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Title: GEOGRAPHIC DISTRIBUTION OF CERATOMYXA SHASTA IN THE COLUMBIA RIVER BASIN AND SUSCEPTIBILITY OF SALMONID STOCKS

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Four objectives were examined in this study, (1) the geographic distribution of the infective stage of Ceratomyxa shasta in the Columbia River basin, (2) the susceptibility of selected salmonid strains to infection, (3) the heritability of resistance to C. shasta, and (4) the effects of salt water on the progress of infection.

Rainbow trout susceptible to C. shasta infection were placed at selected locations in the Columbia River and its tributaries. After an exposure period, the fish were observed for signs of ceratomyxosis. Detection of C. shasta from fish exposed at McNary and Little Goose Dams extends the range of the infectious stage of this parasite about 200 miles further up the Columbia River and into the Snake River drainage.

All Columbia River salmonid strains tested were resistant to infection by C. shasta. Two salmonid strains

from outside the Columbia River basin were also examined. Umpqua chinook salmon were resistant to infection, while a low incidence of infection was found in Eagle Lake rainbow trout. A hybrid between a rainbow trout susceptible to infection by C. shasta and a resistant coho salmon, was susceptible to infection.

Experiments with a steelhead trout strain susceptible to C. shasta infection indicated that ceratomyxosis progresses in salt water once the fish has become infected. Coho salmon smolts were resistant to the disease when held in either salt or fresh water following exposure to the infective stage.

Geographic Distribution of *Ceratomyxa shasta* in the Columbia River Basin and Susceptibility of Salmonid Stocks

by

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GEOGRAPHIC DISTRIBUTION OF *CERATOMYXA SHASTA* IN THE COLUMBIA RIVER BASIN AND SUSCEPTIBILITY OF SALMONID STOCKS

INTRODUCTION

Ceratomyxa shasta, a myxosporidan parasite of salmonid fish, is present in the Columbia River basin and is devastating to certain strains of salmonids (Sanders et al., 1970). Currently, the infectious stage of this parasite is known to exist in the Columbia River to the mouth of the Deschutes River. Columbia River tributaries also containing the infectious stage are the Deschutes, Cowlitz, and Willamette Rivers, and LaCamas Lake and Creek (Johnson et al., 1979). Many areas have not been examined for the presence of the infectious stage of *C. shasta*, and changing environmental conditions within the Columbia basin may have extended the geographic range of the infectious stage of this parasite. Knowledge of the exact range of *C. shasta* is valuable, especially in areas where hatcheries exist or are being planned.

The effect of *C. shasta* infections on salmonids entering salt water is not known. Fish infected in fresh water may have reduced survival in salt water. Knowledge of how ceratomyxosis affects salmonids during both the fresh and saltwater phase of their migration is necessary in assessing the impact of this disease on anadromous salmonids.

An effective therapy for ceratomyxosis has not been found, but several investigators have shown that the disease can be controlled using resistant strains of salmonids (Zinn et al., 1977; Buchanan et al., 1983). Therefore it is necessary to determine the resistance or susceptibility of salmonid strains released into waters containing the infective stage of C. shasta. It would also be useful to know how resistance to this parasite is acquired, and if it is a heritable trait.

The purpose of this study was to examine the impact of C. shasta on salmonids in the Columbia River basin. The objectives were determination of; (1) the geographic distribution of the infectious stage of C. shasta within the basin, (2) the resistance to infection of selected strains of salmonids reared in the basin, (3) the heritability of resistance to infection, and (4) the effects of salt water on the progress of the infection.

LITERATURE REVIEW

Introduction

The protozoan *Ceratomyxa shasta* (Noble) is a member of the class Myxosporea, phylum Myxosoa (Levine, 1980). Myxozoans are parasitic organisms with spores of multicellular origin. The class Myxosporea is characterized by having one to eight polar capsules and two valves. Myxosporidans are primarily parasites of fishes and have been found in both freshwater and marine fishes in virtually all tissues and organs of the host (Rogers and Gaines, 1975). Historical description of myxosporidans, myxosporidan reproduction within the host, *C. shasta* ultrastructure, taxonomy, and histology have not been included in this review. Reviews on these subjects have been written by various authors (Noble, 1944; Gould, 1968; Johnson, 1975; Yamamoto and Sanders, 1979; Fendrick, 1980; Amandi, 1984).

Species of *Ceratomyxa* occur widely as parasites of marine fish, mainly infecting the lumens of gall and urinary bladders (Noble, 1950). The description of *C. shasta* by Noble (1950) was the first report of a species from the genus *Ceratomyxa* as a histozoic parasite of freshwater fish. He observed this myxosporidan in rainbow trout (*Salmo gairdneri*) at Crystal Lake Hatchery, Shasta County, California. In Oregon, the first observation of an

infection caused by C. shasta occurred in 1954 in adult spring chinook salmon (Oncorhynchus tshawytscha) (Wood and Wallis, 1954). Since these early reports, C. shasta infections have been diagnosed from many locations in the Pacific Northwest. This parasite is recognized as an important pathogen, resulting in losses of both hatchery-reared and wild salmonids, and contributing to prespawning mortality in adult salmon.

This literature review will be divided into the following sections:

1. Morphology.
2. Pathology.
3. Transmission of disease and life cycle.
4. Geographic distribution.
5. Host range and susceptibility of salmonid strains.
6. Effects of temperature.
7. Control.

Morphology

According to Noble (1950), trophozoites of C. shasta are developed from a zygote through the processes of nucleogony (formation of a multicellular cell from a unicellular cell) and budding. The trophozoite, or vegetative stage, is rounded or ameboid in shape, and has a granular endoplasm. A trophozoite matures to form a sporoblast (pansporoblast) about 20 μ in diameter and usually containing two sporonts. Each sporont is derived

from six cells - two of which are germinative cells, two produce the polar capsules, and two produce the spore valves (Yamamoto and Sanders, 1979). Spores of C. shasta measure about 6 x 14 μ and have broadly rounded ends which are arched posteriorly. A raised suture line joins two shell valves. Two polar capsules, each 1.8 μ in diameter (Noble, 1950), are located near the anterior end of the spore, one to each sporoplasm. Polar filaments are released during spore germination and are thought to function in attachment.

Pathology

Clinical signs of fish with ceratomyxosis were described by Wales and Wolf (1955), Schafer (1968), and Johnson (1975). Infected rainbow trout were initially emaciated, lethargic, sought quiet water, and swam near the surface. The body coloration of trout darkened but Johnson (1975) noted this was rare for salmon. As the disease progressed, the descending intestine and anus became swollen and hemorrhagic and ascites fluid collected in the coelom. Clinical signs of disease in juvenile spring chinook and coho salmon (Oncorhynchus kisutch) and steelhead trout (Salmo gairdneri) were described by Conrad and DeCew (1966). Signs of disease in spring chinook salmon include emaciation, and development of fluid filled blebs and kidney pustules. Infected coho salmon were

emaciated and had enlarged vents which exuded mucus but were seldom inflamed. Steelhead trout exhibited the darkening and abdominal distension described for rainbow trout.

Noble (1950) noted that infections caused by C. shasta appeared more extensive than infections caused by other members of the genus. The following organs were infected: entire alimentary canal, subcutaneous connective tissue, gill capillaries, uriniferous tubules of the kidney, spleen, liver, gall bladder, and gonads. Schafer (1968) reported infections of the eyes of coho salmon. Conrad and DeCew (1966) found C. shasta spores in muscle tissue of spring chinook salmon. Sanders et al. (1970) found the parasite in all visceral organs as well as the eyes, muscle, and gills of salmonids.

Transmission and Life Cycle

The life history of C. shasta, like that of most myxosporidans, is unknown. Natural transmission occurs when susceptible salmonids are exposed to water or sediments containing the infectious stage (Schafer, 1968; Fryer, 1971; Johnson, 1975). But the identity of the infectious stage and its method of infecting salmonids is not known. Neither attempts to transmit ceratomyxosis from fish to fish, nor feeding infected tissue containing spores and trophozoites have resulted in transmission of the disease (Wales and Wolf, 1955; Schafer, 1968; Wood, 1968;

Johnson, 1975). In studies on a related myxosporidan, Uspenkaja (in Walliker, 1968) and Hoffman and Putz (1971), showed that Myxosoma cerebralis spores taken from infected fish did not transmit whirling disease, but when spores were aged 3-6 months in mud the infectious stage was present and transmitted the disease to fish. Similar experiments with C. shasta have failed to result in transmission. But when susceptible fish were exposed to bottom sediments collected from LaCamas Lake, where annual epizootics occur, infections were obtained (Fryer and Sanders, 1970). Ratliff (1983) further demonstrated that in a thermally stratified lake only the fish exposed to the benthos became infected with C. shasta.

Laboratory transmission of ceratomyxosis has been established by intraperitoneal injections and anal intubation of ascites fluid from infected fish (Schafer, 1968; Fryer, 1971; Johnson, 1979). Although the infection route is generally assumed to be oral, there is some evidence that entry may not involve ingestion. In a study on M. cerebralis, Putz and Hoffman (1966) transmitted whirling disease to prefeeding sac fry by exposing them to waters containing the infectious stage. Schafer (1968) suggested that establishment of C. shasta infections in rainbow trout was not dependent on the ingestion of organisms.

The inability to transmit ceratomyxosis between

susceptible fish has led to speculation that an intermediate host may be involved in the life cycle. Spall (1973) and Current (1977) examined the possibility of aquatic insects and invertebrates as intermediate hosts of two Myxosoma sp., and Henneguya exilis. Although both invertebrates and insects were shown to have ingested spores, no disease transmission occurred when they were fed to susceptible fish. Taylor and Lott (1978) were able to transmit M. cerebralis to rainbow trout by feeding infected fish to aquatic birds then exposing uninfected fish to the spores contained in the feces, after aging in mud. They indicated that although birds could disseminate the spores, a maturation period in a mud substrate was still required for transmission.

Recently, Markiw and Wolf (1983) have shown that the spore aging process of M. cerebralis which results in transmission of whirling disease, requires the participation of tubificid oligochaetes. These tubificids harbor an actinomyxidian of the genus Triactinomyxon which reacted by immunofluoresence with anti-M. cerebralis serum (Wolf and Markiw, 1981). Wolf and Markiw (1984) suggested that the spores of M. cerebralis shed from infected fish infect tubificid oligochaetes and that within the tubificid gut, conversion from the myxosporean spore to the actinosporean takes place. After three to four months, sporocytes release the mature actinosporean Triactinomyxon

which transmits whirling disease upon ingestion by susceptible trout.

Geographic Distribution

Fish diagnosed as having infections resulting from *C. shasta* have been reported from certain river systems in northern California, Oregon, Washington, Idaho, and British Columbia. There is a difference between areas where susceptible salmonids become infected and areas which lack the infective stage but where infected fish have been found. It has been demonstrated that migrating adult coho and chinook salmon and steelhead trout become infected with *C. shasta* while passing through the lower Columbia River (Wood, 1968; Sanders et al., 1970). These fish distribute spores as they migrate throughout the drainage. Although the entire watershed to which salmonids have access is potentially infectious for *C. shasta*, this has not been demonstrated and suggests that introduction of spores is insufficient for transmission. Therefore, the infectious stage of this parasite is restricted to a smaller area within the range where infected fish have been reported (Johnson et al., 1979) (Figure 1, Table 1).

Figure 1. Geographic distribution of the infective stage of Ceratomyxa shasta.

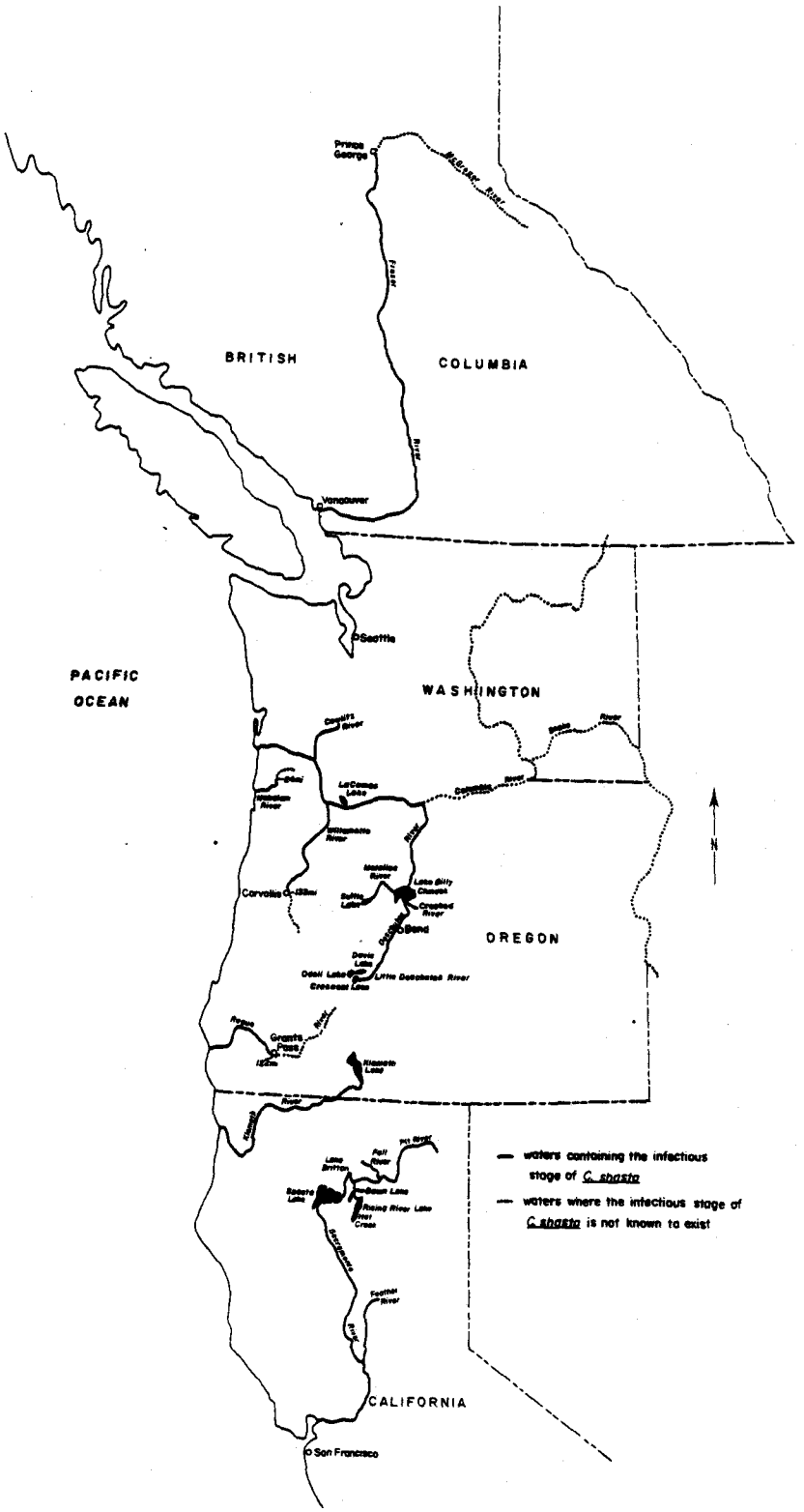

Table 1. Geographic distribution of the infectious stage of *Ceratomyxa shasta*.

Location and Watershed	Comments and Reference
Washington	
Cowlitz River	Wood (1968)
LaCamas Lake	Rucker et al. (1953)
Oregon	
Nehalem River	To river mile 24 (Weber and Knispel, 1977)
Columbia River System	
Columbia River	Upstream to confluence with the Deschutes River (Sanders et al., 1970)
Willamette River	To river mile 133 (Johnson, 1975)
Deschutes River and Tributaries	
Deschutes River	Upstream from mouth to just below Wickiup Reservoir (Ratliff, personal communication, 1984)[1]
Little Deschutes River	Sanders et al. (1970)
Suttle, Davis, Odell, and Crescent Lakes	Sanders et al. (1970)
Metolius River	Johnson et al. (1979)
Crooked River	Upstream from mouth 3 miles (Sanders et al., 1970)
Reynolds Lake, Rock Springs Pond, and Haystack Reservoir	Reservoirs or ponds containing Deschutes River water (Johnson et al., 1979)
Rogue River	Upstream about 112 miles (Holt, personal communication, 1983)[2]
Klamath Lake	R. Holt (personal communication, 1979)[2]
California	
Sacramento River System	
Sacramento River	Upstream from mouth to Shasta Lake (D. Manzer, personal communication, 1984)[3]
Feather River	Downstream from Oroville to confluence with the Sacramento River, and in the north fork to Belden Powerhouse (D. Manzer, personal communication, 1984)[3]
Pit River	Entire drainage (D. Manzer, personal communication, 1984)[3]
Shasta, Crystal, Baum, and Britton Lakes	Schafer (1968)
Hat Creek	Schafer (1968)
Rising River Lake	Schafer (1968)
Fall River	Schafer (1968)
Klamath River	Upstream to Klamath Lake (Johnson et al., 1979)
Canada	
Fraser River	To river mile 449 (Ching and Munday, 1984a)

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2. R. Holt, Pathologist, Oregon Department of Fish and Wildlife, Corvallis, Oregon.
3. D. Manzer, Pathologist, California Department of Fish and Game, Sacramento, California.

Host Range and Susceptibility

It is generally recognized that only salmonids are susceptible to *C. shasta*. Infections by this parasite have occurred in the following salmonid hosts.

Common name	Scientific name	Investigator
Rainbow trout	*Salmo gairdneri*	Noble, 1950
Chinook salmon	*Oncorhynchus tshawytscha*	Conrad and DeCew, 1966
Coho salmon	*Oncorhynchus kisutch*	Conrad and DeCew, 1966
Steelhead trout	*Salmo gairdneri*	Conrad and DeCew, 1966
Brook trout	*Salvelinus fontinalis*	Schafer, 1968
Brown trout	*Salmo trutta*	Schafer, 1968
Atlantic salmon	*Salmo salar*	Sanders et al., 1970
Cutthrout trout	*Salmo clarki*	Sanders et al., 1970
Sockeye salmon	*Oncorhynchus nerka*	Sanders et al., 1970
Chum salmon	*Oncorhynchus keta*	Margolis and Evelyn, 1975

Schafer (1968) and Sanders et al. (1970) noted differences in the susceptibility of these salmonid species. In a study comparing the susceptibility of nine salmonid species, Zinn et al. (1977) found that rainbow, brook, and cutthrout trout, and chum and fall chinook salmon were comparatively more susceptible to ceratomyxosis than coho salmon, and brown trout and Atlantic, sockeye, and spring chinook salmon were least

susceptible. But Wales and Wolf (1955) reported that brook trout were resistant to infection and Schafer (1968) reported they were only moderately susceptible. Reports of the susceptibility of coho salmon have also varied. Schafer (1968) reported that Alsea River coho salmon were resistant to the disease, while Zinn et al. (1977) found them moderately susceptible. These findings suggest that susceptibility may vary within a species.

Johnson (1975) tested four hatchery strains of fall chinook salmon and four strains of rainbow trout for susceptibility to infection by C. shasta. He found that strains from hatcheries located in the Columbia River basin, where the parasite is endemic, were resistant to infection, while strains from locations outside the basin were susceptible.

Zinn et al. (1977) tested the susceptibility of nine hatchery strains of chinook salmon. Groups of fall chinook salmon from areas where the infectious stage of C. shasta did not occur were more susceptible than groups from the Columbia River basin. Spring chinook salmon did not adhere to this pattern. One Columbia River strain of spring chinook salmon was susceptible to ceratomyxosis while one strain from an area where C. shasta is not endemic was resistant.

Buchanan et al. (1983) tested the susceptibility of summer steelhead trout from an Oregon coastal river where

C. shasta has not been shown to exist, and steelhead trout from three Columbia River tributaries. The coastal fish were susceptible to infection while the three Columbia River strains were resistant.

The patterns of resistance found in fall chinook salmon, and rainbow and steelhead trout suggest that ceratomyxosis acts as a selection factor on these salmonids and that the resistance developed by parental stocks is genetically transferred. However, the studies with spring chinook salmon indicate that resistance to infection may develop in stocks that have had no previous exposure to C. shasta (Zinn et al., 1977).

Effects of Temperature

Infection by C. shasta occurs seasonally, appearing in the spring as water temperatures exceed 10°C, and continuing until late fall (Schafer, 1968; Sanders et al., 1970). But in studies to determine the seasonal distribution of C. shasta, infections have been reported at lower temperatures. Weber and Knispel (1977) found C. shasta infections in the Nehalem River in early April, 1976, when the mean water temperature was 7.8°C, and continued to detect ceratomyxosis through mid-December when temperatures reached 6.6°C. The period when mortality was highest began while temperatures were below 10°C and continued until they decreased to 8.9°C. Ratliff (1983)

reported that the beginning of the infective period of C. shasta at Pelton Dam on the Deschutes River was when mean water temperatures were 6.9°C in 1978 and 8.6°C in 1981. Ching and Munday (1984a) reported a high incidence of infections when water temperatures were between 4-6°C in the Fraser River in November, 1982.

Several investigators have examined effects of temperature on the progress of ceratomyxosis. Schafer (1968) observed that C. shasta infections occurred when water temperatures exceeded 10°C; however, a subsequent decrease in temperature did not eliminate an established infection. He also noted that lower temperatures deterred development of the disease. Udey et al. (1974) tested the effects of water temperature on the progress of ceratomyxosis in rainbow trout and coho salmon. Fish were exposed to the parasite then maintained at selected water temperatures ranging from 3.9 to 23.3°C. In rainbow trout, mortality was independent of temperature, but mean time from exposure to death was a function of temperature, increasing from 14 days at 23.3°C to 155 days at 6.7°C. At 3.9°C no deaths occurred but the disease did develop when the fish were moved to 17.8°C water. In coho salmon both mortality and mean time to death were temperature dependent. Mortality ranged from 2% at 9.4°C to 84% at 20.5°C. In these fish, ceratomyxosis seemed to be suppressed by water temperatures of 6.7°C and less.

Control

Because *C. shasta* infections are not transmitted directly between fish, outbreaks of the disease in a hatchery occur as a consequence of introducing the parasite through the water supply. Chemotherapeutic agents which have been tested are not effective in controlling *C. shasta* infections. Although the most effective means of preventing outbreaks in hatcheries is avoidance of water supplies containing the infective stage, this is not always possible.

Two studies evaluated the efficacy of inactivation of the infective stage by water treatment methods. Bedell (1971) tested UV irradiation and chlorination of hatchery water supplies. Both methods reduced the number of *C. shasta* infections, but neither completely eliminated the disease. Sanders et al. (1972) determined that sand filtration in combination with UV irradiation or chlorination of water supplies were effective means of reducing the incidence of disease; however, sand filtration alone was not effective.

The most successful approach for control of ceratomyxosis in both hatchery and wild conditions is the introduction of resistant salmonids (Buchanan et al., 1983). Resistant strains of rainbow and steelhead trout and coho and chinook salmon have been reported (Johnson, 1975; Zinn et al., 1977). The success of management

practices in controlling C. shasta was demonstrated in the Willamette River. When stocking of steelhead trout susceptible to infection was stopped, and resistant fish were introduced, adult returns increased from 0 to 7.5% (Buchanan et al., 1983).

MATERIALS AND METHODS

Experimental Methods

Experimental Animals

All fish used in this study had no history of prior exposure to the infectious stage of *C. shasta*. Fish were obtained from Oregon Department of Fish and Wildlife (ODFW) and United States Fish and Wildlife Service (USFWS) hatcheries. The size of experimental fish was dictated primarily by availability at the time they were needed. In reports by Schafer (1968) and Sanders et al. (1970) it was apparent that the ability of the pathogen to cause infection is not dependent on the age or size of the salmonid.

Fish Holding Facilities

Salmonids were held at the Oregon State University Fish Disease Laboratory (OSU-FDL) in Corvallis, Oregon or the Round Butte Hatchery Isolation Facility (RBH-IF) near Madras, Oregon. The water temperatures at the OSU-FDL and RBH-IF are a constant 12 and 10°C, respectively. Both facilities are supplied with water free of fish pathogens. Fish used to test the effects of salt water on the progress of ceratomyxosis were held in the saltwater facilities at the Oregon State University Marine Science Center Fish Disease Laboratory (MSC-FDL) in Newport, Oregon.

The water supply at the MSC-FDL is UV treated and free of the infective stage of *C. shasta*.

Fish Care

For two weeks prior to exposure to water potentially containing the infective stage of *C. shasta*, all fish were fed an Oregon Moist Pellet diet with 3% terramycin incorporated in the form of TM_{50} (Pfizer). Terramycin was used because it controls many bacterial fish pathogens but is not active against *C. shasta* (Sanders et al., 1972; Udey et al., 1975). Feeding of medicated diet resumed when fish were returned to the laboratory after exposure.

Detection of *Ceratomyxa shasta*

Dead fish were collected daily and either necropsied fresh or were frozen for later examination. Wet mounts of intestinal tract scrapings were examined microscopically at 250-400x for a period of five minutes or until spores were observed. Fish were considered to be infected with *C. shasta* if one or more spores were found. Verification of infection by *C. shasta* by the presence of trophozoites alone was avoided because trophozoites of other myxosporidan species are almost indistinguishable from those of *C. shasta*.

Analysis of Data

Because the patent period for C. shasta is long (an average of 36 days in this study), fish sometimes died from causes other than C. shasta infections before spores could be detected. To adjust for prepatent losses, the percentage of infection was calculated as follows:

$$\frac{\text{Number of fish infected with } C.\ shasta}{\text{Number of fish exposed} \quad \text{minus} \quad \text{Number of fish which died before spores were detected}}$$

Geographic Distribution of Ceratomyxa shasta

A survey of the geographic distribution of C. shasta in the Columbia River basin was conducted by exposing rainbow trout susceptible to C. shasta at selected sites in the watershed, and observing them for signs of ceratomyxosis. Rainbow trout were obtained from Oak Springs and Roaring River Hatcheries. Fish were exposed in 0.074-m^3 cylindrical aluminum liveboxes. Following exposure all fish were transported to either the OSU-FDL or RBH-IF. After 100-120 days, all surviving fish were killed and examined for spores.

In geographic distribution studies conducted in 1983 the exposure period was seven days. In June, exposure sites in the Columbia River were located at the Dalles, John Day, and McNary Dams. Exposure of fish at these dams

was repeated in September with the addition of Ice Harbor Dam on the Snake River and Pelton Dam on the Deschutes River. The Deschutes River location was included as a positive control to demonstrate susceptibility of the test fish. Tributaries of the Columbia River which were tested for the presence of the infectious stage of C. shasta in 1983 included: the Grande Ronde, Imnaha, and Wallowa Rivers, and Lookingglass Creek. These exposures were conducted during July and August.

In 1984 geographic distribution studies were repeated using a 14 day exposure period. Locations in the Columbia River where fish were held were at Bonneville Dam and at selected locations upriver to Priest Rapids Dam. On the Snake River, exposure was at Little Goose Dam instead of Ice Harbor Dam. Exposures were made in June and repeated in September. The Deschutes River location was again included as a positive control. Tributaries where fish were exposed in 1984 included the John Day, Umatilla, and Imnaha Rivers, and the Grande Ronde at a site nearer its confluence with the Snake River than the site used in 1983 (Figure 2).

Susceptibility of Salmonid Strains to Infection by Ceratomyxa shasta

Nine salmonid strains native to the Columbia River basin, and two strains from locations outside the basin

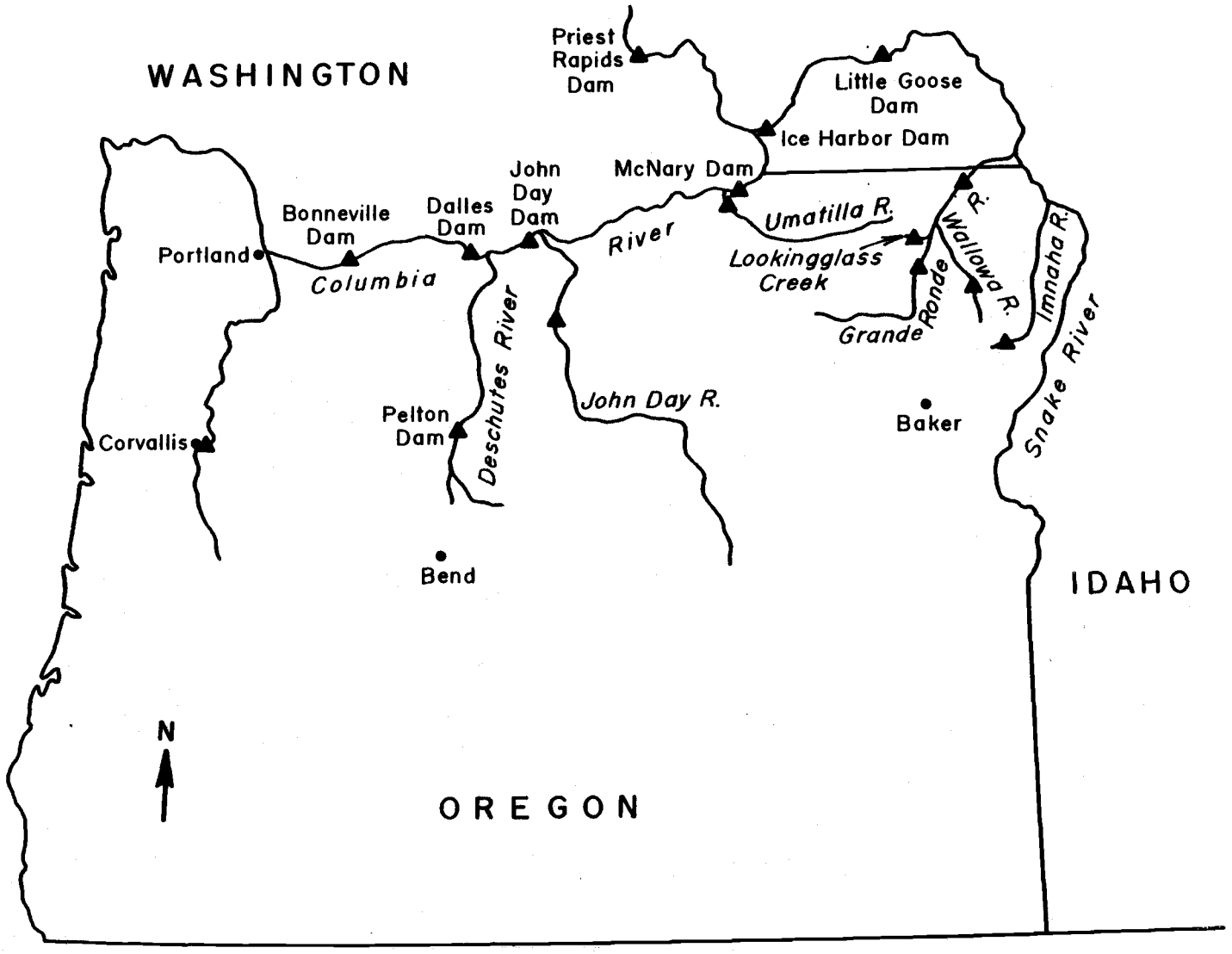

Figure 2. Location of sites where fish were exposed in the Columbia River basin.

Table 2. Salmonid strains tested for resistance to infection by *Ceratomyxa shasta*.

Salmonid Species	Strain	Hatchery Source
Rainbow trout *Salmo gairdneri*		
	Oak Springs	Oak Springs
	Eagle Lake	Klamath
Steelhead trout *Salmo gairdneri*		
	Imnaha	Irrigon
	Willard	Willard
Chinook salmon *Oncorhynchus tshawytscha*		
	Carson	Lookingglass
	Imnaha	Lookingglass
	Lookingglass	Lookingglass
	Upriver Brights	Bonneville
	Lower Columbia River	Bonneville
	Umpqua	Rock Creek
Coho salmon *Oncorhynchus kisutch*		
	Sandy	Sandy
	Willard	Willard

were tested for resistance to C. shasta infection (Table 2). All groups of fish were exposed for five days to the infective stage of C. shasta in the Willamette River near Corvallis, Oregon (Figure 2). Oak Springs rainbow trout were used as a positive control in each group so that incidence of infection could be compared among groups. After exposure, these fish were returned to the OSU-FDL and maintained for 100-120 days.

Resistance of Rainbow Trout ♀ x Coho Salmon ♂ Hybrids to Ceratomyxa shasta

An interspecific cross between a strain of coho salmon resistant to C. shasta infection and a strain of rainbow trout susceptible to infection was made to determine if resistance or susceptibility to this parasite is a genetically transferrable trait. Coho salmon eggs were pooled from three fish collected at Sandy River Hatchery. Rainbow trout eggs were collected from a single parent at the Oak Springs Hatchery. Semen was collected and pooled from ten males at each hatchery. Gametes were transported on ice and fertilized by mixing 10 ml semen per 1000 eggs. Hybrids were created by fertilizing rainbow trout eggs with coho salmon sperm. Coho x coho and rainbow x rainbow fertilizations were also made. Eggs were incubated in the dark in tanks with 12°C flowing water. Dead eggs and yolk sac fry were removed daily.

To verify that the rainbow trout ♀ x coho salmon ♂

was a true interspecific hybrid, the liver isoenzymes of the parental and hybrid species were compared by electrophoresis. Electrophoresis was performed by Dr. G. A. E. Gall, Department of Animal Science, University of California, Davis. In each enzyme studied, the ten hybrid fish examined displayed the isoenzyme bands of both parental species, verifying that the rainbow trout x coho salmon was a true hybrid.

Coho salmon and rainbow trout fry, and rainbow trout x coho salmon hybrids were held at the OSU-FDL until they reached a size suitable for exposure to the infectious stage of C. shasta (about 5 cm.). After a three day exposure period in the Willamette River, they were returned to the OSU-FDL and observed for signs of ceratomyxosis for 120 days, after which time all surviving fish were examined for spores.

Effects of Salt Water on Fish Infected with Ceratomyxa shasta

Salmonid smolts were used to determine the effects of salt water on the progress of ceratomyxosis. Big Creek coho salmon, a Columbia River strain resistant to C. shasta infection, were obtained from Big Creek Hatchery, and Alsea River steelhead trout, a strain susceptible to C. shasta, were from Alsea Hatchery. These fish were held at the OSU-FDL until they reached the smolt stage.

In 1983, two groups of Alsea steelhead trout were exposed to the infective stage of C. shasta in the Willamette River, one group for three days, and the other for five days. In 1984 Alsea steelhead trout and Big Creek coho salmon were exposed for five days. After exposure, these fish were transferred to the OSU-FDL or the MSC-FDL, and held until they were sacrificed 100 days later.

RESULTS

Geographic Distribution of *Ceratomyxa shasta*

In 1983, fish were exposed at selected sites in the main stem of the Columbia River for a period of seven days in June and again in September (Table 3). Infections caused by *C. shasta* were found in 5% of the fish exposed during June at McNary Dam. Infections were also found in 29% of the fish exposed at Pelton Dam on the Deschutes River. The disease was not found in fish held at the Dalles and John Day Dams on the Columbia River, or at Ice Harbor Dam on the Snake River during either exposure period.

During 1984, the exposure period was increased to 14 days. The sites used were varied from those used in 1983 either by changing the location of the site at the dam or by the addition of an exposure site. Infections were found in 45% of the fish exposed at Pelton Dam (Table 3). Two exposure sites at McNary Dam, a site located in the adult fish ladder (upper site) and a site in a juvenile holding tank (lower site), yielded infection incidences of 12.5 and 1.8% respectively during July, but no infections developed from the September exposure. Infections were also found in 16% of the fish at Bonneville Dam, and in 52% of the fish at the Dalles Dam during July. Infection incidences were 0 and 2.6% respectively for the Bonneville and Dalles Dam

Table 3. Incidence of *Ceratomyxa shasta* in susceptible rainbow trout (*Salmo gairdneri*) exposed at selected dams in the Columbia River basin during June and September, 1983[1], and during July and September, 1984[2].

Location and time of exposure	No. of fish recovered[3]	No. of fish infected with C. shasta	Percent of fish infected with C. shasta
Columbia River			
Bonneville Dam			
July, 1984	25	4.0	16.0
Sept., 1984	33	0.0	0.0
Dalles Dam			
June, 1983	56	0.0	0.0
Sept., 1983	50	0.0	0.0
July, 1984	48	25.0	52.0
Sept., 1984	38	1.0	2.6
John Day Dam			
June, 1983	59	0.0	0.0
Sept., 1983	50	0.0	0.0
July, 1984	40	0.0	0.0
Sept., 1984	42	0.0	0.0
McNary Dam			
June, 1983	58	3.0	5.0
Sept., 1983	50	0.0	0.0
July, 1984			
upper site	32	4.0	12.5
lower site	55	1.0	1.8
Sept., 1984			
lower site	44	0.0	0.0
Priest Rapids Dam			
July, 1984	55	0.0	0.0
Sept., 1984	42	0.0	0.0
Snake River			
Ice Harbor Dam			
Sept., 1983	50	0.0	0.0
Little Goose Dam			
July, 1984			
upper site	53	0.0	0.0
lower site	40	1.0	2.5
Deschutes River			
Pelton Dam			
Sept., 1983[4]	96	28.0	29.0
June, 1984	51	23.0	45.0

1. In 1983, fish were exposed for 7 days.
2. In 1984, fish were exposed for 14 days.
3. Number of fish exposed minus deaths caused by handling stress (first 10 days).
4. This group was exposed for 23 days.

exposures in September. The disease was not found at John Day or Priest Rapids Dams on the Columbia River during either exposure. On the Snake River, exposures were made at two locations at Little Goose Dam in July. Fish held in the forebay of the dam (upper site) did not develop the disease, but 2.5% of the fish exposed in juvenile holding facilities (lower site) were infected.

Susceptible rainbow trout were exposed in six tributaries of the Columbia River (Table 4, Figure 1). During 1983, exposures were made in the Imnaha, Wallowa, and Grande Ronde Rivers, and in Lookingglass Creek, a tributary of the Grande Ronde River. Infections caused by C. shasta were not found in fish recovered from any of these sites. In 1984, fish were exposed in the Grande Ronde River at a site nearer its confluence with the Snake River, and in the Imnaha, Umatilla, and John Day Rivers. Again, no infections caused by C. shasta were found.

Susceptibility of Salmonid Strains to Infection by Ceratomyxa shasta

During 1983 and 1984, eleven hatchery stocks of salmonids were exposed in an area of the Willamette River known to harbor the infectious stage of C. shasta (Table 5). Oak Springs rainbow trout were used as a positive control for each exposure group because they are susceptible to infection. Use of the positive control

Table 4. Incidence of Ceratomyxa shasta in susceptible rainbow trout (Salmo gairdneri) exposed in Columbia River tributaries during July and August 1983[1] and 1984[2].

Location and time of exposure	No. of fish recovered[3]	No. of fish infected with C. shasta	Percent of fish infected with C. shasta
Lookingglass Creek			
July, 1983	33	0	0
Grande Ronde River			
July, 1983	18	0	0
July, 1984[4]	32	0	0
Wallowa River			
August, 1983	56	0	0
Imnaha River			
August, 1983	28	0	0
August, 1984	41	0	0
Umatilla River			
July, 1984	32	0	0
John Day River			
July, 1984	7	0	0

1. In 1983, groups were exposed for 7 days.
2. In 1984, groups were exposed for 14 days.
3. Number of fish exposed minus deaths caused by handling stress (first 10 days).
4. The exposure site used in 1984 was closer to the confluence with the Snake River than the site used in 1983.

allowed comparison of the infection incidence among groups of exposed fish. In 1983, at 120 days after the exposure period, all surviving fish were killed and examined for the presence of spores. Because no spores were found this practice was altered the following year. In 1984, all fish surviving 100 days after exposure were considered resistant to infection.

Four strains of upper Columbia River chinook salmon, Carson, Imnaha, Lookingglass, and upriver brights, and one strain of lower Columbia River chinook salmon were resistant to ceratomyxosis, with infection incidences not exceeding 2%. One group of chinook salmon from an area where the infectious stage of C. shasta is not known to exist, the Umpqua chinook salmon, was also resistant to infection.

Both strains of Columbia River coho salmon, the Sandy and Willard, were resistant to infection by C. shasta. But, Willard coho salmon were exposed during a period when levels of the infectious stage of C. shasta in the Willamette River were low, as was demonstrated by a 31% infection incidence in the control rainbow trout.

Two strains of upper Columbia River steelhead trout were tested for resistance. The Imnaha steelhead trout were resistant in each of two exposures. In the 1983 exposure, the Wallowa steelhead trout seemed slightly susceptible to ceratomyxosis, with an infection incidence

Table 5. Susceptibility of rainbow and steelhead trout (Salmo gairdneri), chinook salmon (Oncorhynchus tshawytscha), and coho salmon (Oncorhynchus kisutch) exposed for five days to the infective stage of Ceratomyxa shasta in the Willamette River.

Salmonid stock	No. of fish recovered[1]	No. of fish infected with C. shasta	Percent of fish infected with C. shasta
August 1983 exposures			
Rainbow trout			
Oak Springs[2]	38	28	74
Steelhead trout			
Wallowa	47	5	11
Chinook salmon			
Carson	20	0	0
Imnaha	43	1	2
Lookingglass	49	1	2
Coho salmon			
Sandy	25	0	0
Sept. 1983 exposures			
Rainbow trout			
Oak Springs	43	31	73
Steelhead trout			
Imnaha	49	1	2
Chinook salmon			
Upriver brights	56	1	2

Table 5. (Continued)

Salmonid stock	No. of fish recovered[1]	No. of fish infected with C. shasta	Percent of fish infected with C. shasta
June 1984 exposures			
Rainbow trout			
Oak Springs	45	14	31
Coho salmon			
Willard	55	0	0
August 1984 exposures			
Rainbow trout			
Oak Springs	51	32	61
Chinook salmon			
Umpqua	11	0	0
Upriver brights	32	0	0
Lower Columbia River	48	1	2
Imnaha	52	0	0
Carson	67	0	0
Sept., 1984 exposures			
Rainbow trout			
Oak Springs	62	48	77
Eagle Lake	47	8	17
Steelhead trout			
Imnaha	65	0	0
Wallowa	66	0	0

1. Number of fish exposed minus number of fish which died before spores were detected.
2. Positive control group used in each exposure so infection levels could be compared.

of 11%. But in the 1984 exposure, there were no infections resulting from C. shasta in this group. Rainbow trout control groups had similar infection incidences for both exposure periods (74% in 1983 and 77% in 1984).

One strain of rainbow trout was tested for resistance to infection by C. shasta. Eagle Lake rainbow trout, from Klamath Hatchery, are originally from Eagle Lake, California. These fish were slightly susceptible to C. shasta, with an infection incidence of 17%.

Resistance of Rainbow Trout ♀ x Coho Salmon ♂ Hybrids to Ceratomyxa shasta

Groups of coho salmon resistant to C. shasta, rainbow trout susceptible to C. shasta, and rainbow trout ♀ x coho salmon ♂ hybrids were exposed to the infectious stage of C. shasta in the Willamette River for three days (Table 6). Coho salmon were resistant to infection. Rainbow trout and the rainbow trout x coho salmon hybrid were susceptible. The incidence of infection was 39 and 42%, respectively. Susceptibility seemed to be inherited as a dominant genetic trait. The sex of the hybrids was not determined; therefore, it is not known whether C. shasta susceptibility was sex-linked or autosomal.

Table 6. Susceptibility of coho salmon (Oncorhynchus kisutch), rainbow trout (Salmo gairdneri), and rainbow trout ♀ -coho salmon ♂ hybrids exposed for three days to the infectious stage of Ceratomyxa shasta in the Willamette River.

Salmonid	No. of fish recovered[1]	No. infected with C. shasta	Percent infected with C. shasta
Sandy coho salmon	24	0	0
Oak Springs rainbow trout	28	11	39
Rainbow trout-coho salmon hybrid	19	8	42

1. Number of fish exposed minus number of fish which died before spores were detected.

Effects of Salt Water on Fish Infected with Ceratomyxa shasta

Alsea steelhead trout smolts susceptible to C. shasta infections were exposed to the infectious stage of C. shasta in the Willamette River. In 1983, two groups were exposed, one group for three days and the second for five days. In 1984 the fish were exposed for five days. Half of the fish in each group were transferred to salt water, and half were held in fresh water. The incidence of infection in all groups transferred to fresh water was 100% (Table 7). The freshwater control group demonstrated that the fish were not previously infected with C. shasta. In groups transferred to salt water, some fish died prior to the development of ceratomyxosis. Losses in unexposed, control fish held in salt water indicated that these prepatent losses were caused by inability of the fish to adjust to salt water. Infection incidences in fish surviving the prepatent losses in salt water were lower in two of the three groups than incidences in fish transferred to fresh water (Table 7).

Big Creek coho salmon, which are resistant to ceratomyxosis, were exposed to the infectious stage of C. shasta for five days. Only one fish held in fresh water developed spores, and these spores were found in a muscle lesion rather than in the intestinal tract (Table 7). Neither control fish nor coho salmon exposed to C. shasta

and subsequently transferred to salt water suffered the high prepatent losses found in the steelhead trout. None of the coho salmon transferred to salt water died from *C. shasta*.

Table 7. Effects of salt water on steelhead trout (Salmo gairdneri) and coho salmon (Oncorhynchus kisutch) exposed to the infectious stage of Ceratomyxa shasta.

Salmonid	exposure period (days)	Fresh water			Salt water		
		No. of fish recovered[1]	No. of fish infected	Percent infected	No. of fish recovered[1]	No. of fish infected	Percent infected
1983 exposures[2]							
Alsea steelhead trout	3	21	21	100	6	3	50
	5	23	23	100	13	7	54
	control[3]	19	0	0	11	0	0
1984 exposures[2]							
Alsea steelhead trout	5	18	18	100	9	8	89
	control[3]	25	0	0	16	0	0
Big Creek coho salmon	5	25	1	4	25	0	0
	control[3]	25	0	0	25	0	0

1. Number of fish exposed minus number of fish which died before spores were detected.
2. 25 fish in each exposure group.
3. Control fish were not exposed to the infectious stage of Ceratomyxa shasta.

DISCUSSION

The distribution of the infectious stage of Ceratomyxa shasta in the Columbia River basin has been documented by several investigators (Sanders et al., 1970; Johnson et al., 1979). The parasite was believed to occur in the mainstem of the Columbia River upstream only to its confluence with the Deschutes River. The infectious stage also occurs throughout most of the Deschutes River basin, and in the Willamette River to river mile 133 (Johnson et al., 1979). In this study the distribution of the infectious stage of C. shasta was extended to include the Columbia River upstream to its confluence with the Snake River, and the Snake River to Little Goose Dam. This represents an increase of approximately 200 river miles that are potentially infectious for migrating Snake and upper Columbia River salmonids.

It is not possible to determine whether the increase in the geographic range of the infectious stage of C. shasta documented in the study represents a dissemination of the parasite, or simply an improved method of detection. All locations where fish were exposed in the Columbia and Snake Rivers were included in a study similar to this one conducted during 1966 and 1967, with the exception of sites at Little Goose Dam (Sanders et al., 1970; Sanders and Fryer, 1967). In that study no infections resulting from C. shasta were found at any locations above the Deschutes

River. Groups of fish exposed in the 1966-67 survey were held at the site for the duration of the experiment (60 to 90 days). High non-specific losses during the exposure may have masked the presence of C. shasta infections. In the present study, fish were exposed then returned to a pathogen-free water supply and fed a medicated diet. The decrease in non-specific loss and extension of the holding period to 100 days following exposure may have allowed increased detection of the parasite.

In this survey, incidence of infections seemed dependent on the location of the exposure site and time of exposure. At McNary Dam, where two exposure sites were used in July 1984, infection incidence was 1.8 and 12.5%. The importance of the exposure location was illustrated by Johnson (1975) who found that infection incidence varied by as much as 71% when fish were exposed simultaneously at sites within a 0.1 mile distance in the Willamette River. The change in location of the exposure site may account for not detecting the parasite at the Dalles Dam in 1983, but finding a 52% incidence in 1984.

Percent infection also varied at the same site depending on the season of exposure. Infections in this survey occurred only during June and July, with the exception of a 2.6% infection incidence among fish exposed at the Dalles Dam in September, 1984. This indicates a decrease in the number of infectious units in the Columbia

River over the course of the summer. However, in a seasonal distribution survey conducted by Ching and Munday (1984a) in the Fraser River, the abundance of infectious C. shasta increased in late summer and fall. Weber and Knispel (1977) found that the incidence of infection was high from late April to late November in the Nehalem River, and Johnson (1975) found infection in fish exposed in the Willamette River from spring through early fall. Conversely, Ratliff (1983) found a gradual decrease in the estimated abundance of C. shasta in the lower Deschutes River from June to November. He suggested the increased abundance of the infective stage coincides with the peak of returning adult chinook salmon. Infection of fish during May and June would allow C. shasta to progress to the spore stage of its life cycle before the spawning and death of its host. Spores would be released during periods of low water flow when they could anchor in the river substrate. A similar situation could be hypothesized for the Columbia River, but more information about the seasonal distribution of the parasite in that system is necessary.

Although C. shasta has not been found in the Columbia River above its confluence with the Snake River, it is likely present there in low numbers. This is suggested by the presence of the infective stage at McNary Dam at a higher incidence than found in the Snake River. If C. shasta were not present in either the Yakima or upper

Columbia Rivers, the infectious units from the Snake River would probably be diluted to undetectable levels at McNary Dam. Future surveys of the geographic distribution of C. shasta should include exposure sites in both the Yakima and upper Columbia Rivers.

The extension of the known range of C. shasta in the study may demonstrate a real increase in the distribution of the parasite. Ratliff (1983) suggested that the function of polar filaments is to anchor the spore to the river substrate where it undergoes an "aging" process. Any environmental change which reduces river flow and allows accumulation of silt increases the settling of spores. The building of dams on the Columbia River has resulted in reservoirs which fill this criteria. Thus it is possible that alterations on the Columbia River are responsible for creating an environment favorable to the spread of this parasite.

Although the geographic range of C. shasta was expanded in the mainstem Columbia River and Snake River, infections resulting from C. shasta were not found in any group of fish exposed in Columbia River tributaries. Infected adult salmonids return to the Grande Ronde and Wallowa Rivers, and it is suspected that they also return to the other upriver tributaries where fish were exposed. Although spores are being released into these waters, the presence of the infective stage was not demonstrated. This

phenomenon has been reported by other investigators (Johnson, 1975; Sanders et al., 1970) and further indicates that the infection process requires some factor not present in these tributaries. Wood (1968) proposed the requirement of a lake or reservoir and waters of low velocity, and Ratliff (1983) suggested similar conditions which would allow a favorable environment for the settling and anchoring of spores. The only tributary where fish were exposed that meet these criteria is the Umatilla River. The Umatilla River contains water from McKay and Cold Springs Reservoirs. Although Wallowa River drains from Wallowa Lake, returning adults do not have access to the lake. The absence of the infective stage in these rivers may be because of the low number of returning adults shedding spores in an area suitable for spore attachment, or the absence of some other factor necessary for the development of the parasite.

The presence of the infectious stage of C. shasta in a river system can act as a selection factor on salmonids living in, or passing through it (Zinn et al., 1977; Buchanan et al., 1983). Therefore any fish residing in, or migrating through, the Columbia River should develop resistance to ceratomyxosis through the process of natural selection. All Columbia River strains of chinook and coho salmon and steelhead trout tested were resistant to the parasite; whereas, rainbow trout from outside the Columbia

River basin were susceptible to infection.

One exception among Columbia River strains was the Carson chinook salmon, which were reported by Zinn et al. (1977) to be susceptible to ceratomyxosis. They suggested that development of resistance in these fish may be a result of factors other than exposure of parental stocks. However, Carson chinook salmon were exposed at two separate times during this study and were found resistant to infection, indicating that this strain, like all other Columbia River strains tested, were resistant. The resistance of Columbia River strains makes them good candidates for introduction in water containing the infective stage of C. shasta.

While strains of upriver salmonids are considered resistant to infection, these conclusions were drawn from experiments in which periods of exposure were only 5 days. Longer exposure periods may result in a higher prevelance of infection. Extension of the known range of the infective stage of C. shasta to Little Goose Dam on the Snake River suggests upriver salmonids are exposed to this parasite for a much longer period than previously recognized. Dawley et al. (1984) calculated that during 1983 the average migration rate for yearling chinook salmon and steelhead trout was 18 and 35 km/day in the Columbia River. This means fish migrating from Little Goose Dam to the Columbia River bar would be exposed to the infective

stage of C. shasta for 35 and 18 days, respectively. Coho salmon come primarily from lower Columbia River locations and are not subject to long exposure to the infective stage.

Two strains of salmonids not originating from the Columbia River basin, Umpqua chinook salmon and Eagle Lake rainbow trout, were also tested for resistance to C. shasta. The Umpqua chinook salmon originate from the Umpqua River, a coastal river where the infectious stage of C. shasta is not known to exist. Studies by Zinn et al. (1977) showed this strain of fish was resistant to infection. Results from this study agree, although few fish were recovered. The susceptibility to ceratomyxosis of other salmonids in this river, the Umpqua steelhead trout (Buchanan, 1977) and Smith River coho salmon (R. Holt, Pathologist, ODFW, Corvallis, personal communication), indicates that the infectious stage of C. shasta is not present in this system. The resistance of the Umpqua chinook salmon may be explained by the stocking of resistant chinook salmon. In the 1950's when numbers of returning adult Umpqua chinook salmon were low, Rogue, Imnaha, and Columbia River chinook salmon strains were introduced (J. Bauer, Chief of Fish Culture, ODFW, Portland, personal communication). These are all strains known to be resistant to C. shasta. The resistance of what is now the Umpqua chinook salmon indicates that the run, as

it now exists, may be offspring from survivors of these introductions, and not the native Umpqua chinook salmon.

Eagle Lake rainbow trout were obtained by ODFW from Eagle Lake, California, in 1970 as eggs (Kinunen et al., 1978). These fish are used to stock alkaline lakes and reservoirs in central and southeastern Oregon. The strain has been maintained at Klamath Hatchery where it has had no contact with the infectious stage of C. shasta. If only the history of this strain in Oregon were considered, they would be expected to be susceptible to infection. However, when exposed to the parasite they were resistant, with an infection incidence of 17% compared to an incidence of 77% in control rainbow trout. Eagle Lake has not been studied for the presence of the infectious stage of C. shasta; however, nearby drainages are known to harbor the parasite. This resistance may indicate the presence of C. shasta within the Eagle Lake watershed, or that this strain originated from a nearby drainage containing the infectious stage of C. shasta.

All studies on the resistance of salmonids to C. shasta were conducted with juvenile fish. There is some evidence indicating that resistance of adult salmonids differs from that of juveniles. Sanders (1967) reported that in returning adult Sandy River coho salmon, 78% of the spawned fish and 92% of the prespawning mortality were infected with C. shasta. However, juvenile Sandy River

coho salmon used in this study were resistant to infection. Another example is the Umpqua chinook salmon, which as juveniles were highly resistant to infection; however, 45% of the adults exposed to the infectious stage of C. shasta were infected (Sanders et al., 1970). To further demonstrate the difference between the resistance of juvenile and returning adult salmonids to this parasite, a resistant strain from an area where the parasite is not found should be used (Umpqua chinook salmon). Juveniles and returning adults should be exposed simultaneously to the infective stage of C. shasta and the incidences of infection compared.

The development of resistance to C. shasta in susceptible strains of salmonids would be beneficial for management of stocks. In the past, large numbers of fish were lost when susceptible fish were planted in watersheds containing the infectious stage of C. shasta. In this study an interspecific cross was made between a resistant strain of coho salmon and a susceptible strain of rainbow trout. Infection among exposed rainbow trout ♀ x coho salmon ♂ hybrids was similar to that in the exposed rainbow trout group. This observation demonstrates that susceptibility to C. shasta infections can be genetically transferred. Perhaps if the egg had been from the resistant species, resistance to the parasite may have been transferred. Reciprocal crosses were not made because

other researchers reported a 0% hatching rate for that cross (Chevassus et al., 1983). However, in an experiment where reciprocal crosses were made between resistant and susceptible strains of coho salmon, susceptibility was the dominant factor and did not appear sex-linked (A. Hemmingson, Biologist, ODFW, Corvallis, personal communication). Transference of susceptibility has important management implications. If susceptible strains are inadvertently planted in watersheds containing the infectious stage of C. shasta not only will that stock have poor survival, but it may affect the survival of resident resistant strains.

The dominance of susceptibility as a genetic trait suggests that the resistance of the Umpqua chinook salmon is not derived from the interbreeding of a native susceptible chinook salmon with resistant chinook salmon strains. The strain arising from this cross would be susceptible to infection. Instead, results indicate that the original Umpqua chinook salmon no longer exists and the resistant Rogue and Columbia River strains are what is now known as Umpqua chinook salmon.

The effects of salt water on the progress of a C. shasta infection has an important impact on the survival of anadromous fishes. Few studies have been done concerning this phenomenon. Johnson (1975) reported that infections of C. shasta were prevented at salt concentrations greater

than 15 ppt. He suggested this would protect juvenile salmonids from infection in nursery areas of the Columbia River estuary. However, the fate of fish already infected with the parasite before entering salt water was not determined. Acute ceratomyxosis has been reported in juvenile chum salmon captured off the coast of British Columbia (Margolis and Evelyn, 1975), demonstrating that the disease is not attenuated by salt water. Recently, Ching and Munday (1984b) documented the effects of salt water on infected chinook salmon. After exposing the fish to the parasite for 10 days, the fish were returned to either fresh water or salt water. They found that the disease progressed in salt water and mortality was 100% in both groups. Similar experiments were conducted in this study with steelhead trout and coho salmon. Although survival was poor in steelhead trout transferred to salt water after exposure, two of the three groups held in salt water had a lower incidence of infection than groups transferred to fresh water. All exposed steelhead trout held in fresh water were infected. It appears that migration to salt water may reduce the progress of ceratomyxosis but the extent of attenuation may have been masked in fish overwhelmed by a high number of infectious units. The high incidence of infection and long exposure period used for chinook salmon (Ching and Munday, 1984b) may indicate these fish were also overwhelmed by the

parasite. To determine the effects of salt water on fish groups with a low incidence of infection, shorter exposure periods should be used.

Big Creek coho salmon, a strain resistant to C. shasta, were also tested to determine the effects of salt water on the infection process. Both the exposed fish transferred to fresh water and the fish transferred to salt water were resistant to ceratomyxosis. Although juvenile coho salmon were resistant to infection, spawning adult Big Creek coho salmon suffered a 57% incidence of infection (Sanders, 1967). Because detection of ceratomyxosis relies on identification of the spore stage of the parasite, it is not known if the infective stage is blocked at the site of entry, or becomes established but cannot cause an active infection leading to spore formation. Two possibilities could explain the resistance of juveniles versus the susceptibility of adults that has been demonstrated for some salmonids. First, juveniles may become infected prior to their saltwater migration and maintain the parasite in a dormant state after entering salt water. When the salmonid reenters fresh water to spawn, the parasite becomes active as the fish's resistance deteriorates. Second, juveniles may be resistant to infection, but upon reentering fresh water as adults become infected because of their weakened condition. Ratliff (1983) suggested the success of C. shasta is related to its ability to infect adult salmonids

during a time when destruction of the digestive organs is not necessarily detrimental to the salmonid's longevity. Although this situation would produce a normal host-parasite relationship, the high number of adult prespawning losses due to C. shasta (Sanders, 1967) does not support this and indicates that infections do affect the longevity of adults.

SUMMARY AND CONCLUSIONS

1. Presence of the infective stage of *C. shasta* was demonstrated in the Columbia River to its confluence with the Snake River, and in the Snake River to Little Goose Dam.
2. Five strains of Columbia River chinook salmon, the Carson, Imnaha, Lookingglass, upriver brights, and lower Columbia River; two strains of coho salmon, the Willard and Sandy River, and two strains of steelhead trout, the Imnaha and Wallowa, were all resistant to infection by *C. shasta*.
3. Umpqua chinook salmon, and Eagle Lake rainbow trout were resistant to *C. shasta* even though they are from locations where the presence of the infective stage has not been demonstrated.
4. The susceptibility of a rainbow trout strain to *C. shasta* was genetically transferred in a cross with a resistant strain of coho salmon.
5. Alsea steelhead trout smolts exposed to the infective stage of *C. shasta* died from ceratomyxosis when held in either fresh water or salt water.
6. Big Creek coho salmon smolts held in either salt water or fresh water following exposure, were resistant to the disease.

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