure to *C. shasta*. This strategy would produce the largest selection differential and should produce the most ceratomyxosis resistant F₂ generation. Such an effort would require a large number of rearing tanks and may not be practical.
Even though several authors have cautioned against releasing susceptible salmonids into river systems containing the infectious stage of *C. shasta* (Zinn et al. 1977, Johnson et al. 1979; Buchanan et al. 1983) the ODFW, Idaho Department of Fish and Game, and Washington Department of Fisheries continue to do so (Howell et al. 1985). Of greater concern than the deaths of susceptible stocks is the possibility of genetic degradation of endemic resistant stocks. This work and that of Hemmingsen et al. (in press) with coho salmon demonstrate the intermediate susceptibility to ceratomyxosis of crosses of resistant and susceptible stocks.

If ceratomyxosis susceptible adults are released into a system they may breed with native fish and produce offspring susceptible to ceratomyxosis. An example of increased susceptibility in a wild stock may be Nehalem coho salmon. From 1965 to 1976 1.5 million fry, 40,000 fingerling, 10,000 smolt and 1,200 adult coho salmon from the North Nehalem, Trask and Alsea stocks were released into the Fishhawk Creek system, a tributary to the Nehalem River at river kilometer 106. All of these introduced stocks are susceptible to *C. shasta* (Udey et al. 1975; Zinn 1975; Weber and Knispel 1976). We have no record of coho being released into Cronin Creek, a tributary to the Nehalem at river kilometer 40 and these fish were thought to represent the native Nehalem coho. In 1980, age 0+ coho salmon were exposed to *C. shasta* by placing them in a live box in the Nehalem River (Tom Nickelson, ODFW, unpublished data). The fingerlings were from four sources 1) Trask River coho, 2) the progeny of early spawning wild adult coho from Fishhawk Creek, 3) the progeny of late spawning wild adult coho from Fishhawk Creek and 4) wild fingerlings captured in Cronin Creek. The fingerlings from Fishhawk Creek were intermediate in resistance to ceratomyxosis relative to the introduced Trask River and native Cronin Creek groups (Table 3). Native Fishhawk Creek coho may have interbred
with the introduced susceptible stocks decreasing the resistance of the wild
coho salmon in Fishhawk Creek to ceratomyxosis.

Table 3. The effects of Ceratomyza shasta on four groups of coho salmon
(Tom Nickelson, ODFW, unpublished).

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of fish exposed</th>
<th>Number of dead fish</th>
<th>Number of dead fish infected with C. shasta</th>
<th>Percentage of fish dead and infected with C. shasta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trask</td>
<td>32</td>
<td>23</td>
<td>22</td>
<td>69</td>
</tr>
<tr>
<td>Early Fishhawk</td>
<td>66</td>
<td>24</td>
<td>22</td>
<td>33</td>
</tr>
<tr>
<td>Late Fishhawk</td>
<td>36</td>
<td>9</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>Cronin Creek</td>
<td>55</td>
<td>9</td>
<td>4</td>
<td>7</td>
</tr>
</tbody>
</table>

An example of genetic degradation of a hatchery stock may be winter
steelhead in the Cowlitz River, Washington. Historical records and chromosome
studies indicate that the winter steelhead broodstock used at the Cowlitz
Trout Hatchery (Washington Department of Game) is a combination of native
Cowlitz River fish and the Chambers Creek stock introduced from Puget Sound
(Crawford 1979, Thorgaard 1983). All steelhead stocks native to the Columbia
River that have been tested are resistant to ceratomyxosis (Buchanan et al.
1983; Hoffmaster 1985). Puget Sound is outside the established range of C.
shasta and, therefore, endemic steelhead stocks are probably susceptible to
ceratomyxosis. Losses of winter steelhead reared at the Cowlitz Trout
Hatchery commonly exceed 30% and sometimes exceed 80%, and C. shasta is
thought to be the cause (Tipping et al. 1984).

These examples and susceptibility of the F2 generation of resistant x
susceptible crosses of steelhead in my experiments, even though we exposed the
F1 generation to C. shasta twice, indicate a danger of long term disease
problems in both hatchery and wild populations following introductions of less
adapted stocks.
REFERENCES


Buchanan, D.V., J.L. Fryer, and J.L. Zinn. 1982. Relative susceptibility of four stocks of summer steelhead (Salmo gairdneri) to infections of ceratomyxosis and bacterial diseases found in the Willamette River. Oregon Department of Fish and Wildlife, Information Reports (Fish) 82-7, Portland.


Udey, L.R., J.L. Fryer, and K.S. Pilcher. 1975. Relation of water temperature to ceratomyxosis in rainbow trout (Salmo gairdneri) and coho salmon (Oncorhynchus kisutch). Journal of the Fisheries Research Board of Canada 32:1545-1551.


