LITERATURE REVIEW
CERATOMYXA SHASTA,
INFECTIONS OF SALMONID FISH

PELTON ROUND BUTTE HYDROELECTRIC PROJECT

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INTRODUCTION

Ceratomyxosis is a disease of salmonid fishes caused by the myxosporean *Ceratomyxa shasta*. The parasite has a tropism for the intestinal tissue of the fish and causes high mortalities in susceptible strains of salmonids. The disease was first observed in 1948 in fall-spawning rainbow trout *(Oncorhynchus mykiss)* from Crystal Lake Hatchery, Shasta County, California (Wales and Wolf 1955). The etiological agent was established as a new species by Noble (1950), who described *C. shasta* as the first species of the genus *Ceratomyxa* to parasitize freshwater fish and the only histozoic member of the genus. Other species of *Ceratomyxa* occur in marine fishes and parasitize the lumen of the gall and urinary bladders.

In 1965, *C. shasta* was first identified in juvenile salmonids from Oregon Columbia River hatcheries (Conrad and Decew 1966). All epizootics occurred at hatcheries using water impounded by dams for fish rearing. Epizootics at the Oregon and California hatcheries ceased when the source of rearing water was changed. Although these modifications have decreased losses of fish in many hatcheries, migrating fish are still exposed to the parasite, and the disease contributes significantly to losses in both outmigrating juvenile and returning adult salmon.

Early studies of the parasite examined its distribution and seasonality, explored routes of transmission and compared the resistance of different salmonid species. Schafer (1968) established that the spore of *C. shasta* was not directly infectious for salmonids and was the first to suggest that infection may be initiated by an unidentified life stage. This was recently confirmed by Bartholomew and co-workers (Bartholomew et al. 1997) who demonstrated that completion of the life cycle requires development of alternate life stages in the freshwater polychaete, *Manayunkia speciosa*. The spore released from the polychaete is the infectious stage for the fish (Figure 1).

In fish culture, control of ceratomyxosis is accomplished through avoidance of water sources containing the infective stage, or treatment of the water using ozone, sand filtration, chlorination or ultraviolet irradiation (Bedell 1971; Sanders et al. 1972; Bower and Margolis 1985). No vaccine has been developed and no therapeutant has been effective in preventing or controlling development of ceratomyxosis (Ibarra et al. 1990). The most widely used approach is to raise resistant salmonids for stocking into waters containing the infectious stage of *C. shasta* (Zinn et al. 1977; Buchanan et al. 1983). Resistance to ceratomyxosis, as with all diseases, is relative and can be defined as the ability of a population of fish to limit the rate of infection and disease by *C. shasta* compared to the ability of another population. The ability to resist infection is affected by variables inherent in the pathogen, the environment, and the host.

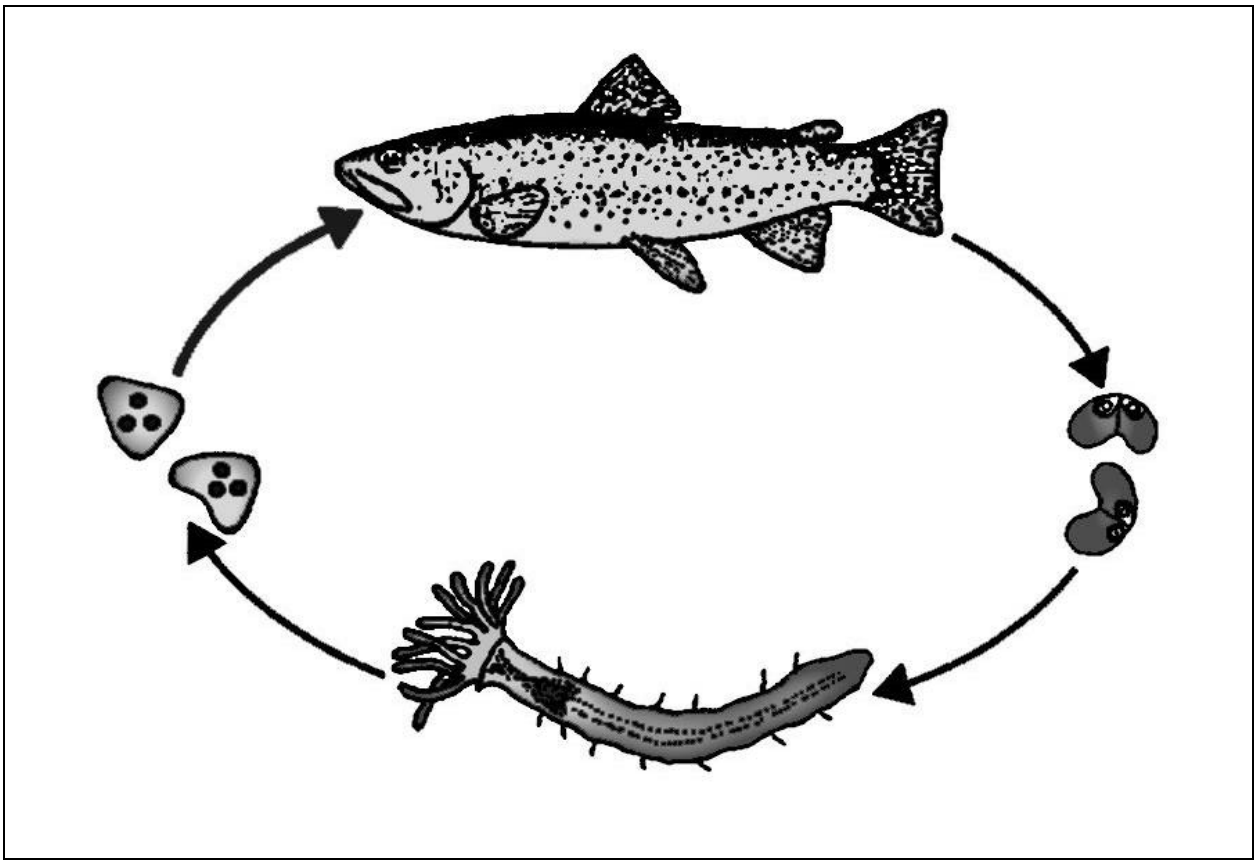

Figure 1. Schematic representation of the life cycle of *Ceratomyxa shasta*. The myxosporean spore is released from the fish and infects the polychaete worm *Manayunkia speciosa*. In turn the worm releases an actinosporean spore, which is able to infect susceptible fish.

GEOGRAPHIC DISTRIBUTION

Ceratomyxa shasta has been identified in salmonids from marine and freshwater environments in northern California, Oregon, Washington, Idaho, Alaska, and British Columbia. Within this broad range, only certain river systems or portions of these systems contain the stage of the parasite infective to salmonids. Waters where infected fish are found do not necessarily contain the infective stage of the parasite (Johnson et al. 1979). This is exemplified in the Columbia River basin, portions of which are located in Oregon, Washington and Idaho. Infected adult coho *(O. kisutch)* and chinook salmon *(O. tshawytscha)* and steelhead trout *(O. mykiss)* migrate and distribute spores throughout the Columbia River drainage, but the infective stage of *C. shasta* has not been demonstrated in many of the tributaries where these fish return to spawn. Within this region *C. shasta* is enzootic throughout much of the Columbia River basin, including the Willamette and Deschutes Rivers, as well as the Klamath and Rogue Rivers (Hoffmaster et al. 1988; Bartholomew et al. 1989a). The reasons for the confinement of this parasite to salmonids of the Pacific Northwest and for its sporadic distribution within this range are unknown. The range of many other fish pathogens has been expanded as a result of shipments of eggs, fish, and intermediate or reservoir hosts. The distribution characteristics of this parasite suggest that the release of spores into the water is, by itself, insufficient to cause disease transmission and indicate that requirements for completion of the life cycle of *C. shasta* are not uniform within these systems.

The presence of the infective stage of *C. shasta* is demonstrated by exposing sentinel populations of susceptible salmonids and observing them for development of the disease and appearance of spores. The distribution of the infective stage has been documented by Johnson et al. (1979), Ching and Munday (1984a), Hoffmaster et al. (1988) and Hendrickson et al. (1989). Comparison of the distribution of *C. shasta* within the Columbia River basin published in 1979 (Johnson et al.) with that reported in 1988 (Hoffmaster et al.) suggests that the parasite has spread. The increase in *C. shasta's* range appears restricted to the mainstem of the Columbia and Snake Rivers and the infective stage of the parasite has not been detected in upriver tributaries. The cause of this expansion is not known, and there are not sufficient data from other river systems to determine if this is an isolated event.

INTERSPECIFIC VARIATIONS IN RESISTANCE

Only members of the Salmonidae are known to be affected by ceratomyxosis, and different species vary in their susceptibility. In the first description of an epizootic it was noted that infections in rainbow trout were serious, but brook *(Salvelinus fontinalis)* and brown trout

(Salmo trutta) held in the same water supply either did not become infected, or the disease was less severe (Schafer 1968). In subsequent epizootics at hatcheries in Oregon, it was again observed that some fish were more susceptible to ceratomyxosis than others. Mortality in chinook *(O. tshawytscha)* and coho salmon *(O. kisutch)* was low, but steelhead trout *(O. mykiss)* were severely affected (Conrad and DeCew 1966). Sanders and co-workers (1970) found certain juvenile rainbow and cutthroat trout *(O. clarki)* and coho and spring chinook salmon extremely susceptible to infection and sockeye salmon *(O. nerka)* less susceptible. Johnson noted that mortality from *C. shasta* was high among brook trout, contradicting the earlier report of Schafer (1968). Chum salmon *(O. keta)* are reported susceptible to *C. shasta* (Margolis and Evelyn 1975; Johnson 1980) and infection has been documented in pink salmon *(O. gorbuscha)*, but the observation was in ocean-caught salmon, and susceptibility relative to other species could not be determined (Bell and Traxler 1985). Atlantic salmon *(S. salar)* have been described as moderately susceptible to ceratomyxosis (Zinn et al. 1977). These reports of species susceptibility are often contradictory and suggested that some factor other than species-level differences may be involved. Nevertheless, species of *Oncorhynchus* are generally susceptible to infection by *C. shasta*.

In addition to his observations that coho salmon were more resistant than rainbow trout, Schafer (1968) also noted that some strains of coho salmon were more resistant than others, the first reference to intraspecies differences in susceptibility. However, these observations were made when little was known about the geographic range of the parasite, and the author did not speculate on the significance of these differences.

In an effort to develop management practices for hatcheries and rivers affected by *C. shasta*, a number of studies were initiated to determine differences in susceptibility to infection of selected species and strains of salmonid (Zinn et al. 1977; Buchanan et al. 1983; Hoffmaster 1985). Because *C. shasta* cannot be cultured, exposure of fish to water that contains the infective stage is the only practical means of inducing ceratomyxosis. Therefore, in these studies, fish were infected by holding them in live cages in enzootic waters. Although the length of exposure, water temperature, season and location of exposure varied, these studies, summarized below, enabled researchers to reach some important conclusions.

Zinn and co-workers (1977) found that strains of fall chinook salmon from areas where the infectious stage of *C. shasta* did not occur suffered significantly higher mortality from ceratomyxosis (88-100%) than strains from areas where the parasite is naturally present (0-13% mortality). The same pattern was true for spring chinook salmon; strains from the Willamette River and upper Columbia River basin where the parasite is enzootic were resistant to infection (Zinn et al. 1977; Hoffmaster 1985). However, the Deschutes strain from the Columbia River basin was only moderately resistant to infection (Ratliff 1981), and the coastal Umpqua River strain from outside the enzootic area was highly resistant (Zinn et al. 1977; Hoffmaster 1985).

These observations led to the suggestion that in spring chinook salmon, resistance to infection may be determined by factors other than prior exposure of the parental stock to the infectious stage of *C. shasta* (Zinn et al. 1977). Increased resistance of the Umpqua chinook may be traced to introductions of Columbia River chinook in the 1950s to supplement the low numbers of returning salmon. The resistance of what is now the Umpqua chinook indicates that the run, as it now exists, may be offspring from survivors of these introductions.

Differences in survival of summer steelhead released into the Willamette River prompted an examination of the relative susceptibility to infection by *C. shasta* of strains from an Oregon coastal river and from three Columbia River tributaries (Buchanan et al. 1983). Steelhead from the coastal river were highly susceptible to *C. shasta* when exposed for either 30 d (95% mortality) or 120 d (89, 90 and 98% mortality during three different exposures). No mortality occurred in any group of steelhead trout from the tributaries of the Columbia River after either a 30 d or a 120 d exposure. Columbia River summer steelhead trout are exposed to *C. shasta* both as smolts emigrating in the spring and as adults returning during in the summer. The authors believed this historical exposure resulted in development of resistance to *C. shasta*, probably through natural selection. They further suggested that mortality caused by *C. shasta* was the reason that introduction of coastal strains of steelhead smolts into the Willamette River from 1966-1975 was unsuccessful. Few if any of the over one million steelhead trout released into the Willamette system during that ten year period are believed to have survived. The following year only *C. shasta*-resistant summer steelhead were introduced, resulting in adult returns of 7.5%.

In contrast, studies of salmon from the Fraser River, British Columbia, Canada, did not find a correlation between resistance and strain origin. Mortality among chinook salmon was moderate to high regardless of whether the strain originated from the lower river where the parasite is enzootic, from central or upper river tributaries, or were of wild or hatchery origin (Ching and Munday 1984a; Ching and Parker 1989). Infection among five steelhead trout strains was similarly variable, with one strain exhibiting high resistance and the others moderate to low resistance. Cutthroat trout from the lower Fraser River appeared resistant (Ching and Parker 1989). All salmon strains from outside the Fraser River drainage that were tested had low resistance to the parasite. The pattern of resistance and susceptibility in salmonids from the Fraser River drainage is much more confused than that reported for strains in the Columbia River drainage. This may, in part, reflect the prolonged exposures used in these studies. As the authors suggest, timing of migration may allow many of these stocks to avoid passing through the lower river during a time when the parasite is most prevalent, and therefore *C. shasta* may not be as important a selection factor in the Fraser River as in the Columbia River. Another explanation is that *C. shasta* has not been present in the Fraser River long enough for resistance to have fully developed (Bartholomew 1998).

VARIATIONS WITHIN POPULATIONS

Infection by *C. shasta* occurs during the freshwater phase of the fish's life cycle. It is not known if infection occurs as juvenile fish migrate to the ocean or as adult salmon reenter fresh water, but Sanders and co-workers (1970) demonstrated that previously unexposed adult salmon can become infected with *C. shasta*. Results of their survey of returning adult fall and winter spawning salmon and steelhead trout indicated that *C. shasta* contributes to prespawning losses. The incidence of *C. shasta* in the more than 1300 adults collected at Columbia River basin hatcheries ranged from 20-75% in coho salmon; 16-43% in spring chinook salmon; 0-65% in fall chinook salmon, and 0-45% in steelhead trout. Other researchers have reported a high incidence of *C. shasta* in adult salmon returning to tributaries of the Snake River, Idaho (Coley et al. 1983; Chapman 1986). Among spring chinook salmon returning to Rapid River Hatchery, Idaho, 94% were infected with *C. shasta* (Coley et al. 1983); however, researchers believed water temperatures were low enough in most years to suppress parasite-induced mortality. In contrast, Chapman (1986) found that summer chinook salmon from the South Fork Salmon River, Idaho that died prior to spawning were infected more often (30 of 46 fish examined: 65%) than those killed during hatchery operations (14 of 76 fish examined: 18%). Although prespawning mortality could not solely be attributed to *C. shasta*, he surmised that it was a primary contributor.

The high incidence of the parasite among adult salmon relative to juveniles of the same strain may result from a general deterioration of immune function due to the physiological stresses associated with spawning. The ability to infect the fish during the terminal phase of its life may also be an efficient strategy for the parasite to maintain its life cycle without seriously depleting the host population, providing death doesn't occur before spawning.

EFFECTS OF TEMPERATURE

Although fish may become infected with *C. shasta* at water temperatures as low as 4°C (Ratliff 1983; Ching and Munday 1984b), progress of the disease is temperature dependent. Udey and co-workers (1975) reported that rainbow trout exposed to the infectious stage of *C. shasta* and held at water temperatures from 6.7 to 23.3°C had little or no ability to resist the infection. Mean time to death was inversely related to temperature, increasing from 14 d at 23.3°C to 155 d at 6.7°C. The decreased time to death with increased temperature most likely results from increased replication rates of the parasite at these higher temperatures rather than decreased immune function on the part of the host. Coho salmon appeared to have a greater capacity to resist infection at the lower temperature, but mean time to death remained temperature dependent. Because *C. shasta* was able to multiply and produce disease in the rainbow trout at all temperatures, the authors propose that the deleterious effect of higher temperatures on coho

salmon may be directed at the defense mechanisms of the host rather than at enhancing the growth and pathogenicity of the parasite.

EFFECTS OF SALINITY

Because mortality may not occur until several months after infection by *C. shasta*, most downstream migrating salmonids enter the ocean before the disease results in death. Acute ceratomyxosis has been reported in juvenile chum salmon *(O. keta)* captured off the coast of British Columbia, Canada (Margolis and Evelyn 1975), indicating that the disease is not attenuated in salt water. To demonstrate this in the laboratory, Ching and Munday (1984b) exposed chinook salmon to the infective stage of *C. shasta* then held the fish in either fresh or salt water. They found that mortality was 100% in both groups. Similarly designed experiments using a susceptible strain of steelhead trout indicate that migration to salt water may reduce the progress of the disease if the fish are not overwhelmed by a large infectious dose (Bartholomew et al. in press).

EFFECTS OF LENGTH OF EXPOSURE

Comparison of exposure studies is difficult because of the inability to quantify the infectious dose to which the fish are exposed as well as the differences in water temperature both during the course of the experiment and between experiments. Ratliff (1981) demonstrated that mortality increased in direct relation to length of exposure. Juvenile Deschutes River fall chinook salmon exposed to the infectious stage of *C. shasta* for 1, 5, 10 and 25 days suffered 2, 18, 40 and 70% mortality, respectively. Yet, in a study by Zinn and co-workers (1977), a coastal strain of fall chinook salmon exposed to *C. shasta* for periods of 5, 66 and 86 days sustained mortalities of 100, 93 and 95%, respectively, indicating that extended exposure periods do not necessarily result in higher rates of infection among susceptible fish. This is likely because the fish encountered a lethal dose of the parasite in the first days of exposure. These studies indicate that resistance to infection may be overwhelmed with prolonged exposure, and therefore, length of migration is an important variable affecting the resistance of the fish. This supposition was the basis for several studies that examined the frequency of infection in migrating salmonids. In each study, juvenile salmon were captured as they migrated out of the river and were held in freshwater until the infection became patent.

Ratliff (1981), in an extension of the study discussed above, collected age-0 fall chinook salmon migrating from the Deschutes River during two consecutive summer seasons. Mortalities associated with *C. shasta* first occurred in fish collected during early May in year one and early

June in year two of the study. Smolts collected prior to May were exposed to low levels of *C. shasta* and were not seriously affected. Mortalities increased in succeeding sample groups to 56% of the early July sample the first year and 90% of the mid-July sample the following year. Because peak migration of wild fall chinook salmon from the Deschutes River occurs in June and coincides with the higher numbers of infected fish, ceratomyxosis is a significant factor affecting their survival in the Deschutes River.

In a similar study examining prevalence of *C. shasta* in salmonids migrating out of the Columbia River, over 2200 juveniles were captured prior to entering the estuary (Bartholomew et al. 1992a). Age-0 chinook salmon were captured in beach seines and age-1 chinook and coho salmon and steelhead trout were taken in purse seines. *Ceratomyxa shasta* was present in 9% of the age-0 chinook salmon collected between late May and early September, with mortality reaching 24% in groups of fish captured in late August. That a total of 11% of the yearling chinook were infected demonstrated the incidence of *C. shasta* did not vary significantly between year classes. The prevalence of infection among the coho salmon and steelhead trout was 5 and 12%, respectively. Lower prevalence in coho salmon would not be expected not unexpected because they originate from the lower Columbia River and therefore have a shorter exposure to the parasite. Mortality caused by *C. shasta* was higher among these outmigrating fish than anticipated from results of live-cage exposures where the infection prevalence among Columbia River salmonids was generally less than 5%. However, the origins of the captured fish were not determined. The authors speculated that prior to construction of the Columbia River dams, salmon from the upper Columbia and Snake Rivers may have avoided ceratomyxosis by migrating through the infectious areas early in the season, before parasite concentrations were high. Changes caused by construction of reservoirs may have extended the duration of fish migration and increased parasite numbers and distribution more rapidly than fish could adapt by developing resistance or avoidance strategies.

Results of unpublished studies conducted by the Oregon Department of Fish and Wildlife on causes of mortality of steelhead smolts in the Willamette River also showed an unexpected high incidence of ceratomyxosis. Outmigrating hatchery and wild winter and summer steelhead smolts were collected from the lower Willamette River during their outmigration each spring for five consecutive years. Between 31 and 88% of the fish captured each year were infected with *C. shasta*. Wild steelhead and the Skamania summer steelhead, a hatchery strain chosen for its resistance to *C. shasta* (Buchanan et al. 1983), were similarly affected (R.A. Holt, Oregon Department of Fish and Wildlife, personal communication). During this 5 year study, conditions in the Willamette River ranged from drought and high temperatures to floods and moderate temperatures. Although the frequency of parasitism fluctuated between years, it was high throughout the study and indicates that *C. shasta* is a primary cause of salmonid mortality in this river.

In contrast to the higher than expected prevalence of *C. shasta* in fish migrating from Columbia River tributaries, Bartholomew and co-workers (1992a) found *C. shasta* in only 3.3% of more than 3500 juvenile chinook salmon collected as they migrated out of the Fraser River. Significant differences in the prevalence of *C. shasta* were associated with both migration route and age of the fish. Age-0 fish had a significantly higher prevalence (5.2%) of *C. shasta* than the age-1 fish (1.5%) collected, probably because of the longer migration period of the younger fish. The authors speculate that the high prevalence of infection reported in earlier experimental exposures (Ching and Munday 1984a; Ching and Parker 1989) may be explained by higher concentrations of the infective stage, extended exposure periods or stress. Although the reason for the dissimilarity in the reported impact of *C. shasta* in the Fraser and Columbia Rivers is unknown, environmental differences such as reduced flow rates and increased water temperature may be important.

GENETICS OF RESISTANCE

Inheritance of susceptibility to *C. shasta* has been demonstrated for coho salmon (Hemmingsen et al. 1986), steelhead trout (Wade 1987) and rainbow trout (Ibarra et al. 1992). In each study, progeny produced from crosses between resistant and susceptible parental strains were intermediate in their susceptibility to infection by *C. shasta*. The contributions to susceptibility to infection by *C. shasta* in the progeny by the male and female parents was similar in two of the studies (Hemmingsen et al. 1986; Ibarra et al. 1991, 1992, 1994), but Wade (1986) reported a strong maternal influence.

Ibarra and co-workers (1992, 1994) also examined the effects of length of exposure on liability to develop ceratomyxosis. They found that the resistant strain showed a low level of mortality regardless of the length of exposure, while mortalities among the susceptible strain and the reciprocal F1's were significantly greater when exposed continuously until death occurred than when exposed for seven days. The mean time to death was also significantly shorter for the susceptible strain than for the reciprocal F1's and the resistant strain. They suggested the reciprocal F1's inherited a mechanism of defense against *C. shasta* from the resistant strain and that this mechanism is sensitive to parasite dose. Results of exposure studies with the F2's and backcrosses showed the F2 intermediate in susceptibility compared with the parental strains and the backcrosses intermediate between the F2 and the corresponding parental strain. Comparison of cumulative mortalities in the continuous exposure treatment showed a linear response among the groups.

Analysis of the genetic components indicated that the alleles for resistance were dominant over the alleles for susceptibility for both survival and time to death, but that the genetic control of susceptibility involves multiple loci. They speculate that resistance to *C. shasta* depends on

two interacting mechanisms; the first involving control of the level of parasitism by a mechanism which affects invasion and/or establishment of the infective stage. This would indirectly result in a longer time to death because lower numbers of parasites would proceed to advanced stages. They propose that a second mechanism may involve the ability of the fish to mount an effective immune response against the parasites that have succeeded in evading the primary defense mechanism.

RESISTANCE MECHANISMS

Chevassus and Dorson (1990) described resistance to a pathogen as a complex phenomenon: penetration of the host may be reduced by a barrier or by the effects of mucous, gastric/intestinal secretions, inactivation by serum components (complement, agglutinins, lysins, lysozyme, C-reactive protein), phagocytic cells, acute phase proteins, killer cells, or induced mechanisms (antibody, interferon). As suggested by Ibarra et al. (1994) the mechanisms important in resistance to *C. shasta* likely affect penetration and early establishment of the parasite, but studies of the host response to infection have been limited by our incomplete knowledge of the parasite's life cycle and the inability to administer a known infectious dose.

Non-specific mechanisms which limit infection by *C. shasta* may also have evolved differently among the resistant strains. Histological examination of survivors of experimentally induced infections by *C. shasta* suggest that resistant fish may be able to more effectively contain the parasite, as evidenced by granulomas surrounding degenerative stages of the parasite (Ibarra et al. 1992). However, Bartholomew et al. (1989b) did not observe granulomatous reactions in resistant fish naturally exposed to *C. shasta* and reported that in fish of a resistant strain which succumbed to infection, the infection appeared similar to that in the susceptible fish.

Little work has been done to identify the role of specific immune responses against *C. shasta*. Bartholomew et al. (1989b) were unable to detect antibodies against the trophozoite or spore stages of the parasite in susceptible rainbow trout by either western blotting or fluorescent antibody techniques. No attempt was made to study the response against the infectious stage, which was unknown at that time. The ability of resistant fish to produce specific antibody against *C. shasta* has not been investigated. Further studies comparing the mechanisms of resistance between susceptible and resistant strains and their progeny are needed to determine which components are important in the development of host resistance to *C. shasta*.

PATHOLOGY AND HOST RESPONSE

Clinical signs of ceratomyxosis vary depending on the level of infection and the tissues and organs affected. Infected juvenile salmon typically become anorexic, lethargic, and darken in color. The vent is usually swollen and hemorrhaged, and the abdomen may be distended with ascites. Exopthalmia is common in fish with ascites (Schafer 1968). Acutely infected fish may die before clinical signs develop.

Internally, the intestinal tract of infected juvenile rainbow trout becomes swollen and hemorrhaged and the intestinal contents mucoid with caseous material lining the intestine and pyloric caeca (Conrad and Decew 1966). The entire digestive tract, the liver, gall bladder, spleen, gonads, kidney, heart, gills, and skeletal muscle may become diseased, hemorrhaged, and necrotic (Wales and Wolf 1955). Infected adult chinook salmon may have nodular lesions in the intestine accompanied by gross lesions in the liver, kidney, spleen, and muscle. Grossly thickened intestinal walls and pyloric caeca, and large abscessed lesions in the body musculature have been reported in infected adult coho salmon (Wood 1979).

In histological examinations, the parasite is first detected in its vegetative, trophozoite stage between the epithelial cells of the posterior intestine. As the parasite multiplies in the mucosa and submucosal layers of the intestine, the host reacts with a vigorous inflammatory response, consisting mainly of a lymphocytic infiltration . Appearance of trophozoites in the liver closely follows their presence in the intestine. The route of invasion is probably venous blood which enters the hepatic portal system directly from the alimentary tract. In the later stages of infection, the parasite penetrates the muscularis and serosa of the intestine and is released into the peritoneal cavity. It also invades other organs via the bloodstream. Trophozoites do not begin sporulation until the tissues are heavily infected and necrotic (Bartholomew et al. 1989b). In scanning electron microscopic studies of heavily infected tissues the structural damage caused by the parasite is evident. The secondary mucosal folds are destroyed and the primary folds severely eroded in areas. Spores and trophozoites are numerous, as are inflammatory and red blood cells (Bartholomew et al. 1989b).

The progress of ceratomyxosis is temperature dependent, with mean time to death inversely correlated to temperature (Udey et al. 1975). The histopathology of the infection parallels this; the first sign of infection appears between days 12 and 18 post-exposure in fish maintained at 12°C, and at seven days in fish held at 18^{o}C (Yamamoto and Sanders 1979; Bartholomew et al. 1989b). In contrast to these observations, Lom (1969) and Dykova and Lom (1978) noted a regression of infection by *Henneguya* species as temperatures increased and suggested that antibodies were more effective in the host response at these temperatures. However, no specific antibody response was detected in rainbow trout heavily infected with *C.*

shasta (Bartholomew et al. 1989b). If an immune response is directed against *C. shasta*, it appears ineffective in controlling the infection.

DIAGNOSIS

Diagnosis requires that spores be found and identified by their size, shape, and location. Spores of *C. shasta* are 14 to 23 mm long and 6 to 8 mm wide at the suture line. The spores are prolonged laterally and contain two refractile polar capsules, each with a coiled extensible filament (Noble 1950). Trophozoites are multicellular, variable in shape, and mature to form a sporoblast that usually contains two spores. Because of the variability in size and shape of the trophozoites and the similarity of this stage to other myxosporeans, observation of trophozoites by light microscopy is not sufficient for diagnosis. Consequently, serological techniques using monoclonal antibodies have been developed (Bartholomew et al. 1989a). The monoclonal antibody was produced against the trophozoite and sporoblast stages of *C. shasta*, reacts specifically with these stages of the parasite, and does not cross-react with trophozoite or spore stages of other myxosporeans. Use of the monoclonal antibody and a fluorescein or enzyme-conjugated secondary antibody enables reliable detection and is especially useful in diagnosing early or acute infections in which sporulation has not occurred. To demonstrate this, the standard method for identification of spores (Thoesen 1994) was compared with an indirect fluorescent antibody test using the monoclonal antibody. When both methods were used on replicate samples collected from prespawning adult mortalities, there was a 25% increase in detection sensitivity using the serological technique.

Newer diagnostic approaches are based on molecular techniques and include polymerase chain reaction assays (PCR). A simple protocol for processing fish tissues and performing the PCR assay has allowed the detection levels to approach 50 fg of purified genomic *C. shasta* DNA. This procedure is quick and reliable. It may allow for the detection of early or subclinical infections in wild and artificially cultured salmon (Palenzuela 1999).

TRANSMISSION AND LIFE CYCLE

Speculation on the involvement of a second host for development and transmission of myxosporean parasites arose from the difficulties encountered in establishing infections of experimental fish in the laboratory. However, when Markiw and Wolf (1983) presented the first evidence for transmission of the myxosporean *Myxobolus cerebralis* via an alternate host, their research was met with skepticism. The life cycle that was proposed linked the 2 classes of Myxozoa, the Actinosporea and the Myxosporea, in a single life cycle in which the myxosporean

spore released from the fish host infects the oligochaete *Tubifex tubifex*. Culmination of parasite development in the oligochaete in turn resulted in a spore of the actinosporean *Triactinomyxon*. Infection of salmonids occurs by contact with the waterborne *Triactinomyxon* following its release from the infected tubificid. This landmark research was subsequently confirmed by in vivo transmission studies (El-Matbouli and Hoffman, 1989; Hedrick et al. 1989). The pattern of myxosporean transmission requiring an associated actinosporean developmental cycle in an aquatic oligochaete has subsequently been demonstrated or suggested for at least 14 freshwater myxosporean species (Kent et al. 1994).

Recent studies on the molecular phylogeny of the Myxosporea (Smothers et al. 1994; Siddall et al. 1995; Schlegel et al. 1996) have not only drastically changed the taxonomy of this group of organisms but have also resulted in techniques for confirming myxosporean life cycles using the small subunit ribosomal RNA gene sequence (18S rDNA). This approach was used by Andree et al. (1997) to compare the alternating myxosporean and actinosporean stages of *M. cerebralis*. The application of this technology was used to complete the life cycle of *C. shasta* by identifying the actinosporean life stage and the unique alternate host required for its development. (Bartholomew 1997)

The actinosporean life stage of *C. shasta*, a myxozoan parasite of salmonids, and the annelid worm which serves as its alternate host, were identified in laboratory transmission experiments and their roles were confirmed using molecular techniques. Infection by the parasite occurred in susceptible fish which were either exposed to or force fed the freshwater polychaete, *M. speciosa*, infected with the actinosporean. These observations were confirmed using the polymerase chain reaction with primers designed from the *C. shasta* 18S rDNA sequence. DNA was amplified from polychaetes harboring the actinosporean which caused infection in the fish, but not from uninfected polychaetes. Amplified DNA from an infected polychaete was sequenced, and its homology with the 18S rDNA sequence of *C. shasta* spores verified the proposed life cycle.

Developmental stages of the tetrahedral actinosporean were observed in a heavily infected polychaete by electron microscopy. The mature parasite was present within the epidermal layer of the polychaete but not present in the intestinal epithelium, which has been shown to be the site of development for other actinosporean species (El-Matbouli and Hoffman 1989; Ruidisch et al. 1991; Kent et al. 1993; Yokoyama et al. 1995). Because infection of the salmonid host typically occurs when the fish encounters the actinosporean in the water column rather than by direct ingestion of the infected worm, there must be some mechanism for the parasite's release from the polychaete. Other Actinosporea which infect the intestinal mucosa of their annelid host are thought to be released into the gut and shed into the water; therefore, an actinosporean developing in the epidermis may require a different strategy. One possible method is rupture of the epidermal layer, resulting in release of the actinosporean, but also death of the alternate host.

A second strategy would be development of the actinosporean within a cell type which extends through the epidermis and opens at the cuticular surface. Ultrastructural examination of an uninfected polychaete revealed a large number of secretory cells containing ovoid secretory granules. This cell type is greatly reduced in the infected worm tissues, where the parasites were observed to develop within large vacuolated cells resembling mature pyriform glands (Jouin, 1992). Development in a secretory cell could provide an alternate strategy for release, allowing the highly elastic actinosporean to be discharged via a secretory pore and thus permitting the host to survive.

Morphological characteristics of the proposed actinosporean stage of *C. shasta* most closely resemble those species of the genus *Tetractinomyxon* (Ikeda 1912). This genus is described as a primitive type with a uninucleate tetrahedral endospore, three uninucleate polar capsule cells, and one binucleate sporoplasm. The described species of *Tetractinomyxon* were identified from marine sipunculids and, until this report, represented the only actinosporeans found in a host other than an oligochaete. The developmental stages observed here by electron microscopy are similar to those described by Ikeda (1912) who observed a sporoblast with eight nuclei, three epispore nuclei, one endospore nucleus, three polar capsule cell nuclei, and a sporoplasm nucleus. He noted that the binucleate condition of the sporoplasm develops after spore formation is complete. This is in agreement with our observation of a uninucleate sporoplasm in the developmental stages.

The unique characteristics of the actinosporean genus *Tetractinomyxon* and their similar morphology to members of the myxosporean genus *Trilospora* Noble, 1939, have led to speculation this group is more closely related to the myxosporean life stage, with the sipunculid host being the equivalent of the marine fish host (Kent et al. 1994). Results of this study indicate that members of the *Tetractinomyxon* may indeed represent the actinosporean life stage of myxosporeans. Therefore, the family name should be suppressed as suggested for the other actinosporean groups, and they should be considered to be members of the order Multivalvulida within the class Myxosporea (Kent et al. 1994). However, as evidenced by the recent proposals of Siddall and co-workers (1995) to reclassify the Myxozoa because of their affinities to parasitic cnidaria, and by Schlegel and co-workers (1996) to link the Myxozoa to the bilateria, it is likely there will be major revisions within this group, and speculations on the status of the genus *Tetractinomyxon* may be superseded by higher order taxonomic changes.

The two actinosporean spores identified are the first reported observation of actinosporean infections in polychaete worms and, although the host is a freshwater species, it is presumed that actinosporeans may also infect marine polychaetes (Bartholomew 1998). At least one actinosporean has been described from a marine oligochaete, *Sphaeractinomyxon stolci* (Caullery and Mesnil, 1904; Marques, 1984), but the possible alternate hosts for the numerous marine species of myxosporea have largely been a subject for debate.

MANAGEMENT IMPLICATIONS

Results of strain resistance studies have demonstrated that resistance to infection by *C. shasta* is one of the primary traits to consider when introducing new salmonid strains or species into a drainage system where this myxosporean is enzootic. Relocation of salmonids from areas where *C. shasta* is not enzootic into areas where the parasite is established is not likely to be successful, and studies on the genetics of resistance argue that these introductions may adversely affect the survival of resident resistant strains if interbreeding occurs (Currens et al. 1997). One of the most important outcomes of research on resistance to *C. shasta* has been to eliminate stocking enzootic regions with susceptible strain of fish.

Further reduction of the impact of this parasite will require a closer look at the resistance mechanisms which have evolved through selective pressures. Recent advances in techniques for identifying genes connected with specific traits will be useful in characterizing disease resistance markers for stock identification and selective breeding programs. Determining the component(s) of the immune system which effectively prevent infection in resistant strains may lead to methods for stimulating those responses in susceptible strains of salmonids. Equally important is the application of this knowledge to management of waters with native salmonid populations. Identifying environmental factors and management practices that adversely affect the resistance of these strains will be necessary in developing watershed plans to protect natural production.

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