FISH DIVISION
Oregon Department of Fish and Wildlife

Relative susceptibility of four stocks of summer steelhead (*Salmo gairdneri*) to infections of ceratomyxosis and bacterial diseases found in the Willamette River.
Relative susceptibility of four stocks of summer steelhead (Salmo gairdneri) to infections of ceratomyxosis and bacterial diseases found in the Willamette River

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ABSTRACT

All of the summer steelhead (Salmo gairdneri) from an Oregon coastal river (Siletz stock) died when they were either exposed continuously to waters containing the infectious stage of Ceratomyxa shasta, or exposed for 30 days followed by transfer to pathogen-free well water. Ninety percent of the Siletz mortalities were found to contain C. shasta spores. Three Columbia River stocks (Skamania, Clearwater, and Deschutes) were resistant to ceratomyxosis during a 120 day exposure or when transferred to pathogen-free well water after exposure for 30 days. Differences in survival of the Columbia River fish were attributed to the effect of temperature (up to 21°C) and bacterial diseases. Skamania summer steelhead were more susceptible to these effects than Deschutes or Clearwater stocks. We recommend that no coastal stocks of steelhead be released into the Columbia River basin or waters of other basins where the infectious stage of C. shasta is known to occur without first testing that stock's susceptibility to ceratomyxosis.

INTRODUCTION

Ceratomyxosis is the suspected cause for differences in survival of Siletz (coastal) and Skamania (Columbia River) summer steelhead (Salmo gairdneri) that have been released in the Willamette River, Oregon since the early 1970's. The infectious stage of Ceratomyxa shasta is present in the lower Columbia River basin (Sanders et al. 1970), but it is not limited to this system (Schafer 1968 and Johnson et al. 1979). No chemotherapeutic agents have been developed to control this parasite (Sanders et al. 1972; Wood 1974; Udey et al. 1975).

Zinn et al. (1977) tested nine salmonid species and found all were susceptible to C. shasta, although no deaths occurred in Atlantic salmon (S. salar). They also found that chinook salmon (Oncorhynchus tshawytscha) derived from outside the Columbia River drainage were highly susceptible to C. shasta whereas
chinook salmon from the Columbia River system had a lower incidence of ceratomyxosis. Rainbow (*S. gairdneri*) and cutthroat trout (*S. clarki*) tested were extremely susceptible to *C. shasta* (Sanders et al. 1970); however, preliminary experiments by Johnson (1975) indicated stock difference in the susceptibility of rainbow trout.

We used long term exposures to test the susceptibility of a coastal stock of summer steelhead (Siletz) and three Columbia River stocks (Skamania, Clearwater, and Deschutes) to ceratomyxosis. The objective of the study was to develop management recommendations governing the use of steelhead stocks in waters that contain the infectious stage of *C. shasta*.

**MATERIALS AND METHODS**

Siletz, Skamania, and Deschutes fish were reared in Oregon at Oak Springs, Roaring River, and Round Butte hatcheries, respectively. Clearwater fish were reared at Dworshak National Fish Hatchery in Idaho. None of the test fish had been previously exposed to *C. shasta*. About 240 smolts, averaging 60 to 80 g each, from each stock were held in 1.5 m$^3$ rectangular liveboxes placed in the Willamette River near Corvallis, Oregon. Exposure of fish to water containing the infectious stage is the only practical method of initiating ceratomyxosis (Schafer 1968; Sanders et al. 1970), and the infectious stage of this parasite is present in the Willamette River near Corvallis when water temperatures exceed 10°C (Udey et al. 1975). Susceptible fish can be infected by *C. shasta* after only a 30 minute exposure to contaminated waters; however, longer exposure can produce greater numbers of infected fish (Sanders et al. 1972; Ratliff 1981).

The Willamette River harbors many bacterial fish pathogens as well as *C. shasta*. One experiment was designed to determine the isolated effects of Ceratomyxosis and the other experiments studied cumulative effects of increasing
temperature, other fish pathogens, and Ceratomyxosis on the four stocks. About 60 fish from each stock were marked by fin removal and placed into each of four liveboxes so that each livebox contained about 240 animals. Four fish died in the first 3 days of exposure and were assumed to be handling mortalities. Also, some fish died in the liveboxes during periods of turbid water conditions and all may not have been recovered when mortalities were collected. The actual numbers recovered and examined are reported.

All the fish were exposed to Willamette River water for 30 days (April 21 to May 20, 1974). Water temperatures ranged from 10.5 to 14.5°C during this period. All fish were fed a supplementary daily ration of Oregon Moist Pellet (OMP) equal to 1.0% of their initial body weight. Terramycin in the form of TM₅₀ (Pfizer) was incorporated at the 3% level into the diet as a prophylactic measure against bacterial pathogens during initial exposure. No chemotherapeutic agent has been found effective in preventing or arresting ceratomyxosis (Sanders et al. 1972).

After the 30th day of exposure, one livebox group was transferred to the Oregon State University Fish Disease Laboratory and held in fish pathogen-free well water at 13.4°C in 1,000-l tanks until termination 133 days later (October 31, 1974). The remaining three livebox groups were held for 90 additional days in the Willamette River at the normal fluctuating temperatures which ranged from 11.1 to 21.6°C. Oregon Moist Pellet diet with TM₅₀ was discontinued in one group after the first 30 days. The other two groups were fed a maintenance ration with TM₅₀ added for a total of 120 days.

Dead fish were collected daily, and either necropsied fresh or frozen for later examination. Wet mounts of intestinal scrapings were examined microscopically (400 X) for c. shasta spores. Preparations containing two or more c. shasta spores were considered positive (Udey et al. 1975). All surviving fish
were examined in the same manner for sublethal infections of ceratomyxosis upon termination of the experiment. Because *Aeromonas hydrophila*, *Aeromonas salmonicida* and *Flexibacter columnaris* are present in the Willamette River system most of the dead fish that were negative for *C. shasta* spores were further examined by streaking small fragments of kidney tissue on brain heart infusion (BHI) agar and on *Cytophaga* agar. Gross observations of the resulting bacterial colonies and pigmentation on BHI agar were noted. A dark brown pigment produced by bacterial colonies on the BHI agar was the characteristic used to indicate fish that were infected with *A. salmonicida*.

RESULTS

Only three Siletz fish from the test group that was later transferred to pathogen-free well water died during the 30-day exposure to Willamette River water. These fish were examined and no *C. shasta* spores were found. Siletz fish began dying 50 days after initial exposure and by day 77 all were dead (Fig. 1). Over 95% of the Siletz mortalities were infected with *C. shasta* spores (Table 1). All of the Siletz fish that died after day 50 were infected. Total mortality for the similarly exposed and transferred Deschutes, Skamania, and Clearwater stocks was 1.6%, 8.5%, and 9.1%, respectively. 163 days after the initial exposure to Willamette River water. None of these mortalities were infected or showed signs of ceratomyxosis. However, two Skamania and five Clearwater fish were believed to be infected with a bacterial disease at death. All survivors of these three stocks were sacrificed at the termination of the experiment and found to be negative for ceratomyxosis.

Two test groups were continuously held in Willamette River water for 120 days and fed OMP with TM50. Mortality increased in one group of Siletz stock on the 50th day of exposure and continued until all were dead at day 72. The other test group of Siletz fish began dying on day 54 and mortalities continued
Fig. 1. Survival of four stocks of summer steelhead exposed for 30 days to Willamette River water then transferred to constant temperature well water (13.4°C) in Spring 1974.
Table 1. Mortalities of four stocks of summer steelhead initially exposed to the infectious stage of *Ceratomyxa shasta* in the Willamette River under three test conditions.

<table>
<thead>
<tr>
<th>Test condition</th>
<th>Stock</th>
<th>No. fish exposed</th>
<th>Total deaths</th>
<th>Percent total mortality</th>
<th>No. dead fish infected with <em>C. shasta</em></th>
<th>Percent dead fish infected with bacterial diseases&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. dead fish infected with bacterial diseases&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Percent dead fish infected with bacterial diseases&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transferred to well water after 30 days</td>
<td>Siletz</td>
<td>61</td>
<td>61</td>
<td>100.0</td>
<td>58</td>
<td>95.1</td>
<td>0</td>
<td>0.0</td>
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<tr>
<td></td>
<td>Skamania</td>
<td>59</td>
<td>5</td>
<td>8.5</td>
<td>0</td>
<td>0.0</td>
<td>2</td>
<td>40.0</td>
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<tr>
<td></td>
<td>Clearwater</td>
<td>55</td>
<td>5</td>
<td>9.1</td>
<td>0</td>
<td>0.0</td>
<td>5</td>
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<td>Deschutes</td>
<td>61</td>
<td>1</td>
<td>1.6</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Held 120 days in Willamette River and fed TM&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Siletz</td>
<td>60</td>
<td>60</td>
<td>100.0</td>
<td>59</td>
<td>98.3</td>
<td>0</td>
<td>0.0</td>
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<tr>
<td></td>
<td>Skamania</td>
<td>59</td>
<td>27</td>
<td>45.8</td>
<td>0</td>
<td>0.0</td>
<td>21</td>
<td>77.8</td>
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<td>10.3</td>
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<td>5</td>
<td>8.5</td>
<td>0</td>
<td>0.0</td>
<td>3</td>
<td>60.0</td>
</tr>
<tr>
<td>Held 120 days in Willamette River and fed TM&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Siletz</td>
<td>55</td>
<td>55</td>
<td>100.0</td>
<td>49</td>
<td>89.1</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Skamania</td>
<td>54</td>
<td>26</td>
<td>48.1</td>
<td>1</td>
<td>3.8</td>
<td>16</td>
<td>61.5</td>
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<td></td>
<td>Clearwater</td>
<td>59</td>
<td>12</td>
<td>20.3</td>
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<td>50.5</td>
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<td>9</td>
<td>15.5</td>
<td>0</td>
<td>0.0</td>
<td>6</td>
<td>66.7</td>
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<tr>
<td>Held 120 days in Willamette River and not fed for 90 days</td>
<td>Siletz</td>
<td>58</td>
<td>58</td>
<td>100.0</td>
<td>52</td>
<td>89.6</td>
<td>0</td>
<td>0.0</td>
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<tr>
<td></td>
<td>Skamania</td>
<td>60</td>
<td>55</td>
<td>91.7</td>
<td>0</td>
<td>0.0</td>
<td>41</td>
<td>74.6</td>
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<td>51</td>
<td>86.4</td>
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<td>0.0</td>
<td>38</td>
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<td>18</td>
<td>81.8</td>
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<sup>a</sup>*Aeromonas hydrophila* and *Aeromonas salmonicida* were the principal bacterial pathogens detected. *Flexibacter columnaris* was not isolated from any dead fish.<br>

<sup>b</sup>This water was pathogen-free.
until all fish were dead at day 70. Water temperatures during the exposure period fluctuated from 10.5 to 18.0°C. Ninety-four percent of the mortalities from the two test groups of Siletz fish were infected with spores of \textit{C. shasta} (Table 1). All Siletz fish that died after 50 days of exposure were infected with \textit{C. shasta}.

Skamania fish fed OMP with TM\textsubscript{50} and held continuously in Willamette River water experienced substantial mortalities (47\%) but these mortalities did not occur until the 80th exposure day (Fig. 2). Water temperatures in the Willamette were over 16°C during this period. Mortality of the Skamania fish continued until the 105th exposure day and appeared to level off when water temperatures began decreasing (Fig. 2). Only one dead fish was infected with \textit{C. shasta}, while 37 of the fish that died were infected with either \textit{A. salmonicida} or \textit{A. hydrophila}. No \textit{E. columnaris} colonies were observed on any of the \textit{Cytophaga} agar cultures. Only 12\% and 15\% of the Deschutes and Clearwater fish fed OMP with TM\textsubscript{50}, respectively, died during the test. These stocks appeared resistant to both \textit{C. shasta} and bacterial diseases found in the Willamette River at temperatures above 16°C when they were fed OMP with TM\textsubscript{50}.

All survivors were examined for \textit{C. shasta} spores at the termination of the experiment. Two fish from the Skamania stock were heavily infected with \textit{Coratomyxa} spores yet these fish were alive and apparently healthy after 120 days of exposure. No Deschutes or Clearwater fish were infected.

All Siletz fish died in the livebox group exposed continuously to Willamette River water and not fed either OMP or OMP with TM\textsubscript{50}. Approximately 90\% of the Siletz mortalities were infected with \textit{C. shasta} spores (Table 1). All Siletz fish that died after the 50th exposure day were infected with \textit{Coratomyxa} spores. The percentage and timing of ceratomyxosis on the Siletz fish was not affected when OMP and TM\textsubscript{50} was discontinued after day 30. Resistance of the three
Fig. 2. Survival of four stocks of summer steelhead exposed for 120 days to Willamette River water and fed Oregon Moist Pellet containing TM50. These figures are the combined survival rates from two test groups.
Columbia River stocks to *C. shasta* was not affected when OMP and TM$_{50}$ was discontinued, but twice as many died of each stock than those that were fed OMP and TM$_{50}$. Only five Skamania fish survived this experiment. Substantial numbers of Skamania fish began dying on exposure day 75 and continued dying through exposure day 105 (Fig. 3). No Skamania mortalities were infected with *C. shasta* spores, but 75% were infected with *A. salmonicida* or *A. hydrophila*. Only eight Clearwater fish survived the experiment. Clearwater mortalities did not begin until the 100th exposure day when river temperatures were 20°C and over (Fig. 3). No Clearwater mortalities were infected with *Ceratomyxa* spores, but 75% were infected with *A. salmonicida* or *A. hydrophila*. Sixty-three percent of the Deschutes fish survived this test (Table 1). No Deschutes mortalities were infected with *Ceratomyxa* spores. Of the 22 fish that died, 82% were infected with bacterial diseases. No *C. shasta* spores were found in survivors from the three Columbia River stocks.

**DISCUSSION**

We found Siletz steelhead (coastal stock) were highly susceptible to ceratomyxosis while the three Columbia River stocks were resistant. These data agree with Zinn et al. (1977) who found fall chinook stocks derived from coastal rivers where the disease is not known to occur were highly susceptible to infection while stocks located within the Columbia River basin were resistant to ceratomyxosis. This should explain why previous workers found varying levels of susceptibilities in the same species of salmonids (Johnson 1975; Schafer 1968; Sanders et al. 1970; and Wales and Wolf 1955). However, Zinn et al. (1977) noted that all salmonid species tested were to some degree susceptible to ceratomyxosis. Ratliff (1981) found that 47% to 77% of Deschutes spring chinook were susceptible to ceratomyxosis. In our experiments, none of the Deschutes or Clearwater fish were infected after 120 days of exposure to
Fig. 3. Survival of four stocks of summer steelhead held for 120 days in the Willamette River. After the first 30 days, these fish were not fed Oregon Moist Pellet or protective TM50.
*C. shasta.* When we discontinued feeding OMP with TM₅₀ more Columbia River steelhead died from other causes but their resistance to ceratomyxosis was not affected. The use of terramycin did not enhance or inhibit the progression of ceratomyxosis in steelhead.

Zinn et al. (1977) found some *C. shasta* spores in surviving Atlantic salmon after a 43-day exposure. They believed that the Atlantic salmon would have eventually died had their experiment been allowed to continue. We found two Skamania steelhead which were alive yet infected with large numbers of *C. shasta* spores when the experiments were terminated. All mortalities caused by *C. shasta* in our experiment occurred between days 50 and 87. These two survivors were held another month in river water temperatures exceeding 21°C. It is quite possible these fish would have continued to survive.

When susceptible rainbow trout were exposed to *C. shasta* then transferred to a constant temperature laboratory, Udey et al. (1975) found that the mean time to death of fish with ceratomyxosis was 40 to 45 days at 15.0°C and 56 to 57 days at 12.2°C. They believed that the mean time from exposure to death was a function of temperature. Three of our exposures were at fluctuating temperatures, and 50% of the fish susceptible to ceratomyxosis died between 55 and 60 days (Figs. 2 and 3). The mean Willamette River temperature was 13.0°C through exposure day 60. However, these temperatures were taken once each day near the time of maximum river temperature, and the Willamette River averages at least 1°C lower from maximum in its 24-hour diurnal range. These data essentially agree with the temperature effect to ceratomyxosis reported by Udey et al. (1975).

Though the three Columbia River stocks were equally resistant to *C. shasta* under increasing temperatures and long-term exposures of 120 days, the combined effects of temperatures and bacterial diseases present caused differences in
survival among stocks held continuously in the river. *A. salmonicida* and *A. hydrophila* were the principal bacterial pathogens detected. A total of 34% of the Columbia River stocks succumbed to *A. salmonicida* as determined by brown pigmentation on streaked BHI agar. No *F. columnaris* colonies were observed. Apparently, Skamania fish are more susceptible to these effects than Deschutes or Clearwater. Other workers have found stock resistance to ulcer disease and furunculosis inbrook trout (Wolf 1953; Snieszko et al. 1959; Ehlinger 1964). Also, Snieszko (1974) noted that stress caused by temperature affects the response of fishes to an invasion by pathogens. We were unable to definitively identify which bacteria were killing fish but suspect that *A. hydrophila* and *A. salmonicida* were major contributors.

The State of Oregon has released over one million steelhead smolts from coastal stocks into the Willamette River system from 1966 to 1975 (Buchanan 1977). These fish originated from the Alsea River, Cedar Creek and Siletz River. Only one production group from these smolts was marked so the resulting adult returns could be determined. This group consisted of 37,000 summer steelhead smolts from the Siletz River released in April 1971. No adult returns have been recorded from this release. Since salmonids from the Alsea River, Cedar Creek and Siletz River are susceptible to ceratomyxosis (Udey et al. 1975; Zinn et al. 1977; this paper, respectively), we believe that few, if any, of these fish survived.

The infectious stage of *C. shasta* is present in the Columbia River basin and in portions of California but is not limited to these systems. Margolis and Evelyn (1975) found that *C. shasta* is present in southern British Columbia and Johnson et al. (1979) noted that the Rogue River in southern Oregon contained *C. shasta*. To prevent fish losses similar to those we reported in Oregon, we recommend that 1) studies be continued to determine the exact range
of the infectious stage of *C. shasta; 2) no coastal steelhead be released into the Columbia River basin or other areas where the infectious stage has been identified without first testing that stock's susceptibility to ceratomyxosis; and 3) further studies be conducted to definitively determine salmonid stock differences in resistance to bacterial diseases found in the Columbia River basin.

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