DISSERTATION

EFFECTS OF ENVIRONMENTAL STRESSORS ON JUVENILE TROUT EXPOSED TO *MYXOBOLUS CEREBRALIS*

Submitted by

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In partial fulfillment of the requirements

for the degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring 1999

COLORADO STATE UNIVERSITY

March 10, 1999

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY GEORGE J. SCHISLER ENTITLED EFFECTS OF ENVIRONMENTAL STRESSORS ON JUVENILE TROUT EXPOSED TO *MYXOBOLUS CEREBRALIS* BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

EFFECTS OF ENVIRONMENTAL STRESSORS ON JUVENILE TROUT EXPOSED TO *MYXOBOLUS CEREBRALIS*

Declines in survival of young-of-the-year rainbow trout in some Colorado rivers have been attributed to the presence of *Myxobolus cerebralis*, the causative agent of salmonid whirling disease. A study was initiated to determine if environmental stress factors could be playing a role in year-class disappearances in the Upper Colorado River.

Gas saturation levels were monitored throughout a 40 kilometer reach of the Upper Colorado River during the summer and fall of 1995 to identify possible sources of gas supersaturation. Water discharged from the spillway of Windy Gap Reservoir was found to be the main source of man-made supersaturation, while photosynthetic activity of aquatic plants was determined to be the natural source of supersaturation in the study area.

Clinical signs of whirling disease and gas bubble trauma were recorded for 1656 rainbow (*Oncorhynchus mykiss*) and 3060 brown trout (*Salmo trutta*) young-of-the-year collected from 1994 through 1996 in the Upper Colorado River. Higher proportions of both rainbow and brown trout were moribund in late summer and fall than in spring and early summer months. Proportions of moribund fish of both species decreased with distance downstream of Windy Gap Reservoir.

A controlled laboratory experiment was conducted to test the hypothesis that chronic low levels of gas supersaturation affect growth, morbidity, and mortality of fingerling rainbow trout infected with *M. cerebralis*. Fingerling rainbow trout, exposed

and unexposed to *M. cerebralis* were held in gas saturations of 110, 107.5, 105, and 100 - 102.5% for 22 weeks. Significant effects on growth, morbidity and survival were found due to *M. cerebralis* infection. Gas saturation levels tested did not significantly affect growth, morbidity or survival of fish in this experiment.

A second laboratory experiment was conducted to test the effects of elevated water temperature, bacterial pathogens, and gas supersaturation on *M. cerebralis*-infected rainbow trout. Fingerling rainbow trout were exposed to all combinations of these stressors for six months. Mortality was significantly affected by exposure to *M. cerebralis* ($P = 0.0002$) and elevated water temperature ($P = 0.0002$). Lesser effects were observed due to elevated gas saturation and exposure to *Flavobacterium psychrophilum*, the causative agent of bacterial coldwater disease. Morbidity was significantly affected by exposure to *M. cerebralis* ($P = 0.0001$), and *M. cerebralis -F. psychrophilum* interactions ($P = 0.0556$). Mortality of fish was significantly increased ($P = 0.0001$) with addition of stress factors.

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Acknowledgements

I would like to thank my advisor, Dr. Eric P. Bergersen, for his guidance, support, and friendship throughout this study. My committee members deserve a great deal of thanks: Dr. David Bowden for his statistical expertise and valued presence throughout my graduate career. Dr. Steven Flickinger for his encouragement and indispensable input. Dr. Gary White for statistical advise and for challenging me to surpass my own expectations. Pete Walker for his unique insight and inspiration.

Dr. Ron Hedrick and Terry McDowell of the University of California-Davis provided triactinomyxons for the laboratory experiments. Pat Davies provided much needed laboratory space and resources. Steve Brinkman, Trina Lynch, Cheryl Dion, and Matt McIntyre helped with care of fish in the laboratory experiments. Larry Harris provided funding and moral support. Barry Nehring was a great source of information and expertise.

I would like to especially thank my parents, George R. and Frances J. Schisler for their never-ending assurance, commitment, support, and strength. Thank you so much for everything you have done.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1:

Myxobolus cerebralis and environmental factors influencing onset of disease in fishes

Background

Salmonid whirling disease, caused by *Myxobolus cerebralis*, was first diagnosed in the United States in 1958, after its suspected introduction via frozen European rainbow trout (*Oncorhynchus mykiss*) in 1956 (Hoffman 1990). Since then the parasite has spread throughout most of the range of salmonids in the United States, most recently into the Rocky Mountain West. While the parasite has been thought to be fairly innocuous in wild populations of trout (Colorado River Wildlife Council 1988), year classes of rainbow trout in rivers such as the Colorado in Middle Park, Colorado, and the Madison in Montana are currently thought to be disappearing, due at least in part, to whirling disease. Large numbers of juvenile brown trout (*Salmo trutta*) in the Colorado River are also exhibiting signs of whirling disease, which is highly unusual given the presumed resistance of brown trout to the disease.

M. cerebralis has a two host life cycle (Figure 1.1), including its salmonid host and an aquatic oligiochaete, most likely *Tubifex tubifex* (Markiw and Wolf 1983). Spores of the parasite, when ingested by *T. tubifex*, multiply and develop in the gut lining of the oligochaete. Triactinomyxons (the life stage of the parasite infective to fish) are produced 3 to 4 months post-infection (Wolf and Markiw 1984). The triactinomyxons float freely in the water column after their release from their host, and can survive in this form for 3-4 days at 12.5° C (Markiw 1992b). When the triactinomyxon encounters an appropriate salmonid host, it attaches to the epidermis, buccal cavity lining or the gill filaments of the fish (Markiw 1989; El-Matbouli et al. 1995). The triactinomyxon sporoplasm migrates into the epidermis where the sporoplasm cells reproduce mitotically. The cells then migrate via the nervous system to the host cartilage, multiplying mitotically several more times. Once inside the cartilage, the parasite forms a plasmodium, composed of vegetatively reproducing nuclei and generative cells (El-Matbouli et al. 1995). The cartilage is digested

by the plasmodium (Garden 1992), often causing external signs of whirling disease including blacktail, spinal deformities, cranial deformities, shortened opercula, shortened lower jaws, opercular cysts, degenerated fins, and "whirling" behavior (Halliday 1976). Mature spores are formed in the cartilage of infected fish from 4 to 6 months post-infection (Hoffman and Putz 1969; Tidd and Tubb 1970). After the fish dies, the spores are once again available to *T. tubifex*, although it has been suggested that the mature spores can also be released from the fish in the feces (Uspenskaya 1964; Rydlo 1971) and from opercular cysts (Taylor and Haber 1974). The mature spores are highly resistant to environmental conditions, and may withstand temperatures as low as -20 degrees C and passage through the digestive systems of mallard ducks (*Anas platyrhynchos*) and northern pike (*Esox lucius*) without loss of viability (El-Matbouli and Hoffman 1991).

Hatchery Experience

Outbreaks of whirling disease have been reported in aquaculture operations almost everywhere salmonids are propagated artificially (Hoffman 1990; Halliday 1976). These outbreaks are usually associated with rearing juvenile fish in earthen raceways or ponds where large numbers of *T. tubifex* reside, resulting in high levels of triactinomyxon exposure (Hoffman 1990; Halliday 1974). Control measures for managing *M. cerebralis* in hatcheries include construction of concrete raceways, and use of spore-free water (such as from wells or springs) for the rearing of fry and fingerlings (Hoffman 1990; Halliday 1974). Sand-charcoal (Hoffman et al. 1962) and ultraviolet light filters (Hoffman 1974, 1975) have also been used to eliminate the parasite from hatchery water supplies. These control measures have reduced the incidence of clinical signs of the disease in hatcheries to the point that it is considered quite manageable in fish culture operations (Graff 1996).

Wild Trout Experience

Overt outbreaks of whirling disease have, until recently, not been observed in natural populations of salmonids (Bogdanova 1970; Halliday 1974, 1976; El-Matbouli et al. 1992). The eastern states of New Hampshire, New Jersey, Connecticut, Massachusetts, and New York all have naturally reproducing populations of wild salmonids, many of which have been exposed to hatchery fish infected with *M. cerebralis*. None of these states has reported adverse effects of whirling disease on wild trout populations, although not all the states have examined the issue extensively (Hulbert 1996).

Among the midwestern/eastern states of Pennsylvania, Michigan, Ohio, Virginia, West Virginia, and Maryland, none has reported a decline of wild trout as a result of whirling disease, even though the parasite has been present in the region since as early as 1956 (Graff 1996). Additionally, no clear evidence of significant impacts to wild trout populations have been found in California, Nevada, Oregon, and Idaho, despite the wide distribution of *M. cerebralis* in those states (Modin 1996).

The effect of whirling disease on naturally reproducing populations of salmonids is thought to be much different in some Rocky Mountain States (Wiley 1996). Losses of year-classes of rainbow trout in the Colorado, Cache la Poudre, Gunnison, Rio Grande, and South Platte rivers in Colorado are thought to be occurring as a result of whirling disease (Nehring 1996). Heavy losses of rainbow trout yearling and younger age-classes have reduced the adult rainbow trout population by 90% in sections of the Madison River of Montana (Vincent 1996). Free-ranging rainbow and brown trout exhibited severe signs of whirling disease in the Fremont River of Utah when first discovered there in 1991 (Walker and Nehring 1995). Subsequent salmonid eradication in the river in hope of eliminating the parasite prevented the evaluation of effects the parasite would have had on the population.

Conditions influencing survival of *M. cerebralis* infected fish

Four conditions have been shown to influence the amount of morbidity and mortality among trout exposed to *M. cerebralis*: species of salmonid, age of the fish at time of infection, number of triactinomyxons to which an individual fish is exposed, and water temperature. All four of these conditions are likely influencing rainbow trout survival in areas where whirling disease is thought to be causing year class losses.

Species Differences

Of the salmonids tested by O'Grodnick (1979) for relative infection by *M. cerebralis*, rainbow trout were the most susceptible, followed by sockeye salmon (*Oncorhynchus nerka*), brook trout (*Salvelinus fontinalis*), chinook salmon (*Oncorhynchus tshawytscha*), brown trout (*Salmo trutta*), and coho salmon (*Oncorhynchus kisutch*). Lake trout (*Salvelinus namaycush*) were determined to be completely refractory to infection by the parasite. Rainbow trout also exhibit signs of the disease more frequently and with more severity than other salmonid species (O'Grodnick 1979).

Age at infection

Severity of *M. cerebralis* infection decreases with age of the fish at time of exposure (Hoffman and Byrne 1974; Markiw 1992). The earliest rainbow trout may become infected with *M. cerebralis* is 2 to 3 day-old sac fry (Putz and Hoffman 1966; Markiw 1991). Markiw (1991) observed mortalities of up to 90% among newly hatched fish exposed at this age. However, her experiment was not conducted with replication, so the high mortalities observed may have been the result of factors other than *M. cerebralis*. Hedrick (pers. comm. 1996) has infected rainbow trout as young as 2 - 3 days old with heavy doses of triactinomyxons and has not observed significantly greater mortalities than

uninfected control fish.

Although very young fish are more susceptible to *M. cerebralis* infection, adult rainbow trout of up to 3.5 years of age can become infected with *M. cerebralis*. Larger numbers of triactinomyxons are required to result in infection in older fish, and signs of the disease and numbers of spores produced per fish are lower in older fish (Markiw 1992a).

Number of Infective Units

Severity of *M. cerebralis* infection and spore burden for rainbow trout have been strongly correlated with relative dosing of triactinomyxons (Markiw 1992a). Data from her experiment revealed that low numbers of triactinomyxons ($\leq$ 10) per fish resulted in no infections by *M. cerebralis* , while a sharp increase in proportions of infected fish was found with intermediate levels of triactinomyxons. All fish exposed at 10,000 and 100,000 triactinomyxons per fish became infected with the parasite (Figure 1.2). Numbers of spores produced by exposed fish followed a similar pattern, with less than 10 triactinomyxons per fish producing no spores, followed by a rapid increase in numbers of spores produced with exposures of 100 to 1000 triactinomyxons, and spore production leveling off between 10,000 to 100,000 triactinomyxons per fish (Figure 1.3).

Water Temperatures

M. cerebralis develops more rapidly and is more pathogenic at higher water temperatures (Halliday 1973). This temperature-related factor in myxosporidean host-parasite relations has been observed in many other myxosporidians (Hoshina 1952; Leith and Moore 1967; Sanders et al. 1970; Lom 1970). Like any organism, *M. cerebralis* has an optimum temperature for development. Bogdanova (1960) found that cold water (3 - 7 °C) slows the development of the disease. Halliday (1973) found that spore maturation rate

and number of rainbow trout with signs increased from 7 - 17° C. Schaperclause (1931) found that water temperatures of 20 - 25° C resulted in only mild cases of the disease. This indicates that temperatures that are optimal for juvenile rainbow and brown trout growth are also those at which the parasite develops most rapidly and is most virulent.

The Role of Stressors in Disease Resistance

The association of stressors and reduced resistance to disease has been noted by many authors (Wedemeyer 1974; Mazeaud et al. 1977; Ellis 1981; Maule et al. 1989). Increased susceptibility of stressed fish to disease is caused by reduced immune response brought on by elevated plasma cortisol (McLeay 1975; Ellsaesser and Clem 1986; Maule et al. 1987; Tripp et al. 1987). When a fish is stressed, inter-renal cells in the head kidney of the fish secrete cortisol at higher rates than normal (Patino et al. 1987). Elevated plasma cortisol is associated with decreased levels of circulating lymphocytes (McLeay 1975, Barton et al 1987) and changes to hematopoietic tissues and cells in fish (Peters and Schwarzer 1985). These changes result in reduced production of new blood cells and increased susceptibility to disease.

Presence of an etiological agent in a population or individual fish must be distinguished from actual occurrence of the disease. While a fish may have been exposed to or harbor an organism that can cause disease, overt development of disease does not always occur. For a disease to manifest itself (Figure 1.4), a susceptible host must be exposed to the disease agent under environmental conditions that favor the agent (Cockburn 1963; Snieszko 1974) . In some cases, more than one pathogen must be present for overt development of the disease to occur (Figure 1.5). This interactive relationship that leads to disease is especially important among fishes, whose health is more affected by their habitat and environment than that of warm-blooded animals (Snieszko

1973).

Several authors have likened the stress response in fish to that of the General Adaptation Syndrome (GAS) among mammals (Wedemeyer 1980; Schreck 1981; Pickering 1981). The General Adaptation Syndrome in mammals is a concept that describes the change in health of an animal as a response to stressful conditions, usually associated with fright, discomfort, or pain. This syndrome develops in three stages, the "Alarm Reaction", the "Stage of Resistance", and the "Stage of Exhaustion" (Selye 1950). During the alarm reaction stage, many physiological changes such as increased cortisol levels, sodium retention, potassium excretion, hyperglycemia, and increased blood pressure occur. These characteristics disappear or are reversed in the stage of resistance when adaptation to the stress has occurred. Animals that become conditioned to the stress or are removed from the stress do not progress to the stage of exhaustion. However, animals may lose their conditioning to the stress factor, and progress to the stage of exhaustion if the stress is too severe or long lasting.

Stress from a variety of different factors have been associated with fish disease. Most notable are the chemical, biological, physical, and procedural factors presented by Snieszko (Wedemeyer et al. 1976) that are thought to be strong contributors to reduced disease resistance in hatchery reared fish. Chemical stressors can include metals such as zinc and copper that can cause damage to gills and fins, and external lesions. Pesticides can also cause instances of fin rot. Other water quality variations such as changes in pH or water hardness require physiological compensation, which is stressful to fish. Excessive nitrogenous and other metabolic wastes can compromise the ability of fish to function properly physiologically. Chemical composition of the fish's diet can also influence its physiological state.

Pathogenic and biological stressors include high population densities of fish leading

to crowding, and stress due to attack by micro and macroorganisms. Crowding of fish in aquaculture operations leads to accumulation of ammonia and increased oxygen demand, and danger of disease outbreaks increases proportionally. Micro and macroorganisms of various kinds, including pathogenic and non-pathogenic species are a constant source of stress for fish, especially when they occur in the water supply in high numbers. Commensuals and parasites of the skin can cause lesions and provide focal points for infection. Those occurring on the gills can cause reduced gas exchange, resulting in respiratory distress, and can also become focal points for further infection.
Physical factors contributing to stress in fish are temperature, dissolved gas, light, and sound. Water temperature affects fish metabolic rate, immunologic response, reproduction, dissolved oxygen present in water, biological oxygen demand, toxicity of pollutants, and the growth and development rate of pathogens and parasites. Depletion of oxygen has a strong stress effect on fish, causing hypoxia and changes in blood pH of fish. Excess oxygen or nitrogen (gas supersaturation) can result in formation of gas emboli in the gills and skin that become sites for bacterial invasion.

Procedural conditions causing stress in hatchery-reared fish can include handling, transport, stocking, and disease treatment. Stress caused by these factors are self-evident, and present a constant problem for fish culturists. Snieszko's (Wedemeyer et al. 1976) main point was that any environmental stress, no matter the source, coincides strongly with onset of fish disease. Given a fish culture situation where fish are usually reared at high densities under unnatural conditions, one would expect to see fish disease becoming an important problem, especially as it relates to stress. Organisms may become more pathogenic in situations where high host densities occur over several generations. Disease in wild fish has not been investigated as thoroughly, and the link between disease and stress in wild fish has not been well established.

Although wild and hatchery fish share many of the same stressors, wild fish are subjected to some stressors unique to natural environments. Fish from wild stocks have been shown to be more sensitive than domesticated strains with regard to their cortisol response (Woodward and Strange 1987), which indicates that stressors may cause much more damaging effects among wild fish (Pickering 1989).

Spawning stress is a common phenomenon among wild fish, and is the stressor most often associated with wild fish and disease. Hooking and handling by anglers is another common form of stress in wild salmonids that may lead to greater susceptibility to bacterial or fungal infections leading to indirect mortality (Schisler 1995). Pollutants from industrial or agricultural sources can cause physiological disturbances resulting in stress (Pickering 1989). Overwintering stress due to loss of fat reserves and low dissolved oxygen levels allow fish to become more susceptible to disease in the spring as a result of their weakened condition (Snieszko 1973). Immune response of fish is also compromised at low water temperatures (Manning and Nakanishi 1996). Wide seasonal or diurnal temperature fluctuations have been associated with disease in hatcheries (Meyer 1970), and one would expect to see a similar, if not more severe effect in the wild. Eutrophication and wide fluctuations in pH and dissolved oxygen are also sources of stress for wild fish (Fry 1969). Nitrogen and oxygen supersaturation can cause stress and even outright death. Snieszko (1973) stated, "It is estimated that more than 50% of the fishes (in the Columbia River) may be lost due to gas embolisms." Wild fish are further subjected to day to day stressors in the form of predator avoidance, territory protection, foraging competition, and others. Fish may be more susceptible to disease in one natural environment than another, depending on the number or type of stressors to which the fish are exposed.

I hypothesized that the combination of *Myxobolus cerebralis* and stressors present in some Colorado rivers have caused the population level effects in Colorado that are not

observed in other regions of the United States. The studies presented in the following chapters were designed to identify and evaluate some of the potential stressors to which fingerling rainbow and brown trout are exposed in the Upper Colorado River in an effort to determine why such devastating impacts from *M. cerebralis* are observed in that particular drainage.

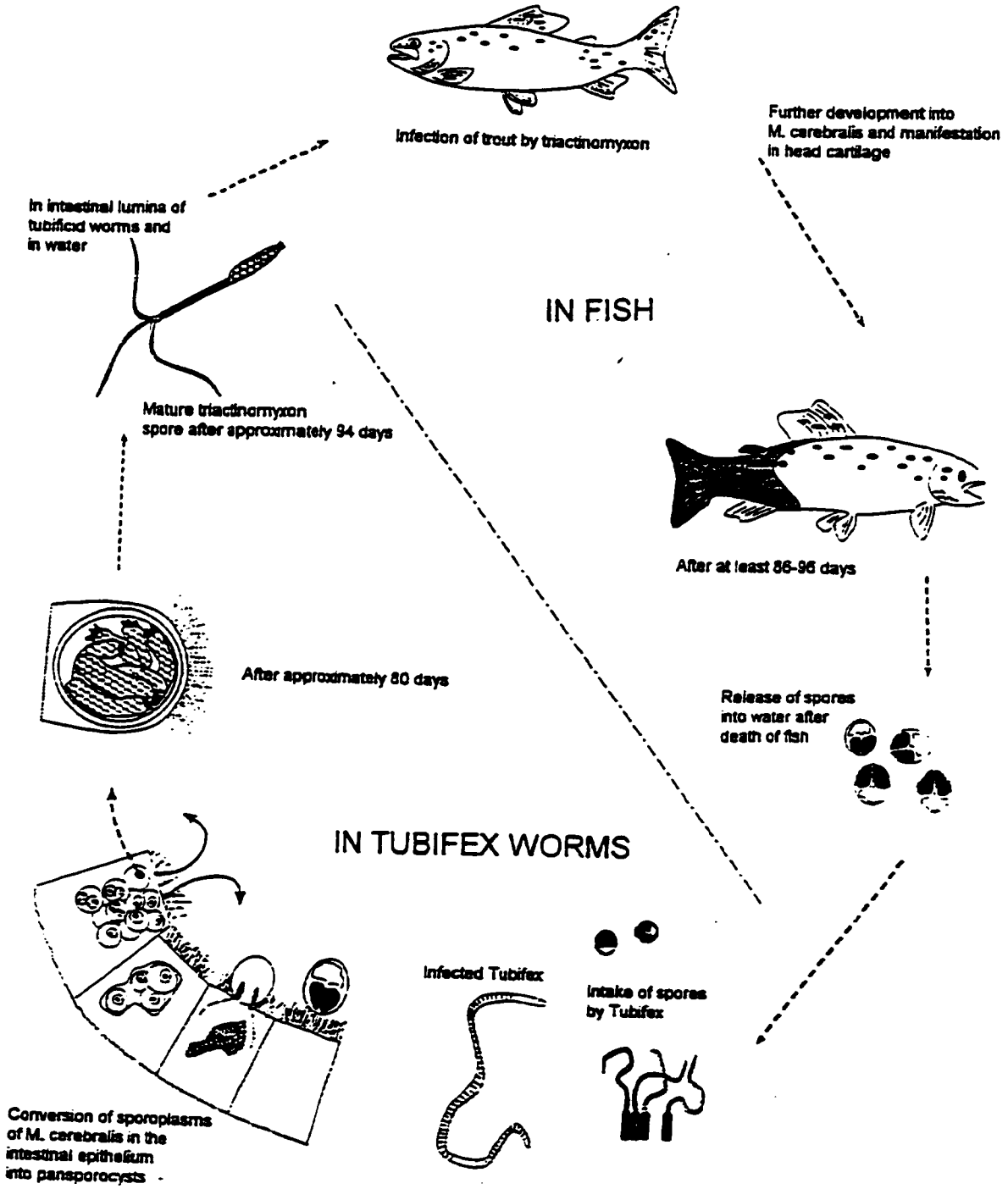

Figure 1.1. Life cycle of *Myxobolus cerebralis* (from Walker and Nehring 1995).

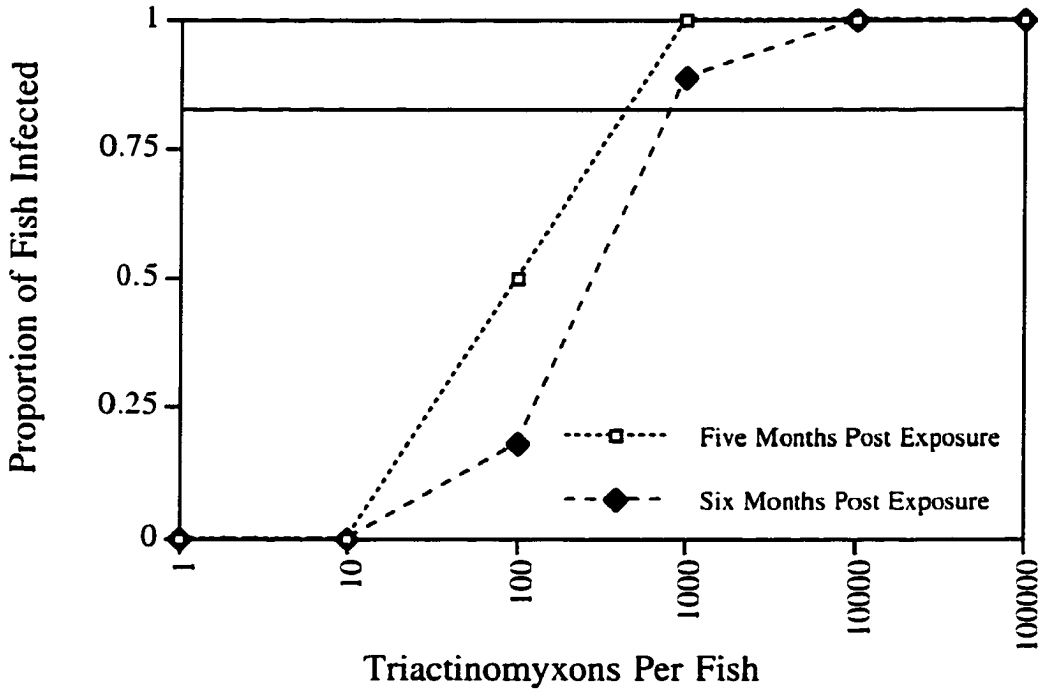

Figure 1.2. Markiw's (1992a) results for proportion of fish successfully infected as a function of number of triactinomyxons per juvenile rainbow trout. The horizontal line represents the proportion of juvenile rainbow trout in the Colorado River determined to be infected with *M. cerebralis* from histological sectioning in 1994 (Walker and Nehring 1995).

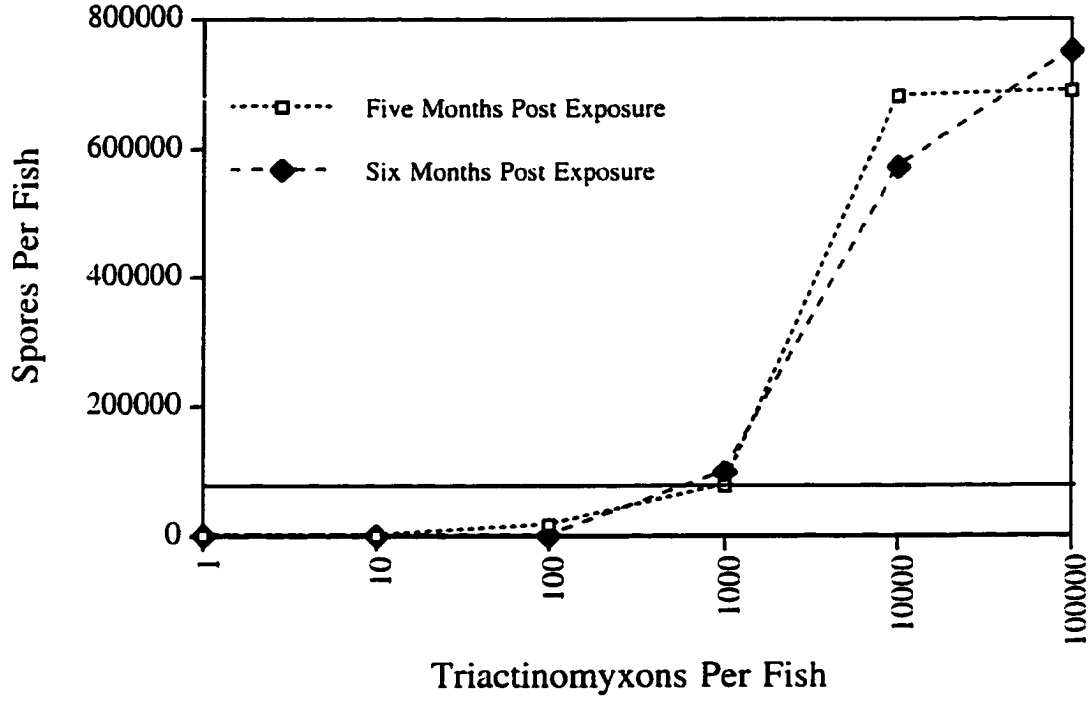

Figure 1.3. Markiw's (1992a) results for average spore loadings as a function of number of triactinomyxons per juvenile rainbow trout. The horizontal line represents the numbers of spores present in juvenile rainbow trout collected from the Colorado River in 1994 (Walker and Nehring 1995).

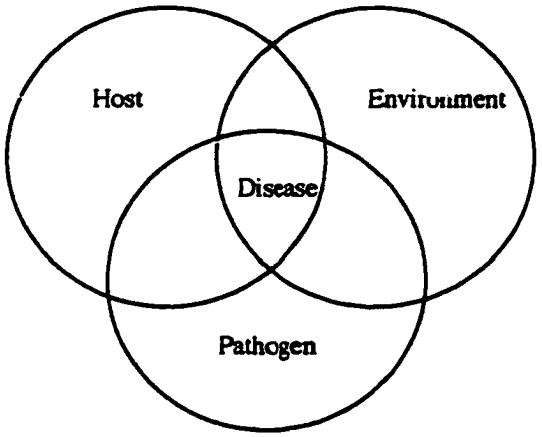

Figure 1.4. Snieszko's (1974) conceptual model for the occurrence of disease. According to the model, the host and pathogen must appear together in an environmental situation that is stressful to the host in order for the disease to occur.

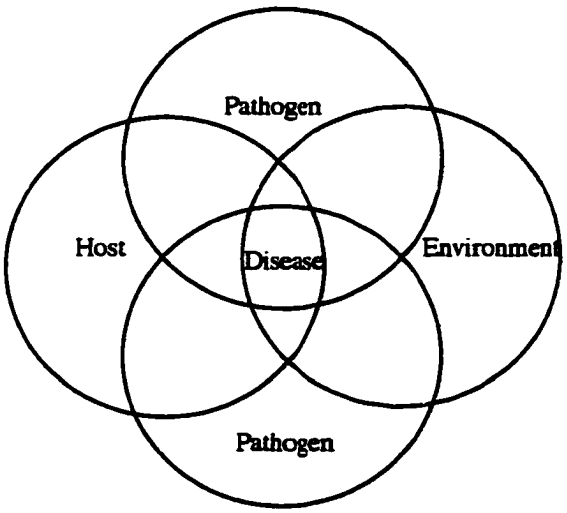

Figure 1.5. In some situations, a fish must be exposed to more than one pathogen in a stressful environment for disease to occur (Wedemeyer et al. 1976).

References

Barton, B. A., C. B. Schreck, and L. D. Barton. 1987. Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions and stress responses in juvenile rainbow trout. Diseases of Aquatic Organisms 2:173-185.

Bogdanova, E. A. 1960. An endemic disease of salmonids in Sakhalin island. Doklady Akademii Nauk SSSR 134: 1501-1503

Bogdanova, E. A. 1970. On the occurrence of whirling disease of salmonids in nature in U.S.S.R. Journal of Parasitology. 56, Proceedings of the 2nd International Congress of Parasitology Abstract No. 719.

Cockburn, A. 1963. *The Evolution and Eradication of Infectious Diseases*. John Hopkins Press, Baltimore, Maryland.

Colorado River Wildlife Council 1988. Whirling Disease Management in North America, an Emergency Conference. Denver, CO. April 12 - 14, 1988. Fish Disease Subcommittee. 20 p.

Ellis, A. E. 1981. Stress and the modulation of defense mechanisms in fish. In *Stress and Fish* . A. D. Pickering [ed.], pp. 147 - 169. London and New York: Academic Press.

El-Matbouli, M., Fischer-Scherl, T., and Hoffmann, R. W. 1992. Present knowledge on the life cycle, taxonomy, pathology and therapy of some myxosporea spp. important for freshwater fish. Annual Review of Fish Diseases 3: 367-402.

El-Matbouli, M. and R. W. Hoffman 1991. Effects of freezing, aging, and passage through the alimentary canal of predatory animals on the viability of *Myxobolus cerebralis* spores. Journal of Aquatic Animal Health 3:260-262.

El-Matbouli, M., R. W. Hoffman and C. Mandok. 1995. Light and electron microscopic observations on the route of the triactinomyxon-sporoplasm of *Myxobolus cerebralis* from the epidermis into rainbow trout cartilage. Journal of Fish Biology 46: 919-935.

Ellsaesser, C. F., and L. W. Clem. 1986. Haematological and immunological changes in channel catfish stresses by handling and transport. Journal of Fish Biology 28:511-521.

Fry, F. E. J. 1969. Some possible physiological stresses induced by eutrophication. In *Eutrophication: Causes, Consequences, Correctives* pp. 531-536. Washington: National Academy of Science

Garden, O. 1992. The Myxosporea of fish: A review. British Veterinary Journal. 148: 223-239.

Graff, D. R. 1996. Whirling Disease: The midwestern/eastern experience. "Where do we go from here?": Proceedings of the Whirling Disease Workshop, Denver, CO. February 6 - 8, 1996.

Halliday, M. M. 1973. Studies on *Myxosoma cerebralis*, a parasite of salmonids. II. The development and pathology of *Myxosoma cerebralis* in experimentally infected rainbow trout (*Salmo gairdneri*) fry reared at different water temperatures. Nordisk Veterinaermedicin 25: 349-358.

Halliday, M. M. 1974. Studies on *Myxosoma cerebralis*, a parasite of salmonids. III. Some studies on the epidemiology of *Myxosoma cerebralis* in Denmark, Scotland and Ireland. Nordisk Veterinaermedicin 26: 165-172.

Halliday, M. M. 1976. The biology of *Myxosoma cerebralis*: The causative organism of whirling disease in salmonids. Journal of Fish Biology 9: 339-357.

Hoffman, G. L. 1966. Effects of whirling disease. The Progressive Fish-Culturist 28:151.

Hoffman, G. L. 1974. Disinfection of contaminated water by ultraviolet radiation, with emphasis on whirling disease (*Myxosoma cerebralis*) and its effect on fish. Transactions of the American Fisheries Society 103: 541-550.

Hoffman, G. L. 1975. Whirling disease (*Myxosoma cerebralis*): Control with ultraviolet irradiation and effect on fish. Journal of Wildlife Diseases 11: 505-507.

Hoffman, G. L., C. E. Dunbar, and A. Bradford. 1962. Whirling disease of trouts caused by *Myxosoma cerebralis* in the United States. Special Scientific Report. Fisheries No. 427. 15pp. U. S. Department of the Interior.

Hoffman, G. L. 1990. *Myxobolus cerebralis*, a worldwide cause of salmonid whirling disease. Journal of Aquatic Animal Health 2:30-37.

Hoffman, G. L., and C. J. Byrne. 1974. Fish age as related to susceptibility to *Myxosoma cerebralis*, cause of whirling disease. The Progressive Fish-Culturist 36:151.

Hoffman, G. L. and R. E. Putz. 1969. Host susceptibility and the effect of aging, freezing, heat, and chemicals on spores of *Myxosoma cerebralis*. The Progressive Fish-Culturist 33: 95-98.

Hoshina, T. 1952. Notes on some myxosporidian parasites of fish of Japan. Journal of the Tokyo University of Fisheries 39: 69-89.

Hulbert, P. 1996. A survey of whirling disease impacts and control strategies in five eastern states. "Where do we go from here?": Proceedings of the Whirling Disease Workshop, Denver, CO. February 6 - 8, 1996.

Leith, D. A. and K. D. Moore. 1967. Pelton Pilot Hatchery Progress Report. November 1966 through October 1967. Fish Commission of Oregon Research Division.

Lom, J. 1970. Some aspects of the host-parasite relation in myxosporidians of the genus *Henneguya*. Journal of Parasitology. 56, Proceedings of the 2nd International Congress of Parasitology Abstract No.800.

Manning, M. J. and T. Nakanishi. 1996. The Specific Immune System: Cellular Defenses. In *The Fish Immune System: Organism, Pathogen, and Environment*. G. Iwama and T. Nakanishi (eds.) Academic Press Inc. San Diego, California. pp. 159-206.

Markiw, M. E. 1989. Salmonid whirling disease: Myxosporean and actinosporean stages cross-react in direct fluorescent antibody test. Journal of Fish Diseases 12: 137-141.

Markiw, M. E. 1991. Whirling disease: Earliest susceptible age of rainbow trout to the triactinomyxid of *Myxobolus cerebralis*. Aquaculture 92: 1-6.

Markiw, M. E. 1992. Experimentally induced whirling disease. I. Dose response of fry and adults of rainbow trout exposed to the triactinomyxon stage of *Myxobolus cerebralis*. Journal of Aquatic Animal Health 4: 40-43.

Markiw, M. E. 1992. Experimentally induced whirling disease. II. Determination of longevity of the infective triactinomyxon stage of *Myxobolus cerebralis* by vital staining. Journal of Aquatic Animal Health 4: 44-47.

Markiw, M. E. and K.Wolf 1983. *Myxosoma cerebralis* (Myxozoa: Myxosporea) etiologic agent of salmonid whirling disease requires tubificid worm (Annelida: Oligiochaeta) in its life cycle. Journal of Protozoology 30:561 -164.

Maule, A. G., C. B. Schreck, and S. L. Kaattari. 1987. Changes in the immune system of coho salmon (*Oncorhynchus kisutch*) during the parr-smolt transformation and after implantation of cortisol. Canadian Journal of Fisheries and Aquatic Science 44:161-166.

Maule, A. G., R. A. Tripp, S. L. Kaattari, and C. B. Schreck. 1989. Stress alters immune function and disease resistance in chinook salmon (*Oncorhynchus tshawytscha*). Journal of Endocrinology 120:135-142.

Maule, A. G., Tripp, R. A. , Kaattari, S. L. and C. B. Schreck. 1989. Stress alters immune function and disease resistance in chinook salmon (*Oncorhynchus kisutch*) during parr-smolt transformation and after implantation of cortisol. Canadian Journal of Fisheries and Aquatic Science 44:161-166.

Mazeaud M. M., Mazeaud, F. and E. M. Donaldson. 1977. Primary and secondary effects of stress in fish: some new data with a general review. Transactions of the American Fisheries Society 106:201-212.

McLeay, D. J. 1975. Variations in the pituitary-interrenal axis and the abundance of circulating blood-cell types in juvenile coho salmon, *Oncorhynchus kisutch*, during stream residence. Canadian Journal of Zoology 53:1882-1891.

McLeay, D. J. 1975. Sensitivity of blood cell counts in juvenile coho salmon (*Oncorhynchus kisutch*) to stressors including sublethal concentrations of pulp mill effluent and zinc. Journal of the Fisheries Research Board of Canada 32:2357-2364.

Meyer, F. P. 1970. Seasonal fluctuations in the incidence of disease on fish farms. Symposium on the Diseases of Fishes and Shellfishes. American Fisheries Society Publication No. 5. 526 pp.

Modin, J. 1996. Whirling disease impacts on wild salmonid populations in four western states: California, Nevada, Oregon and Idaho; and the current status of the parasite in Washington."Where do we go from here?": Proceedings of the Whirling Disease Workshop, Denver, CO. February 6 - 8, 1996.

Nehring, B. R. 1996. Whirling disease in feral trout populations in Colorado. "Where do we go from here?": Proceedings of the Whirling Disease Workshop, Denver, CO. February 6 - 8, 1996.

O'Grodnick, J. J. 1979. Susceptibility of various salmonids to whirling disease (*Myxosoma cerebralis*). Transactions of the American Fisheries Society 108: 187-190.

Patino, R., J. M. Redding, and C. B. Schreck. 1987. Interrenal secretion of corticiosteriods and plasma cortisol and cortisone concentrations after acute stress and during seawater acclimation in juvenile coho salmon (*Oncorhynchus kisutch*). General Comparative Endocrinology 68:431-439.

Peters, G., and R. Schwarser. 1985. Changes in hemopoietic tissue of rainbow trout under the influence of stress. Diseases of Aquatic Organisms 1:1-10.

Pickering, A. D. 1981. The concept of biological stress. In *Stress and Fish* . A. D. Pickering [ed.], pp. 1 - 7. London and New York: Academic Press.

Pickering, A. D. 1989. Environmental stress and the survival of brown trout, *Salmo trutta*. Freshwater Biology 21: 47-55.

Rydlo, M. 1971. Nachwies von Sporen den *Myxosoma cerebralis* in verschiedenen Organen drehkranker Regenbogenforellen. Zeitschrift Gesamte Fischerei 24(7):97-99.

Sanders, J. E., J. L. Fryer, and R. W. Gould. 1970. Occurrence of the myxosporidian parasite *Ceratomyxa shasta* in salmonid fish from the Columbia River Basin and Oregon Coastal Streams. A symposium on Diseases of Fishes and Shellfishes. S. F. Snieszko [ed.] American Fisheries Society. Washington D. C. Special Publication No. 5.

Schaperclause, W. 1931. Die Drehkrankheit in den Forellenzucht und ihre Bekampfung. (Whirling Disease in trout farming and its control). Zeitschrift fur Fischerei. 29:521-567.

Schisler, G. J. 1995. Survival of rainbow trout (*Oncorhynchus mykiss*) caught and released on scented artificial baits. M. S. Thesis. Colorado State University.

Schreck, C. B. 1981. Stress and compensation in teleostean fishes: Response to social and physical factors. In *Stress and Fish*. A. D. Pickering [ed.], pp 295-321. Academic Press, London.

Selye, H. 1950. Stress and the general adaptation syndrome. British Medical Journal 1: 1383-1392.

Snieszko, S. K. 1973. Recent advances in scientific knowledge and developments pertaining to diseases of fishes. In *Advances in Veterinary Science and Comparative Medicine* Volume 17. Academic Press, New York.

Snieszko, S. K. 1974. The effects of environmental stress on outbreaks of infectious diseases of fishes. Journal of Fish Biology 6:197-208.

Taylor, R. E. L. and M. H. Haber. 1974. Opercular cyst formation in trout infected with *Myxosoma cerebralis*. Journal of Wildlife Diseases 10:347-351.

Tidd, W. M. and R. A. Tubb. 1970. Investigations of whirling disease in Ohio. Journal of Parasitology. 56, Proceedings of the 2nd International Congress of Parasitology Abstract No. 632.

Tripp, R. A., A. G. Maule, C. B. Schreck, and S. L. Kaattari. 1987. Cortisol mediated suppression of salmonid lymphocyte responses *in vitro*. Developmental and Comparative Immunology 11:565-576.

Uspenskaya, A. V. 1964. The ecology and distribution of the pathogen causing 'Twist disease' in trout. *Myxosoma cerebralis* (Hofer, 1903; Plehn, 1905). In *Stream Fisheries of the Soviet Union*. Biol. Abstr. No. 30816.

Vincent, R. E. 1996. Whirling disease - The Montana experience, Madison River. "Where do we go from here?": Proceedings of the Whirling Disease Workshop, Denver, CO. February 6 - 8, 1996.

Walker, P. G. and R. B. Nehring. 1995. An investigation to determine the cause(s) of the disappearance of young wild rainbow trout in the upper Colorado River, in Middle Park, Colorado. Colorado Division of Wildlife, Brush, Colorado.

Wedemeyer, G. A. 1974. Stress as a predisposing factor in fish disease. United States Department of the Interior, Fish and Wildlife Service Publication FDL-38, Washington D.C.

Wedemeyer, G. A., Meyer, F. P., and L. Smith. 1976. Physiological factors in fish diseases. In *Diseases of Fishes. Book 5: Environmental Stress and Fish Diseases.* Snieszko, S. F. and H. R. Axelrod [eds.] pp. 72-88.

Wedemeyer, G. 1980. Environmental stress as a cause of fish disease. Aquamed, Tavolek, Redmond, Washington. 3pp.

Wolf, K. and M. E. Markiw. 1984. Biology contravenes taxonomy in the Myxozoa: New discoveries show alternation of invertebrate and vertebrate hosts. Science 225:1449-1452.

Woodward, C. C. and R. J. Strange. 1987. Physiological stress responses in wild and hatchery-reared rainbow trout. Transactions of the American Fisheries Society 116: 574-579.

Wiley, R. W. 1996. Perspective on whirling disease in the Rocky Mountains. "Where do we go from here?": Proceedings of the Whirling Disease Workshop, Denver, CO. February 6 - 8, 1996.

CHAPTER 2:

Identification of Gas Saturation Sources in the Upper Colorado River, Middle Park, Colorado

In late August, 1994, young-of-the-year rainbow (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) were sampled by Colorado Division of Wildlife personnel from three locations in the Upper Colorado River as part of an ongoing investigation into the disappearance of young-of-the-year rainbow trout in the drainage (Walker and Nehring 1995). One of the factors contributing to the disappearance of these trout is thought to be whirling disease, caused by *Myxobolus cerebralis*, a parasitic myxosporidean. *M. cerebralis* destroys the cartilage of juvenile fish, resulting in a wide variety of clinical signs, including cranial deformities, deformed lower jaws and opercula, blacktail, spinal deformities, disintegration of the fins, opercular cysts, and "whirling" behavior (Halliday 1976).

Along with typical signs of whirling disease among young-of-the-year trout sampled in 1994, many of the fish exhibited signs of gas bubble trauma (GBT). Signs of GBT observed included exopthalmia (protrusion of the eye from its socket) and emboli (bubbles of gas) in the gill lamellae, behind the eye, and in the kidneys (Walker and Nehring 1995). Preliminary sampling at various locations throughout the drainage revealed gas saturation levels ranging from 97.2 to 110.5% (Δ P = -16 to 54 mm Hg). Given the severity of GBT signs among trout sampled and the moderately high TGP readings in the same sampling locations, the present study was initiated to identify sources and levels of supersaturation in the drainage. This information was needed to determine whether or not GBT could be negatively affecting the rainbow trout population.

Gas bubble trauma, also known as gas bubble disease, is an environmentally induced physiological condition that occurs among fish residing in water that is supersaturated with atmospheric gasses. Supersaturation occurs when total gas pressure (TGP) in a body of water exceeds compensation pressure (PCOMP). TGP is defined as the sum of the gas pressures present in water, including water vapor pressure. PCOMP is

equal to barometric pressure plus hydrostatic pressure. While barometric pressure changes with regard to elevation and weather patterns, hydrostatic pressure increases with increased water depth (about 76 mm Hg per meter). Blood and tissue pressures and surface tension also contribute to compensation pressure, but are minute and rarely measured (Bouck 1980). The difference between TGP and PCOMP is referred to as ΔP. GBT should not occur when ΔP is less than zero (water is not saturated), nor when ΔP is equal to zero (water is at equilibrium). If ΔP is greater than zero, water is supersaturated, and signs of GBT may begin to develop.

Supersaturation can arise from a variety of different human and natural sources. For supersaturation to occur, water and atmospheric gasses must be forced together under pressure, or the capacity of water to hold gasses in solution must be reduced. Water and gasses are often mixed under pressure in deep plunge pools below spillways of dams or waterfalls when gasses are entrained in falling water. The Columbia River System has had a long history of gas supersaturation problems related to the numerous hydroelectric dams present in the drainage (Ebel 1969; Beiningen and Ebel 1970; Meekin and Allen 1974a; Meekin and Allen 1974b).

In hatchery systems, water and gas can be mixed unintentionally under pressure when air is drawn into water supplies through leaky pipes (Marsh and Gorham 1905; Harvey and Smith 1961). Warm water has lower capacity to hold dissolved gasses in solution than cold water, so warming of saturated water without corresponding loss of gas can also result in supersaturation. This is often the case when two saturated bodies of water with different temperatures mix (Adair and Hains 1974). Loss of hydrostatic pressure also reduces the ability of water to hold gasses in solution. This occurs when water is released from deep bottom releases of dams, or escapes from aquifers via wells or springs (Matsue et al. 1953). Photosynthetic activity has also been reported to cause

supersaturation when the production of oxygen from aquatic plants or algae exceeds the amount of oxygen escaping from the water into the atmosphere (Boyd et al. 1994; Woodbury 1941).

The Environmental Protection Agency (1977) suggested 110% saturation (Δ P = about 76 mm Hg at sea level) as a safe upper limit for supersaturation for freshwater and marine aquatic life. This criterion has been criticized by some researchers for being too high to protect fish from GBT in hatcheries or other situations where fish are restricted to shallow water (Ebel et al. 1979; Bouck 1980). Arguments have also been made that the EPA criterion is too restrictive in natural systems because fish have been known to avoid supersaturated water (Stevens et al. 1980), and fish with access to deep water can sound to increase hydrostatic pressure and avoid developing GBT.

Methods

The Colorado River from Granby Reservoir downstream to 5 kilometers west of Parshall, Colorado was chosen as the study area (Figure 2.1). This stretch of river has supported viable self-sustaining populations of rainbow and brown trout for over 50 years. Four impoundments occur in the drainage, including Granby Reservoir, Willow Creek Reservoir, Windy Gap Reservoir, and Williams Fork Reservoir. Granby Reservoir, Willow Creek Reservoir, and Windy Gap Reservoir are all part of the Northern Colorado Water Conservancy District's Windy Gap Project. This series of reservoirs is used to provide water to municipalities on the eastern slope of the Continental Divide in Colorado. Yearly precipitation and demand for water by these municipalities dictates the amount of water released downstream. A pump station at Windy Gap Reservoir returns water to Granby Reservoir via a 9.6-kilometer pipeline. No power generating turbines or other mechanisms are used at these reservoirs, and water levels remain fairly constant in the

impoundments. At the time the study was initiated, I hypothesized that there was either a point source of supersaturation somewhere in the drainage, or that some seasonal event was causing supersaturation, leading to the GBT signs observed in the young-of-the-year trout in 1994. I designed a sampling protocol I felt would provide adequate sample sizes over the course of the field season to identify the sources and extent of gas supersaturation in the drainage.

Forty-seven locations were designated throughout the study area to be sampled for gas saturation levels. Seven of these locations were chosen as sampling sites where gas saturation levels would be monitored on a regular basis. They included the outflows of the four reservoirs in the drainage, as well as the confluence of the Colorado River and Willow Creek, the confluence of the Colorado River and the Fraser River, and the confluence of the Colorado River and Williams Fork River. These fixed sampling sites were chosen because they were considered possible sources of supersaturation, and were located in fairly widely spaced locations throughout the drainage, which allowed good general coverage of the study area. Saturation levels were measured at the outflows directly below each of the dams. Because Windy Gap Reservoir has two separate release locations that operate simultaneously (the spillway release and the fishway release), saturation levels were measured at both locations. Three measurements were taken at each of the three confluences sampled. These included one slightly upstream of the confluence in the tributary arm, one slightly upstream of the confluence in the Colorado River arm, and one measurement directly in the center of each confluence.

The remaining mainstem of the Colorado River was divided into four, 10-kilometer sections (A1 - A4), which in turn were each divided into 10, 1-kilometer subsections. The subsections were sampled randomly, based on their alphanumeric designation, for gas saturation levels. Twenty-four-hour measurements were also made at

randomly chosen sampling sites to monitor diurnal fluctuations in TGP.

Gas saturation levels were measured using Common Sensing Inc. Model TBO-L totai dissolved gas and oxygen monitors. The monitors measure TGP and oxygen pressure separately, and calculate saturation levels based on water temperature and barometric pressure. Twenty-four hour measurements were recorded with Common Sensing Inc. DL3 dataloggers attached to Model TBO-L monitors, and powered by 12-volt batteries. Saturation levels were recorded at each of the seven fixed sampling sites, and four of the remaining 40 random sampling sites (subsections) per sampling day. Locations of sample sites were assigned alphanumeric codes to simplify location descriptions (Figure 2.1).

Analysis of variance (ANOVA) testing was conducted separately for saturation data collected from the dams and confluences. Because samples were collected at different times of day on each sampling day for each location, I felt it was appropriate to treat each sample as an individual replicate in this analysis. A two factor ANOVA was conducted on the dam outflow data to test monthly (June, July, August, and September plus October) and location effects on saturation levels. A three-factor ANOVA was conducted on the data from the confluence sampling that tested the effects of confluences, sites within the confluences (arms of the confluences), and month of sampling. Interaction effects of month and sampling location were tested in both ANOVAs for the dam and confluence data.

A multiple regression analysis was conducted on the random subsection samples using month of the year, time of day, and stream kilometer as parameters. As in the ANOVA tests, data from September and October were combined. Time of day was included as a parameter, with most random samples taken from 0800 hours to 1800 hours. The stream kilometer parameter was defined as 1 to 40 starting at the head of the study area at Granby Reservoir to the bottom of the study area near Parshall, Colorado. A set of 45

different models was created using these factors and all two-way interaction effects. Akaike's Information Criterion (AIC) (Akaike 1973) was used to choose a model that best fit the data without overparameterization. This model was then used to describe the saturation dynamics in the drainage.

Results

Ranges of gas saturation levels for each of the seven fixed sampling locations and each of the four randomly sampled stream sections were summarized (Table 2.1). Mean saturation values and standard deviations by month were summarized for sampling locations (Table 2.2). These results indicate that saturation levels in section A2 were higher than the other sections, and that during June and July, daily saturation levels at two of the regular sampling locations (below Willow Creek Reservoir -R2, and below the spillway of Windy Gap Reservoir -R3(S)) were higher than the other locations.

The highest mean saturation levels among the dams (Table 2.2) were found at the Windy Gap Reservoir spillway during the months of June ($\Delta P = 54.9$) and July ($\Delta P = 62.1$). Saturation levels decreased at this location after the dam stopped spilling in early August, and at that time became comparable with those in the rest of the drainage. Most of the inflow into Windy Gap Reservoir after July was either pumped back up to Granby Reservoir or released through the fishway release. The Willow Creek Reservoir outflow also had high saturation levels in June and July, and the highest mean saturation during the August and September - October sampling ($\Delta P = 22.2$). The Williams Fork Reservoir outflow (R4) had mean ΔP values below 0 from August through October. These low saturations were presumably due to stratification of the reservoir, resulting in anoxic conditions at the bottom of the reservoir. Because the outflow of the reservoir is at the base of the dam, low saturation levels were reflected in the outflow measurements. The

ANOVA for the dam outflows (Table 2.3) reflected the differences between the dams ($P < 0.0001$) and months ($P < 0.0001$), and verify a month x dam interaction ($P < 0.0001$). The null hypothesis of no temporal or spatial effects on saturation levels was rejected for the dam outflow data.

Differences in saturations were apparent between arms of each of the confluences (Table 2.2). Saturation levels at the Willow Creek confluence were consistently higher in the Willow Creek arm and center of the confluence than in the Colorado River arm. In the Fraser River confluence, lower saturations were found in the Fraser River arm than the center of the confluence or the Colorado River arm in every month except during the September - October sampling period. None of the sites within the Williams Fork confluence were consistently higher or lower than the other sites throughout the sampling period. The results of the ANOVA for the confluences rejected the null hypothesis of no spatial or temporal effects (Table 2.4). Significant differences were found between the months of sampling ($P = 0.0175$), between the sites within the confluences ($P = 0.0122$), and a month by confluence interaction was found as well ($P = 0.0001$).

The multiple regression model chosen with AIC (Table 2.5) for the mainstem subsection sampling contained the river kilometer, river kilometer squared, time of day, and time of day squared as continuous variables. The months of June through July, and the months of August through October were included as class variables. An interaction between river kilometer and river kilometer squared with the month variables was included as well. The second degree polynomials for both river kilometer and time of day reflect the increase in saturation levels in the system up to an inflection point, followed by a subsequent decrease. For example, estimated saturations increased from 800 hours up to 1400 hours, then declined again until 1800 hours (Figures 2.2 and 2.3). Saturation levels were similarly affected by river location during the months of June and July. Estimated

saturation levels increased downstream to about stream kilometer 20, where they decreased to the lower end of the sampling area (Figure 2.2). This spatial change in saturation levels was not as pronounced during the months of August through October, after water stopped flowing over the Windy Gap Reservoir spillway (Figure 2.3). The model provides a rough overview of the dynamics of the system, and rejects the null hypotheses of no diurnal, temporal, and spatial effects on saturation levels in the drainage.

Data from the 24-hour sets supported the regression model results of significant diurnal fluctuations in most locations. An example of this diurnal fluctuation (Figure 2.4) was recorded below Granby Reservoir. While nitrogen saturation values remain relatively stable throughout the 24-hour period, oxygen saturation levels increase dramatically during mid-day, driving up the total gas saturation levels. As with the multiple regression model, the saturation levels increased from 800 hours until 1400 hours and decreased until the end of the day. Total gas saturation did not follow an obvious diurnal fluctuation in some sampling locations. A 24-hour measurement at the spillway outflow of Windy Gap Reservoir is an example of this (Figure 2.5). Total gas saturation in this case was driven by both oxygen and nitrogen supersaturation caused by water falling over the spillway of the dam throughout the entire sampling period. In most other cases where a detectable saturation level of over 100% occurred, the oxygen component, which was driven up by photosynthetic activity, was the cause of supersaturation. In some cases, respiration caused saturation values to drop below 100% at night (Figure 2.6).

Discussion

Gas saturation levels observed in the drainage were not unusually high for a natural system, especially in the latter months of the study. Bouck (1976) noted that no external symptoms of GBT could be found among wild fish exposed to saturation levels of up to

110%, quite contrary to the occurrence of the disease among fish in raceways at lower saturation levels, and observations by Walker and Nehring (1995) of fingerling rainbow and brown trout with signs of the disease in this drainage.

Results of the gas saturation measurements from fixed sampling points, random sampling points and 24-hour sets all indicate that supersaturation in the study area is the result of a combination of natural and man-made causes. Diurnal fluctuations in oxygen saturations were apparent from both random sampling, and 24-hour measurements. Heavy mats of *Spirogyra* and other algae were observed throughout much of the study area, and were probably the major contributors to oxygen production. I concluded that the source of natural supersaturation in the sampling area was oxygen production caused by aquatic plants. The high saturation values at the outflow of Willow Creek Reservoir and the Willow Creek arm of the Colorado River confluence are most likely a result of the warmer water temperatures and apparent high productivity of that particular tributary. The Fraser River arm of the Fraser-Colorado River confluence, on the other hand, was typically colder with faster moving water that did not allow much growth of aquatic plants. This was reflected in its lower saturation measurements than the center of the confluence and the Colorado River arm of the confluence.

During the latter months of the sampling period the raw data and the regression model indicate lower saturation levels throughout much of the drainage. ANOVA results for the dam and confluence data also revealed significant monthly effects on saturation levels. In areas where oxygen was the major contributor to saturation, the lower saturation levels were probably due to shorter day length and a die-off of aquatic plants. Although weather conditions were not recorded, lower saturation values were almost always observed on cloudy days. In future studies where saturation values may be driven by photosynthetic activity, inclusion of cloud cover in a multiple regression model such as the

one used in this study may improve fit of the model and explain more variability in the data.

The man-made source of supersaturation in the study area was Windy Gap Reservoir. Supersaturation measurements from the spillway outflow were the highest among all the dam outflows measured, and measurements from the fishway were the third highest of the outflows. In addition, both mean monthly saturation values and the regression model of the mainstem Colorado River show an increase in supersaturation below the reservoir and a gradual decrease further downstream as the supersaturation subsided. Supersaturation at the reservoir was caused by entrainment of atmospheric gasses in the water falling over the spillway, and to a lesser extent water released from the fishway outflow. The gradual decrease in supersaturation downstream was most likely caused by loss of gasses to the atmosphere and dilution with increased water volume. Twenty-four hour measurements at Windy Gap Reservoir confirmed that high continuous levels of saturation existed there while water was pouring over the spillway during June, July, and the beginning of August. Decreased saturation levels in the absence of spill were reflected by significantly lower saturation levels in the multiple regression model for the months of August through October.

Results of my study suggest that elevated gas supersaturation caused by Windy Gap Reservoir could act as an additional stressor leading to higher mortalities among *M. cerebralis* infected rainbow trout. The following chapter describes the distribution of clinical signs of disease among young-of-the-year rainbow and brown trout in the Upper Colorado River, further implicating Windy Gap Reservoir as an indirect cause of high mortalities of rainbow trout in the drainage.

Table 2.1. Location codes and ranges for total gas saturation levels (Δ P) at the seven fixed sampling locations and four randomly sampled subsections of the Upper Colorado River from June through October, 1995.

LOCATION	ABB.	*n*	RANGE
Granby Reservoir Outflow	R1	59	-1, 38
Willow Creek Reservoir Outflow	R2	56	8, 51
Windy Gap Reservoir Spillway	R3 (S)	55	-4, 77
Windy Gap Reservoir Fishway	R3 (F)	54	0, 36
Williams Fork Reservoir Outflow	R4	56	-27, 43
Willow Creek Confluence (Willow Creek Arm)	C1 (WC)	52	0, 77
Willow Creek Confluence (Colorado Arm)	C1 (CO)	55	0, 40
Willow Creek Confluence (Center)	C1 (M)	48	-2, 57
Fraser Confluence (Fraser Arm)	C2 (FR)	54	2, 52
Fraser Confluence (Colorado Arm)	C2 (CO)	55	-7, 47
Fraser Confluence (Center)	C2 (M)	53	-3, 45
Williams Fork Confluence (Williams Fork Arm)	C3 (WF)	54	5, 37
Williams Fork Confluence (Colorado Arm)	C3 (CO)	53	2, 40
Williams Fork Confluence (Center)	C3 (M)	51	4, 41
First 10 Km Mainstem Colorado	A1	56	2, 47
Second 10 Km Mainstem Colorado	A2	56	6, 53
Third 10 Km Mainstem Colorado	A3	57	0, 42
Fourth 10 Km Mainstem Colorado	A4	57	4, 42

Table 2.2. Mean total gas saturation values (Δ P) and standard deviations for sampling locations in the Upper Colorado River for the months of June through October, 1995.

	JUNE		JULY		AUGUST		SEPT. - OCT.	
LOCATION	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
R1	4.43	5.77	6.18	6.10	13.66	10.95	11.92	6.99
R2	25.71	6.03	35.50	7.76	36.21	11.38	22.23	6.27
R3 (S)	54.92	20.96	62.07	5.44	36.00	23.45	16.25	10.80
R3 (F)	16.43	8.61	18.14	9.30	21.30	9.48	18.67	9.07
R4	10.57	16.70	21.81	18.11	-13.00	11.86	-9.36	10.20
C1 (WC)	19.57	8.82	33.75	22.18	23.69	9.02	24.17	11.21
C1 (CO)	18.21	7.14	16.56	8.47	17.23	6.94	17.00	11.12
C1 (M)	18.93	7.34	31.54	15.23	22.42	8.67	18.45	14.74
C2 (FR)	10.57	5.41	13.25	5.34	19.69	7.69	25.64	13.03
C2 (CO)	18.35	9.14	20.50	13.29	21.29	10.72	24.73	8.00
C2 (M)	16.21	8.16	18.13	10.24	24.00	9.07	23.27	11.38
C3 (WF)	21.64	5.93	14.73	3.43	25.27	8.70	13.00	6.06
C3 (CO)	21.46	7.25	15.37	5.40	25.87	9.88	16.77	7.39
C3 (M)	21.00	3.18	15.66	3.90	28.28	10.37	18.77	5.69
A1	18.35	9.39	22.93	13.68	18.31	8.63	23.23	13.28
A2	36.15	10.24	33.80	12.22	28.28	9.20	20.75	10.33
A3	23.21	6.87	25.25	7.64	20.20	10.67	15.16	9.14
A4	19.85	6.08	14.68	6.80	24.54	10.44	19.46	10.97

Table 2.3. ANOVA results for the dam outflow data collected at five outflow sites in the Upper Colorado River during the months of June through October, 1995. Data from September and October were combined for this analysis.

SOURCE	DF	TYPE III SS	MEAN SQUARE	*F* VALUE	*P* > *F*
DAM	4	56119.0366	14029.7592	99.05	0.0001
MONTH	3	10070.5911	3356.8637	23.70	0.0001
DAM x MONTH	12	21832.0719	1819.3393	12.84	0.0001

Table 2.4. ANOVA results for the tributary confluence data collected at three separate confluences and three locations (forks) within each confluence. Months included June through October, 1995. Data from September and October were combined for this analysis.

SOURCE	DF	TYPE III SS	MEAN SQUARE	*F* VALUE	*P* > *F*
MONTH	3	1371.3689	457.1229	4.3747	0.0175
CONFLUENCE	2	439.0625	219.5312	0.5536	0.6016
MONTH x CON	6	5476.9495	912.8249	8.7326	0.0001
FORK (CON)	6	2380.7134	396.7855	3.8029	0.0122
MONTH x FORK (CON)	18	1882.4959	104.5831	1.1698	0.2825

Table 2.5. Gas saturation modeling results for 40 kilometers of the mainstem Upper Colorado River during the months of June through October, 1995.

PARAMETER	DF	ESTIMATE	STD ERR	CHISQUARE	$P>$ CHSQ
INTERCEPT	1	-92.7242	12.8789	51.8361	0.0001
RIVER KILOMETER	1	0.0335	0.2944	0.0129	0.9094
TIME OF DAY	1	17.9383	1.9403	85.4707	0.0001
MONTH	1	-8.9566	3.5916	6.2186	0.0126
RIVER KILOMETER^2	1	-0.0017	0.0070	0.0587	0.8086
TIME OF DAY^2	1	-0.6742	0.0731	85.0967	0.0001
KILOMETER x MONTH	1	1.9444	0.4166	21.7875	0.0001
KILOMETER^2 x MONTH	1	-0.0495	0.0100	24.2935	0.0001
SCALE	1	8.8750	0.4202	.	.

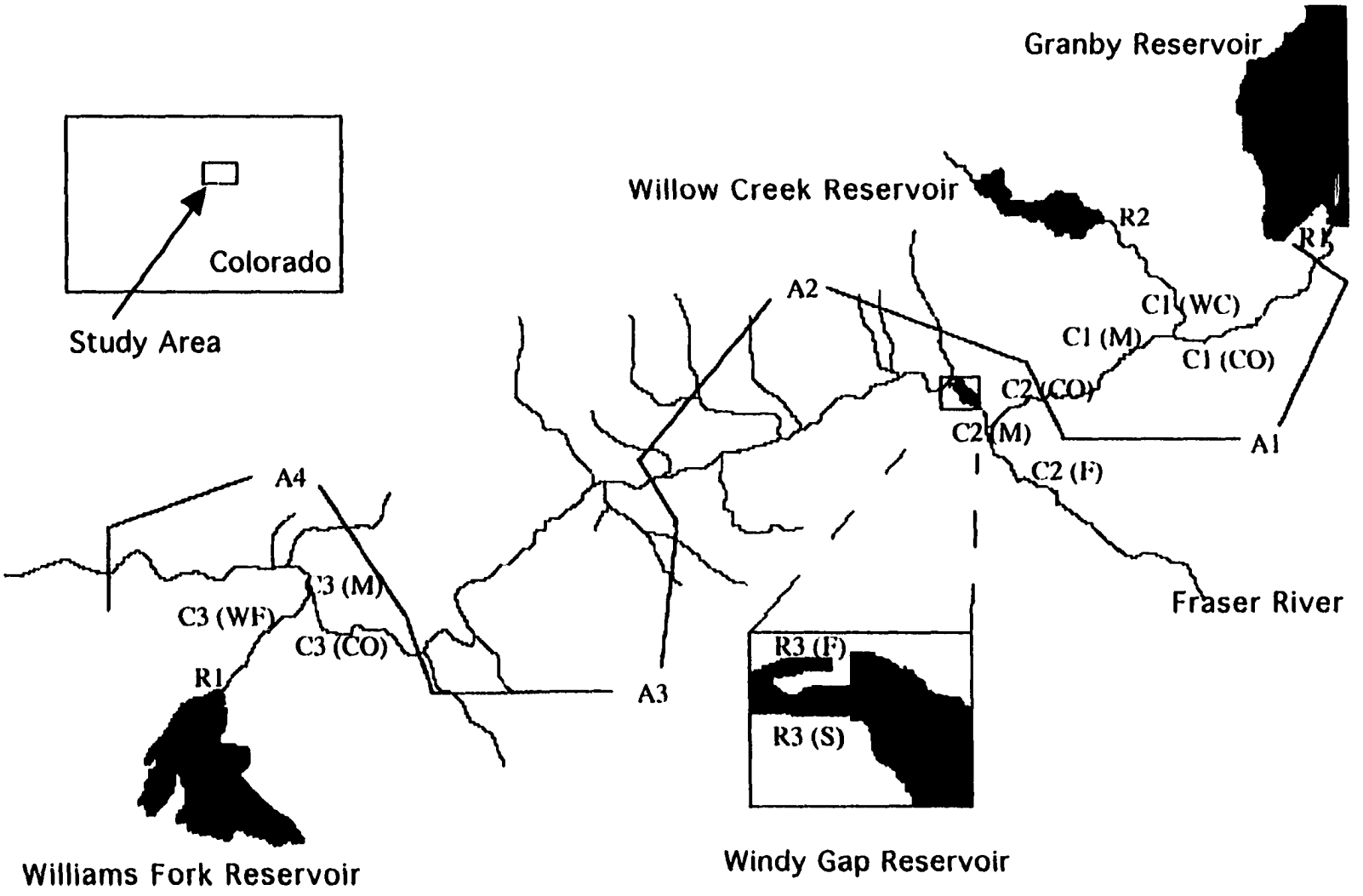

Figure 2.1. Study area and location codes for the Upper Colorado River in Middle Park, Colorado.

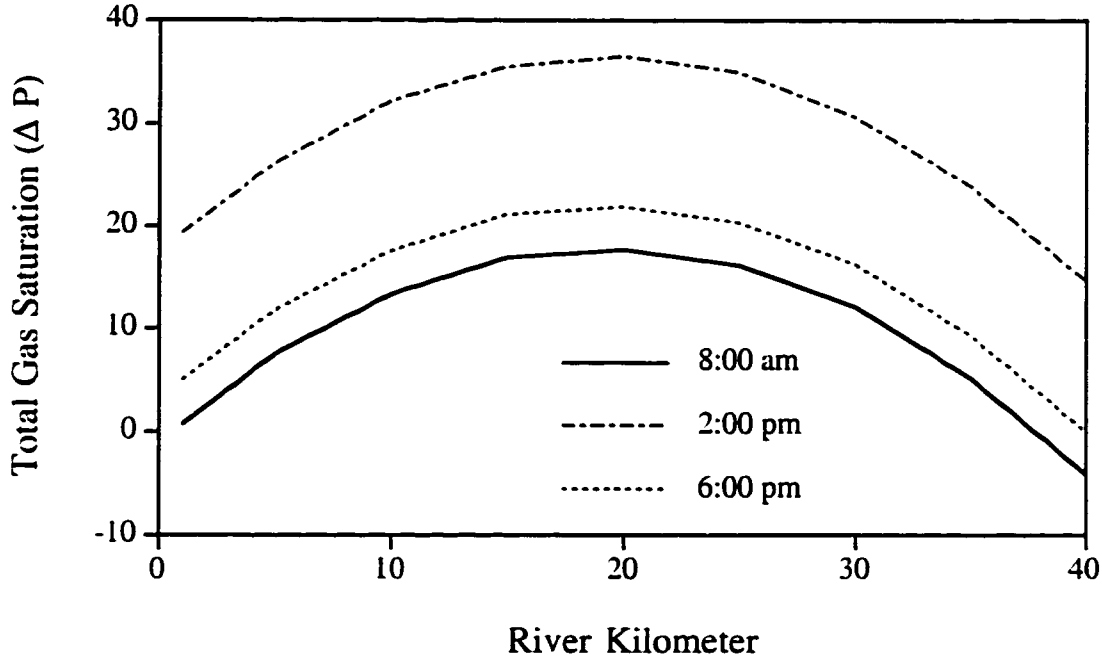

Figure 2.2. Total dissolved gas saturation by time of day and river kilometer for the months of June and July as predicted by the multiple regression model for the Upper Colorado River. River kilometer represents distance downstream of Granby Reservoir.

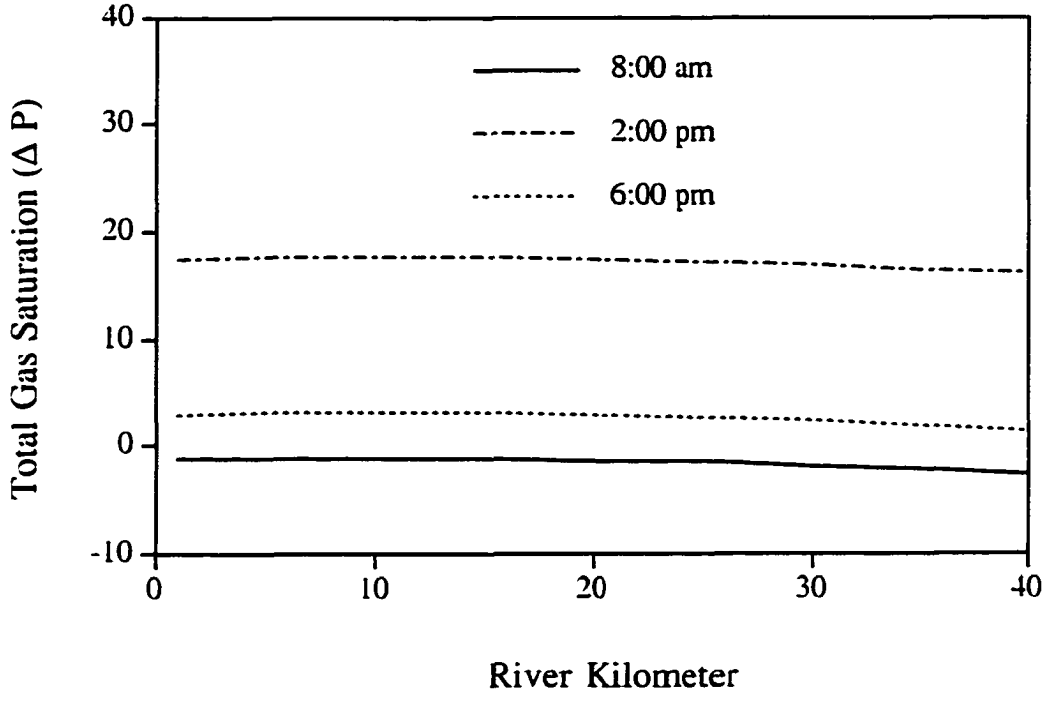

Figure 2.3. Total dissolved gas saturation level by time of day and river kilometer for the months of August through October as predicted by the multiple regression model for the Upper Colorado River. River kilometer represents distance downstream of Granby Reservoir.

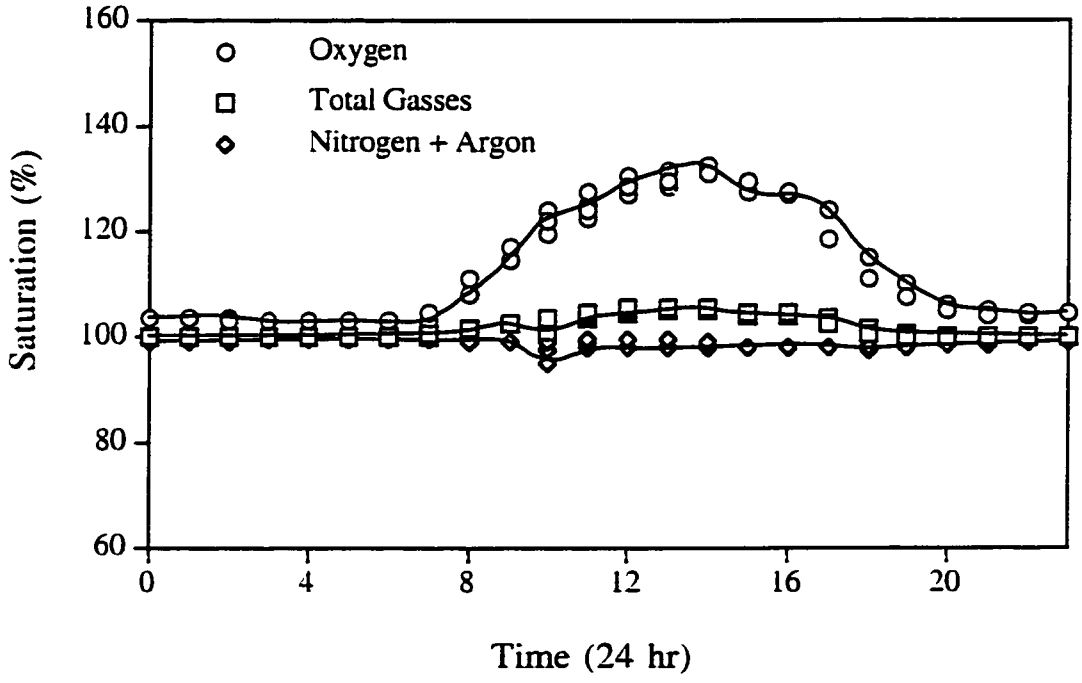

Figure 2.4. Measurements over 24-hours of oxygen, nitrogen, and total gas saturation (%) taken at 30-minute intervals below Granby Reservoir from August 21 to August 28, 1995. Solid lines are computer-generated splines of data points.

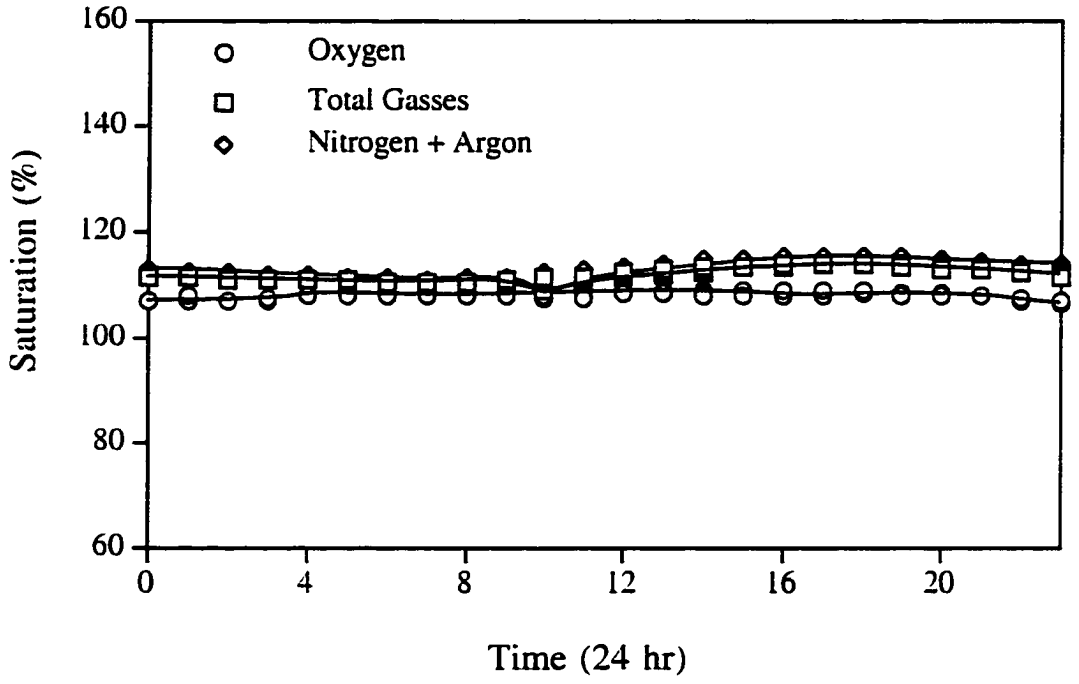

Figure 2.5. Measurements over 24-hours of oxygen, nitrogen, and total gas saturation (%) taken at 30-minute intervals at the spillway of Windy Gap Reservoir from July 28 to July 29, 1995. Solid lines are computer-generated splines of data points.

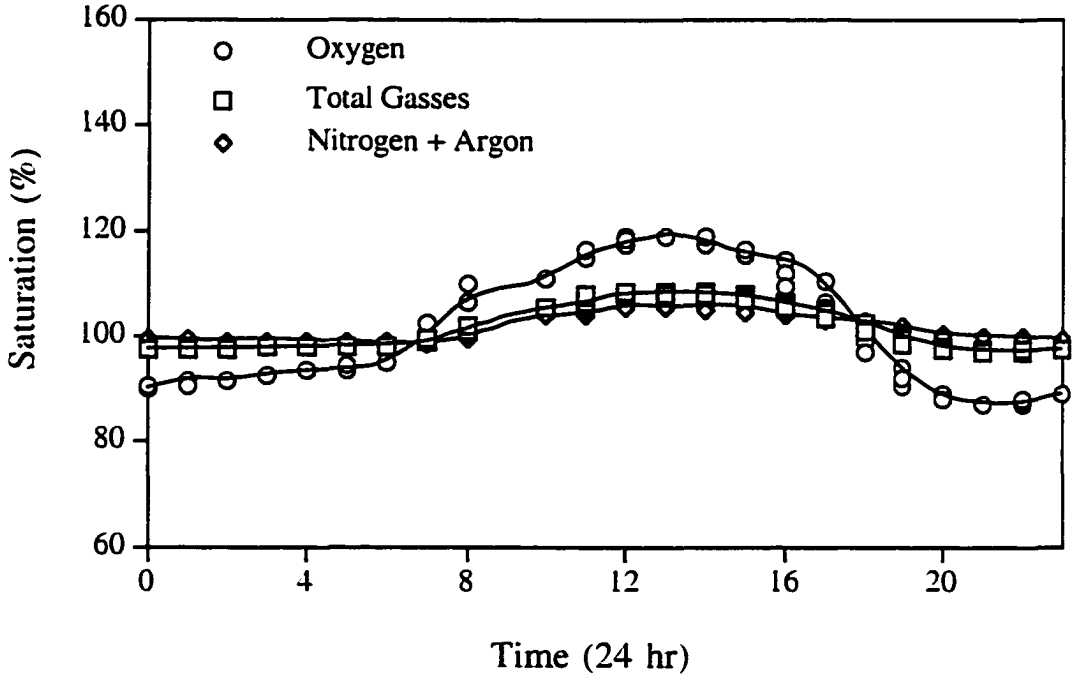

Figure 2.6. Measurements over 24-hours of oxygen, nitrogen, and total gas saturation (%) taken at 30-minute intervals at the center of the Fraser River confluence from August 31 to September 1, 1995. Solid lines are computer-generated splines of data points.

References

Adair, W. D., and J. J. Hains. 1974. Saturation values of dissolved gasses associated with the occurrence of gas-bubble disease in fish in a heated effluent. Pages 59-78 In J. W. Gibbons and R.R. Sharitz, editors. *Thermal ecology*. United States Atomic Energy Commission Contribution 030505, Washington, District of Columbia, USA.

Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. *Second International Symposium on Information Theory*, B. N. Petrov and F. Csaki (eds), 267-281. Budapest: Akademiai Kiado.

Beiningen, K. T., and W. J. Ebel. 1970. Effect of John Day Dam on dissolved nitrogen concentrations and salmon in the Columbia River, 1968. Transactions of the American Fisheries Society. 99:664-671.

Bouck, G. R. 1976. Supersaturation and fishery observations in selected alpine Oregon streams. In *Gas Bubble Disease*. D. H. Flikeisen and M. J. Schneider. Technical Information Service, U. S. Department of Commerce. Springfield, Virginia.

Bouck, G. R. 1980. Etiology of Gas Bubble Disease. Transactions of the American Fisheries Society. 109:703-707.

Boyd, C. E., B. J. Watten, V. Goubier, and R. Wu. 1994. Gas supersaturation in surface waters of aquaculture ponds. Aquaculture Engineering 13:31-39.

Ebel, W. J. 1969. Supersaturation of nitrogen in the Columbia River and its effect on salmon and steelhead trout. United States National Marine Fisheries Service Fishery Bulletin 68:1-11.

Ebel, W. J., K. T. Beiningen, G. R. Bouck, W. R. Penrose, and D. E. Weitkamp. 1979. Gasses, total dissolved, pp. 113-118. In R. V. Thurston, R. C. Russo, C. M. Fetterolf Jr., T. A. Edsall, and Y. M. Barber Jr. [ed.] *A review of the EPA Red Book: quality criteria for water*. Water Quality Section, American Fisheries Society, 5410 Grosvenor, Bethesda, MD.

Environmental Protection Agency. 1977. *Quality criterion for water*. Office of Water and Hazardous Materials. U. S. Environmental Protection Agency. Washington D. C. 256 p.

Halliday, M. M. 1976. The biology of *Myxosoma cerebralis*: the causative organism of whirling disease of salmonids. Journal of Fish Biology 9:339-357.

Harvey, H. H. and S. B. Smith. 1961. Supersaturation of the water supply and occurrence of gas bubble disease at Cultus Lake Trout Hatchery. Canadian Fish Culturist 30:39-46.

Marsh, M. C., and F. P. Gorham. 1905. The gas disease in fishes. Report of the United States Bureau of Fisheries (1908) 28:891-906.

Matsue, Y., S. Egusa and A. Sacki. 1953. On nitrogen gas contents in flowing water of artesian wells and springs. Bulletin of the Japanese Society of Scientific Fisheries 19:439-444.

Meekin, T. K., and R. L. Allen. 1974a. Effects of controlled spilling at Chief Joseph Dam on dissolved nitrogen supersaturation in the Columbia River. Washington Department of Fisheries Technical Report 12:1-31.

Meekin, T. K., and R. L. Allen. 1974b. Nitrogen saturation levels in the mid-Columbia River, 1965-1971. Washington Department of Fisheries Technical Report 12:32-77.

Stevens, D. G., A. V. Nebeker, and R. J. Baker. 1980. Avoidance response of salmon and trout to air-supersaturated water. Transactions of the American Fisheries Society 109:751-574.

Walker, P. G., and R. B. Nehring. 1995. An investigation to determine the cause(s) of the disappearance of young wild rainbow trout in the upper Colorado river, in Middle Park, Colorado. Colorado Division of Wildlife. Brush, Colorado.

Woodbury, L. A. 1941. A sudden mortality of fish accompanying a supersaturation of oxygen in Lake Waubesa, Wisc. Transactions of the American Fisheries Society 71:112-117.

CHAPTER 3:

Assessment of the Distribution of Clinical Signs of Whirling Disease Among Rainbow and Brown Trout Fingerlings in the Upper Colorado River

M. cerebralis has been known to cause a variety of clinical signs in salmonids, especially among those heavily infected with the parasite. While "whirling" behavior is considered the most common sign, less transient forms of overt signs also occur, including blacktail, spinal deformities, cranial deformities, shortened opercula, shortened lower jaws, opercular cysts, and degenerated fins (Halliday 1976). Gas supersaturation can cause similar signs of disease, including deformed opercula, discoloration, exopthalmia, and spinal deformities. From 1994 through 1996, clinical signs of whirling disease and gas bubble trauma among fingerling rainbow (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) in the Upper Colorado River were recorded during two separate studies. Similar field observations from both studies led me to believe that there were spatial and temporal effects on the presence of signs of the disease. More specifically, I hypothesized that the highest numbers of fingerling rainbow and brown trout with clinical signs were found immediately below Windy Gap Reservoir, with a reduction further downstream. Windy Gap Reservoir, near Granby Colorado, has been implicated as a source of gas supersaturation in the drainage. Windy Gap Reservoir is also a potential point source of large numbers of triactinamyxons because of its shallow depth and silty bottom, which is an ideal habitat for *Tubifex tubifex*, the alternate host for *M. cerebralis*.

Methods

Data from two separate studies were combined to test my hypothesis. Data from one study were collected during routine fry population estimates, which were conducted at 2-week intervals in 1994 and 1995, and at 1-month intervals in 1996. Fry population estimates were performed using three pass removal techniques with a backpack electroshocker. Estimates were made at six sampling locations 2.1 to 25.8 kilometers downstream of Windy Gap Reservoir from the months of July through December in 1995,

and June through October in 1994 and 1996. Both rainbow and brown trout fry were examined for signs consistent with whirling disease, including blacktail, cranial deformities, and spinal deformities. A total of 2546 brown trout and 1459 rainbow trout were captured and examined in this manner over the three year period.

Data from the second study were collected as part of an investigation to locate potential sources of gas supersaturation in the drainage. Rainbow and brown trout fry were collected from 35 separate randomly chosen sampling locations from June through November of 1995, and from June through October of 1996. The study area extended from Granby Reservoir (12 kilometers upstream of Windy Gap Reservoir) down to 30 kilometers below Windy Gap Reservoir. Fry were captured by electrofishing with single pass removals. External signs associated with whirling disease and gas bubble trauma recorded included blacktail, spinal deformities, cranial deformities, deformed opercules and deformed lower jaws, and exopthalmia. A total of 514 brown trout and 197 rainbow trout fingerlings were examined over the 2-year period.

Data from both studies were combined into a single data set consisting of 1656 rainbow and 3060 brown trout. Presence or absence of any sign was used as the response variable. To determine if fish were significantly less affected with increasing distance downstream of Windy Gap Reservoir, I took into account variation due to monthly, yearly, and species differences. A set of 12 logistic regression models were created using river kilometer, month, year, fish species, and likely two-way interactions. A model was selected using AIC (Akaike's Information Criterion) (Akaike 1973) that best fit the data without over-parameterization. Data from sampling points above Windy Gap Reservoir were eliminated from this analysis to simplify the model.

Results

Raw data from the combination of the two studies resulted in a very high proportion of fish exhibiting deformities or clinical signs of disease (morbidity) in all three years of the studies (Figure 3.1). From 1994 to 1996, 60.4 (n = 1098), 56.6 (n = 290) and 62.6 % (n = 268) of rainbow trout were symptomatic. Brown trout also exhibited deformities or signs of disease in high ratios during all three years. Averages of 17.3 (n = 1151), 28.5 (n = 918), and 31.2 % (n = 911) of brown trout were symptomatic in 1994, 1995, and 1996.

Monthly data reflected the course of *M. cerebralis* infection among newly infected fish (Figure 3.2), with a low proportion of fish exhibiting signs early in the sampling months, followed by a rapid increase in the fall. A slight decrease in proportions of fish with signs were observed in the winter months, perhaps due to a die-off of highly infected fish during the stressful overwintering period. The raw data also suggest a decrease in proportions of symptomatic fish as a function of distance downstream of Windy Gap Reservoir.

Parameters fitted to the logistic regression model included species of fish ($P < 0.001$) and month of capture ($P < 0.001$) as class variables, with year of capture ($P < 0.001$) and kilometer downstream of Windy Gap Reservoir ($P < 0.001$) as continuous variables. Monthly data were fit as a class variable because no smooth function could be fit to the data that would explain the month to month variation as well as considering each month individually. This was partially due to the small sample sizes of rainbow trout in the fall and winter months of the studies. Significant interaction effects were also found for species by year of sampling ($P < 0.001$), and species by month of sampling ($P < 0.001$). The significant decrease in symptomatic fish with distance downstream of Windy Gap Reservoir strengthened suspicions of an association of loss of

rainbow trout recruitment with the reservoir (Figure 3.3).

Discussion

Results of this study indicate that morbidity among rainbow and brown trout in the Colorado River does not follow a random pattern. Rather, it is influenced by temporal effects, and distance downstream from a man-made source of gas supersaturation -- Windy Gap Reservoir. Whirling disease has long been associated with disturbed habitat and effects due to human alterations. While Windy Gap Reservoir produces elevated gas saturations, it is quite possible that Windy Gap Reservoir acts as a source of triactinomyxon production due to its shallow depth and silty bottom. Ongoing studies into the peak releases of triactinamyxons in this system will provide conclusive statements about quantity of infective units released from the reservoir, and help identify cause and effect relationships. The concept of point sources as a factor influencing *M. cerebralis* infectivity should be seriously considered by researchers dealing with high rates of infectivity or declines in trout populations due to *M. cerebralis*. Reduction of the parasite to a manageable level in these systems may be dependent on control of infectivity at these sources.

The possible interaction effect of *M. cerebralis* and environmental stressors leading to higher than expected mortalities among rainbow trout remains a question of great interest. The role of gas supersaturation as a factor causing high levels of morbidity and mortality among rainbow trout young-of-the-year was yet undefined. In the following chapter, effect of gas supersaturation was tested in a laboratory setting to determine if elevated saturation levels reduced the survival of *M. cerebralis* infected fish.

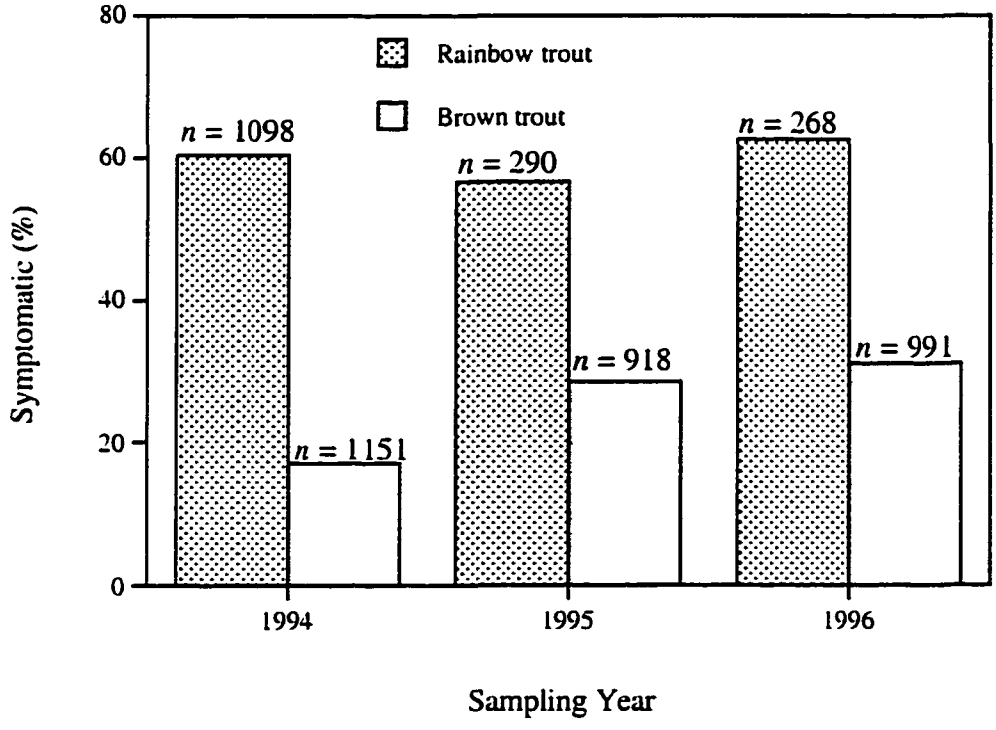

Figure 3.1. Proportions of symptomatic rainbow and brown trout young-of-the-year in the Upper Colorado River for the 1994 - 1996 sampling years.

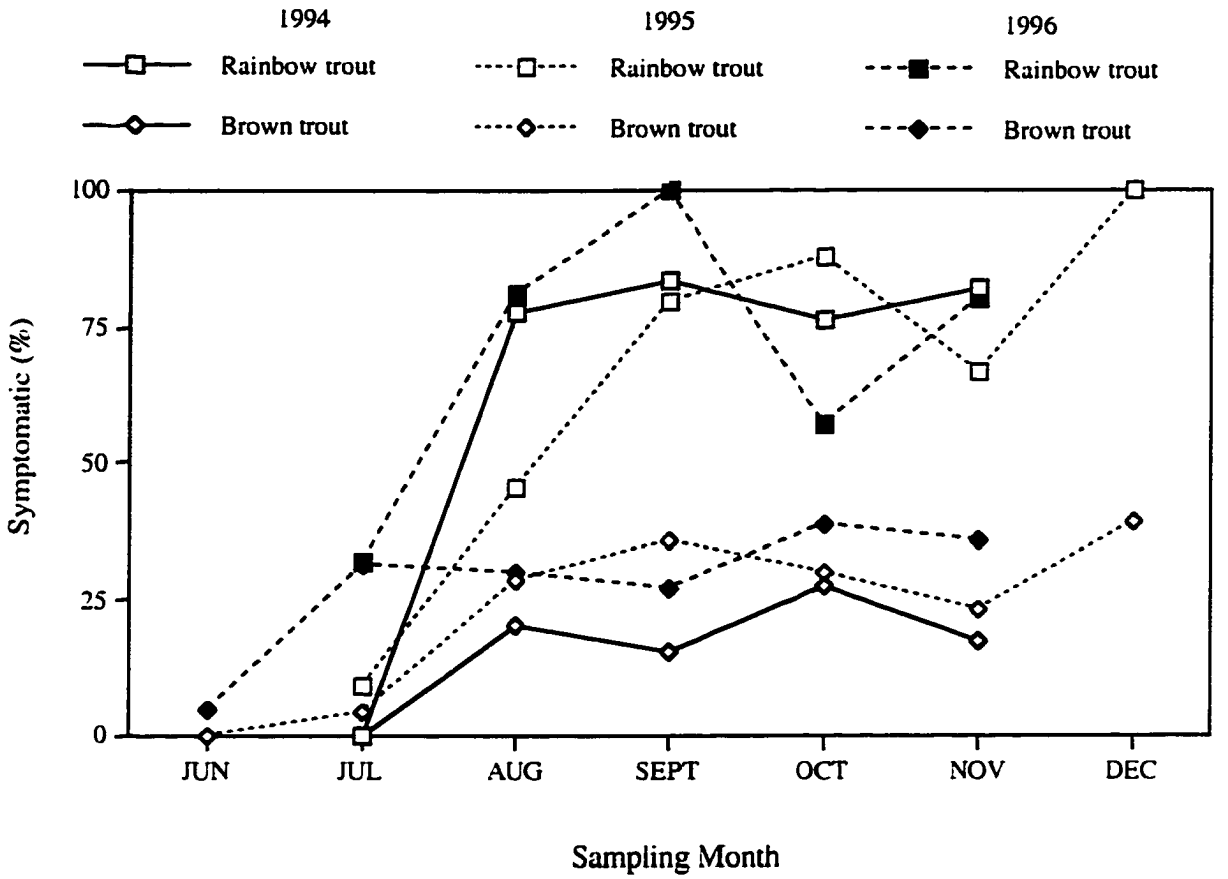

Figure 3.2. Yearly and monthly percentages of symptomatic rainbow and brown trout young-of-the-year in the Upper Colorado River for the 1994 - 1996 sampling years.

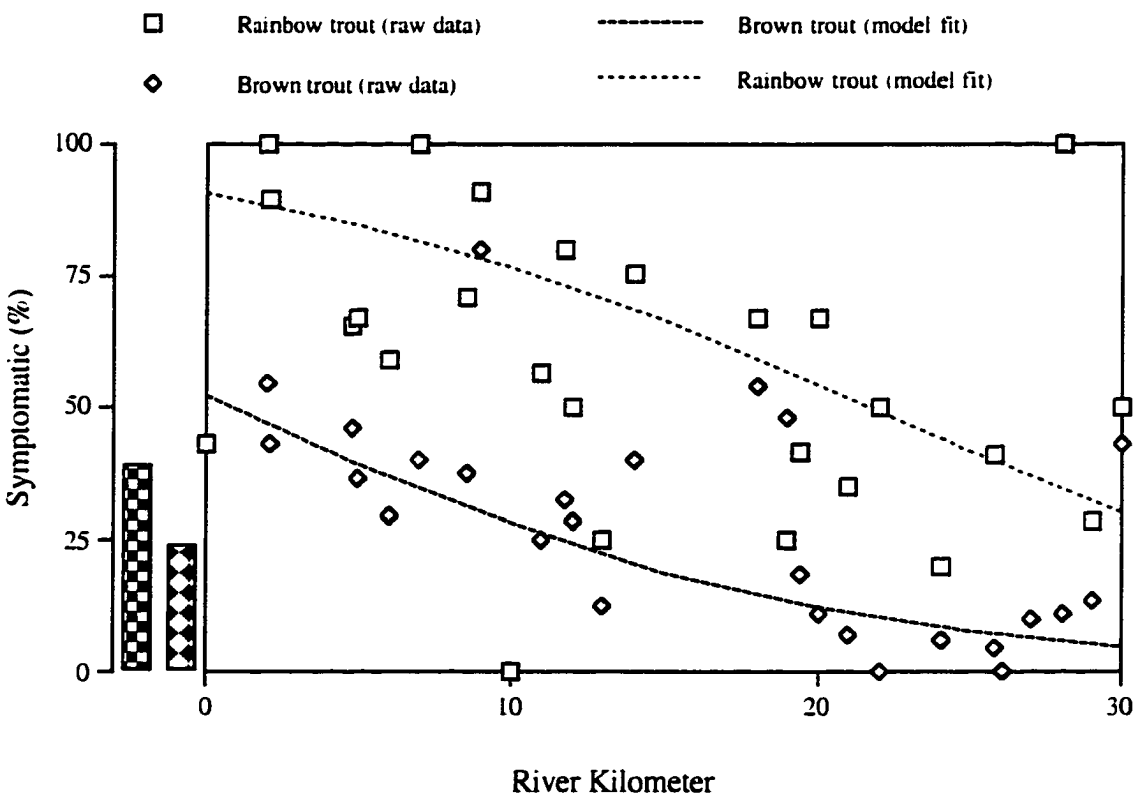

Figure 3.3. Raw data and model fit of percentage of symptomatic rainbow and brown trout young-of-the-year as a function of distance downstream of Windy Gap Reservoir on the Upper Colorado River. Bars on the left side of the graph represent percentages of symptomatic rainbow (box pattern) and brown trout (diamond pattern) above Windy Gap Reservoir.

References

Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. *Second International Symposium on Information Theory*, B. N. Petrov and F. Csaki (eds), 267-281. Budapest: Akademiai Kiado.

Halliday, M. M. 1976. The biology of *Myxosoma cerebralis*: The causative organism of whirling disease in salmonids. Journal of Fish Biology. 9: 339-357.

O'Grodnick, J. J. 1975 Whirling disease (*Myxosoma cerebralis*) spore concentration using the continuous plankton centrifuge. Journal of Wildlife Diseases. 11:54-57.

CHAPTER 4:

Evaluation of Chronic Gas Supersaturation on Growth, Morbidity, and Mortality of Fingerling Rainbow Trout Infected with *Myxobolus cerebralis*

Average gas supersaturation levels of 110% have been recorded in the Colorado River (Chapter 3) and the Gunnison River (Nehring and Thompson 1996), with occasional peaks of supersaturation approaching 115%. Young-of-the-year rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) sampled from the Upper Colorado River in 1994 displayed signs of disease that can occur in juvenile salmonids exposed to chronic low levels of supersaturation including exopthalmia, spinal deformities, and discoloration. Gas bubbles were also observed in the gill filaments and in the kidneys of some fish.

Gas saturation even a few percentage points above 100% has been known to be detrimental to newly hatched fry (Dennison and Marchyshyn 1973). Other signs of gas bubble trauma (GBT) reported between 100 and 110% supersaturation include overinflation of the swim bladder among small fish (Shrimpton et al. 1989), ocular lesions, immunosuppression, reduced growth, bubbles in the intestinal tract (National Marine Fisheries Service 1995), loss of swimming ability (Schiewe 1974), and even death (Bouck 1976). The reported threshold between chronic and acute GBT occurs somewhere between 108 and 110% TGP (Jensen et al. 1986). However, the United States Environmental Protection Agency criterion suggested as a safe upper limit for aquatic life is 110% (USEPA 1976). This criterion is supported by numerous studies indicating that acute gas saturations below 110% do not significantly affect survival of fish (Dawley and Ebel 1975; Nebeker et al. 1980; Jensen 1988). The majority of studies conducted in the past have investigated the effect of acute high levels of supersaturation, usually greater than 115%. Effects of chronic low levels of supersaturation on salmonids have been largely ignored.

This study was initiated to determine the effects of chronic gas supersaturation on *M. cerebralis*-infected fingerling trout. To eliminate the effects of other environmental

stressors that may occur in natural systems, a laboratory experiment was designed to test the hypothesis that chronic low levels of gas supersaturation compromise the growth and survival of fingerling rainbow trout infected with *M. cerebralis*.

Methods

A battery of twenty-four, 96-liter aquaria was set up in the Aquatic Toxicology Laboratory at the Colorado Division of Wildlife Research Station in Fort Collins, Colorado. Cooled well water (12.5 °C) was mixed under pressure with atmospheric gasses to produce water saturated at 118%. The water was then mixed in 300 L headboxes with unsaturated well water to produce total gas saturations of 110% (O_2 = 103%, N_2 + Ar = 112%), 107.5% (O_2 = 103%, N_2 + Ar = 108%), 105% (O_2 = 97%, N_2 + Ar = 107%), and 100 - 102.5% (O_2 = 91.6 - 95%, N_2 + Ar = 102.3 - 103%) total dissolved gas. Saturations were chosen based on saturation levels observed in the upper Colorado River, which ranged from 99.3% (Δ P = -4) to 113.4% (Δ P = 77) below Windy Gap Reservoir during the months of June through October, 1995. Saturations were monitored with Common Sensing TBO saturometers throughout the entire experiment to ensure consistent saturation levels. Water was gravity-fed at a rate of 1 L/min to each aquarium, which would provide adequate flows to maintain 35 fish per aquarium. Three aquaria were designated for *M. cerebralis*-exposed fish, and three for unexposed fish at each saturation level.

Colorado River rainbow trout fry were obtained from the Glenwood Springs rearing unit, a *M. cerebralis*-negative facility, immediately after swim-up for use in the experiment. Number of infective units (triactinomyxons) and exposure time were decided upon by using data from yearling rainbow trout disease testing in the Colorado River. Of surviving yearling rainbow trout sampled, 83% were found to be infected, and myxospore loadings

averaged 71,250 per fish (Walker and Nehring 1995). Markiw (1992) found that infectivity rates and spore loadings of this magnitude could be produced by exposing fish to anywhere from 100 to 1000 infective units. Fry designated for the *M. cerebralis* treatment groups were all exposed at the same time to an estimated 100 to 500 triactinomyxons per fish for 3 hours in standing water to ensure infection. Both the treatment and control groups were held for 10 d after the treatment fish were infected to allow fish to recover from handling. After the 10-d recovery period, 35 fish were placed into individual aquaria five fish at a time, to ensure random assignment of fish to aquaria.

Fish were fed several times daily using guidelines in Piper et al. (1982), and mortalities were removed daily throughout the entire 5 months of the experiment. Mid-way through the experiment (10 weeks post-exposure) three fish from each aquarium were removed for evaluation. These fish were euthanized individually with an overdose of tricaine methanesulphonate (MS-222), weighed, measured, and examined for external signs of whirling disease and gas bubble trauma. Lengths and weights were compared to determine if there were any growth effects due to different treatments. Morbidity data consisted of proportions of fish exhibiting abnormalities, including; cranial deformities, blacktail or discoloration, spinal deformities, deformed opercules, deformed lower jaws, and exopthalmia in each test group. Because of its transient nature, whirling behavior was not recorded in the morbidity data. All four gill arches from the right side of the fish were removed, and examined under a compound microscope (40X) for presence and severity of gas emboli in the gill filaments. Ten filaments from each gill arch were categorized as either containing emboli or not containing emboli. Ratio of filaments containing emboli to those not containing emboli was calculated for each fish in this manner.

Mortalities were evaluated at the end of the experiment to determine if exposure to chronic gas supersaturation and *M. cerebralis* infection affected survival. A sample of three

fish from the remaining surviving fish in each aquarium were weighed, measured, and examined for external signs of gas bubble trauma and whirling disease in a similar manner as in the mid-experiment evaluations. Two-factor analysis of variance (ANOVA) with aquaria used as experimental units was used to compare the mortalities, weights, lengths, proportion of gill filaments containing gas emboli, and numbers of signs of disease in the experiment. Four additional fish from each of the aquaria containing *M. cerebralis* exposed fish were sacrificed for myxospore evaluations. Evaluations were conducted using the pepsin-trypsin digest method described by Markiw and Wolf (1974). An ANOVA was conducted to determine if myxospore numbers were affected by gas saturation level.

Results

Overt signs of whirling disease were apparent in some fish as early as 1 month post-exposure to *M. cerebralis*. Clinical signs of disease were observed in the majority of *M. cerebralis*-exposed fish by 7 weeks post-exposure. A small proportion of fish in the *M. cerebralis*-unexposed groups exhibited signs of disease (Figure 4.1). We later discovered that the fish used in this experiment had been exposed to *Flavobacterium psychrophilum*, the causative agent of bacterial coldwater disease, prior to arrival at our facility. A sub-sample of fish taken from each aquarium at the end of the experiment tested positive for the presence of the organism. Signs of disease observed in the *M. cerebralis*-negative groups were likely caused by bacterial coldwater disease. Average frequency of signs of disease was 0.14 (SD = 0.22) per fish among fish not exposed to *M. cerebralis*. Fish exposed to *M. cerebralis* averaged 1.9 (SD = 0.68) signs per fish. Because of the non-uniformity of variances observed between fish exposed and fish not exposed to *M. cerebralis*, an arcsin square-root transformation was conducted before analysis.

During the first morbidity evaluation event, frequency of signs of disease was significantly affected by exposure to *M. cerebralis* ($F = 79.23$; df = 1, 16; $P = 0.0001$). Gas supersaturation ($F = 0.34$; df = 3, 16; $P = 0.7979$) and interaction effects ($F = 0.47$; df = 3, 16; $P = 0.7078$) were not significant. The most common signs of disease included blacktail, exopthalmia, and cranial deformities. No overt external signs of gas bubble trauma such as gas emboli on the skin or orbit of the eyes were observed among the fish held at any saturation level during the first evaluation.

Mean lengths and weights of fish were not noticeably affected by either *M. cerebralis* infection or by gas saturation level at 10 weeks post-exposure (Figure 6.2). The ANOVA resulted in non-significant effects due to *M. cerebralis* exposure for both length ($F = 0.56$; df = 1, 16; $P = 0.4648$) and weight ($F = 0.03$; df = 1, 16; $P = 0.8654$). Saturation level also had a non-significant effect on length ($F = 0.52$; df = 3, 16; $P = 0.6727$), and weight ($F = 0.97$; df = 3, 16; $P = 0.4309$). Interaction effects with *M. cerebralis* exposure were non-significant for length ($F = 0.65$; df = 3, 16; P = 0.5938), and weight ($F = 0.50$; df = 3, 16; $P = 0.6904$).

Averages of 15.0 to 46.1 % of examined gill filaments contained gas emboli after 10 weeks (Table 4.1). However, proportions of gill filaments with gas emboli were not significantly affected by *M. cerebralis* infection ($F = 2.14$; df = 1, 16; $P = 0.1630$), or gas saturation level ($F = 2.04$; df =3, 16; $P = 0.1485$), and no significant interaction effect was observed ($F = 1.38$; df = 3, 16; $P = 0.2840$).

At 10 weeks post-exposure, mortality among fish exposed to *M. cerebralis* was higher than unexposed fish at all saturation levels, except 100 - 102.5% saturation (Figure 4.3). Average mortality among fish exposed to *M. cerebralis* was 19.7%, while those not exposed averaged 14.7%. A significant effect due to *M. cerebralis* infection was found ($F = 4.89$; df = 1, 16; $P = 0.042$), while neither saturation level ($F = 2.70$; df = 1, 16;

$P = 0.0804$) nor interaction effects were significant ($F = 2.04$; df = 3, 16; $P = 0.1485$).

Frequency of signs of disease at the end of the experiment were much higher among fish exposed to *M. cerebralis* than those not exposed (Figure 4.4). Virtually all fish in the *M. cerebralis* exposed groups exhibited at least one sign of disease at the end of the experiment. An average of 1.78 (SD = 0.62) signs per fish were found among fish exposed to *M. cerebralis*, and 0.11 (SD = 0.22) signs per fish among fish not exposed to *M. cerebralis*. Data were transformed (square-root) because of non-uniform variances. The raw data and ANOVA results indicate signs of disease were affected by *M. cerebralis* infection ($F = 99.85$; df = 1, 16; $P = 0.001$), but not by saturation level ($F = 0.60$; df = 3, 16; $P = 0.6239$). Interaction effects were also non-significant ($F = 0.96$; df = 3, 16; $P = 0.4352$). Reactions to the gas saturations used in this experiment varied from fish to fish. External signs of gas bubble trauma such as severe exopthalmia and obvious bubble formation on the skin in the orbit of the eyes occurred only in a few individual fish in the highest saturation level.

The percentage of gill filaments containing gas emboli averaged from 3.5 to 26.7 % at the end of the experiment (Table 4.1). The interaction effect of saturation level and *M. cerebralis* exposure on emboli was not significant ($F = 3.02$; df = 3, 16; $P = 0.0602$), although a high proportion of filaments contained emboli among *M. cerebralis* exposed fish at 110% saturation. Neither saturation level ($F = 0.57$; df = 3, 16; $P = 0.6403$) nor *M. cerebralis* exposure ($F = 0.19$; df = 1, 16; $P = 0.6692$) had significant effects on proportions of gill filaments containing emboli.

Average weights and lengths of fish at the end of the experiment were lower among fish exposed to *M. cerebralis* at every level of gas saturation tested (Figure 4.5). Average weight was 4.7 g (SD = 1.11) for fish exposed to *M. cerebralis*, and 6.73 g (SD = 1.02) for fish not exposed to *M. cerebralis*. Average length was 76.1 mm (SD = 6.2) for fish

exposed to *M. cerebralis* and 85.3 mm (SD = 4.8) for fish not exposed to *M. cerebralis*. No obvious trend in lengths and weights occurred among saturation levels. The ANOVA resulted in significant effects due to *M. cerebralis* exposure on both length (F = 15.51; df = 1, 16; P = 0.0012) and weight (F = 17.25; df = 1, 16; P = 0.0007) of the fish. Non-significant effects for gas saturation level were observed for length (F = 0.43; df = 3, 16; P = 0.7357) and weight (F = 0.30; df = 3, 16; P = 0.8263). Interaction effects were non-significant for length (F = 0.97; df = 3, 16; P = 0.4328), and weight (F = 0.39; df = 3, 16; P = 0.7622).

Estimated numbers of mature spores varied widely among the fish evaluated. Spore counts averaged 578,578 (SD = 544,675) among all fish evaluated, and ranged from 6,667 to 2,826,667 (Table 4.2). Because of the non-uniform variation in spore counts, a log-transformation was used before analysis. A 1-factor ANOVA resulted in non-significant differences in spore counts among saturation levels (F = 1.11 df =3, 8 P = 0.3266). A sub-sample of one fish from each aquarium containing fish not exposed to *M. cerebralis* was submitted for spore counts as a negative control. No spores were found in any of the fish not exposed to *M. cerebralis*.

At the end of the experiment, mortalities were higher in the *M. cerebralis* exposed groups at every saturation except 110%. This contributed to the interaction effect between saturation level and *M. cerebralis* infection on mortality (F = 2.78; df = 3, 16; P = 0.0750). Saturation level was not found to be a significant contributor to mortality when tested with the ANOVA (F = 1.58; df = 3, 8; P = 0.2326). Average mortality at the end of the experiment was 28.1% for *M. cerebralis* exposed fish and 22.9% for fish not exposed to *M. cerebralis*. The difference in mortality between *M. cerebralis* exposed fish and fish not exposed to *M. cerebralis* was significant (F = 4.27; df =1, 16; P = 0.0553).

Discussion

The results of this study indicate that chronic gas saturation up to 110% does not have a significant effect on morbidity or mortality of fingerling rainbow trout infected with *M. cerebralis*. The limited effect of the increased gas supersaturations supports the notion that 110% saturation is a safe upper limit for salmonids under the conditions of this study.

The results of this experiment indicate that growth of fingerling rainbow trout is significantly affected by *M. cerebralis* infection, but not significantly affected by changes in gas saturation levels from 100 to 110%. Fish infected with *M. cerebralis* in this study appeared to be less efficient feeders. Noticeably more waste products and uneaten food were observed in the bottoms of the aquaria containing *M. cerebralis* infected fish. *M. cerebralis* exposed fish would often exhibit whirling behavior during feeding, resulting in fewer food particles being consumed. *M. cerebralis* exposed fish searched less actively for uneaten food particles after the initial feeding than fish not exposed to *M. cerebralis*. Energy expended during the whirling episodes may also result in a higher metabolic rate, in turn resulting in larger amounts of waste products.

Signs of disease were strongly affected by *M. cerebralis* infection, but not by gas supersaturation. Overt signs of gas bubble trauma did not appear until after 14 weeks of exposure to 110% saturated water. Certain individual fish developed overt signs of gas bubble trauma, while other fish residing in the same aquarium appeared to be normal. Individual fish resistance seems to play a large part in their tendency to develop gas bubble disease.

The presence of bubbles in the gill filaments of fingerling trout was not a good indicator of gas bubble trauma in this experiment. Other authors (Walker and Nehring 1995; Maule et al. 1997) have reported highly variable association between presence of bubbles in gill filaments and the occurrence of gas bubble trauma. An observation that may

explain this variation is the tendency for air to be drawn into the severed end of the gill filaments by capillary action. This would also explain higher numbers of gill filaments containing gas bubbles in the first examination than in the second. Fish were much smaller during the first examination. so relatively more damage occurred to the filaments during excision.

Myxospore loads were not affected by gas saturation level. Although all fish were exposed to the same number of triactinomyxons at the same time, spore counts were quite variable from fish to fish within the same treatment groups. Variability of myxospore load could be affected by numbers of triactinomyxons successfully attacking individual fish. However, as with the onset of signs of gas bubble trauma, total myxospore load appeared to be largely determined by individual fish resistance.

While gas saturation did not significantly affect growth, morbidity or mortality in this experiment, *M. cerebralis* had strong affects on all of these parameters under the conditions of this study. Results of this experiment indicate that gas saturations found in the Upper Colorado River may not be severe enough to explain the total loss of rainbow trout year classes in the river. Factors such as reduced fitness of *M. cerebralis* infected fish are likely affecting the survival of fingerling rainbow trout to a greater degree than gas supersaturation. Altered feeding and predator avoidance behaviors could greatly affect the survival of *M. cerebralis* infected fish in natural systems. Additional experiments should be conducted to determine how these factors affect survival of infected fish.

Because infection by *Flavobacterium psychrophilum* may have compromised the results of this experiment, the decision was made to conduct further testing on gas supersaturation effects. The following chapter describes a second laboratory study designed to test *M. cerebralis* and gas supersaturation effects along with additional stressors of elevated water temperature and *F. psychrophilum* infection.

Table 4.1.- Percentages of rainbow trout fingerling gill filaments containing gas bubbles after 10 and 22 weeks of exposure at four different levels of gas supersaturation.

	10-WEEKS OF EXPOSURE			
	M. cerebralis exposed		Not exposed to *M. cerebralis*	
Saturation (%)	Mean	SD	Mean	SD
<102.5	36.7	19.7	40.1	16.6
105.0	21.4	8.5	30.0	16.9
107.5	16.9	17.7	46.1	12.8
110.0	20.6	14.3	15.0	12.4
	22-WEEKS OF EXPOSURE			
<102.5	10.6	5.3	20.0	20.0
105.0	8.1	6.0	8.3	5.2
107.5	8.9	5.0	15.3	13.2
110.0	26.7	14.0	3.5	1.3

Table 4.2.- Myxospore loadings (in thousands) for *M. cerebralis* exposed fingerling rainbow trout held at four different levels of gas supersaturation for 22 weeks.

SATURATION (%)	NUMBER OF FISH	MEAN	SD	MINIMUM	MAXIMUM
<102.5	4	1001	1268	67	2826
	4	813	432	488	1412
	4	695	286	270	880
105.0	4	324	246	129	668
	4	1335	768	351	2088
	4	199	338	7	705
107.5	4	512	481	174	1223
	4	287	220	55	586
	4	328	205	91	592
110.0	4	465	287	197	844
	4	480	368	97	985
	4	498	174	327	716

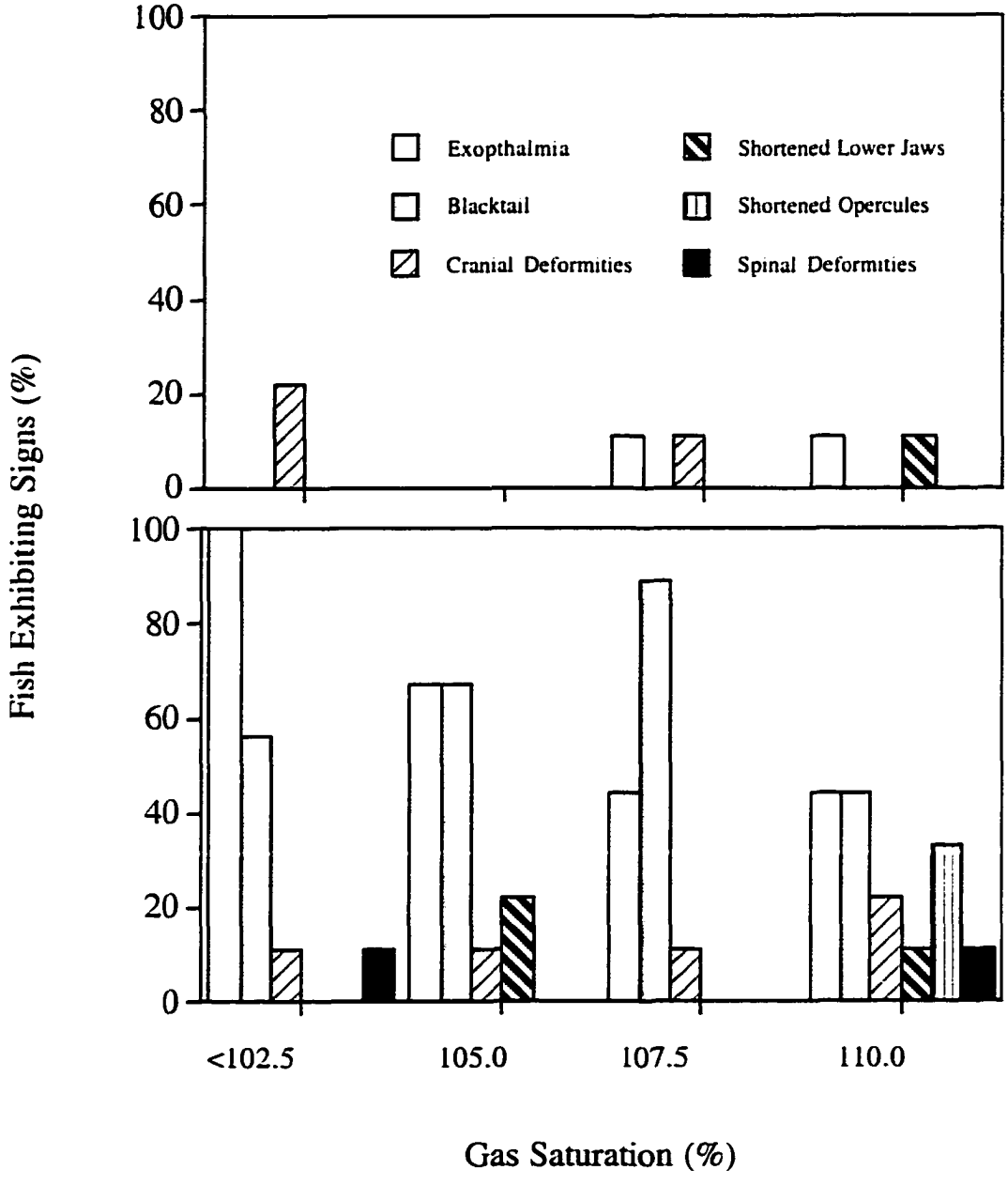

Figure 4.1. - Percentage of fingerling rainbow trout not exposed (a) and exposed (b) to *M. cerebralis* exhibiting signs of disease after 10 weeks at four gas supersaturation levels.

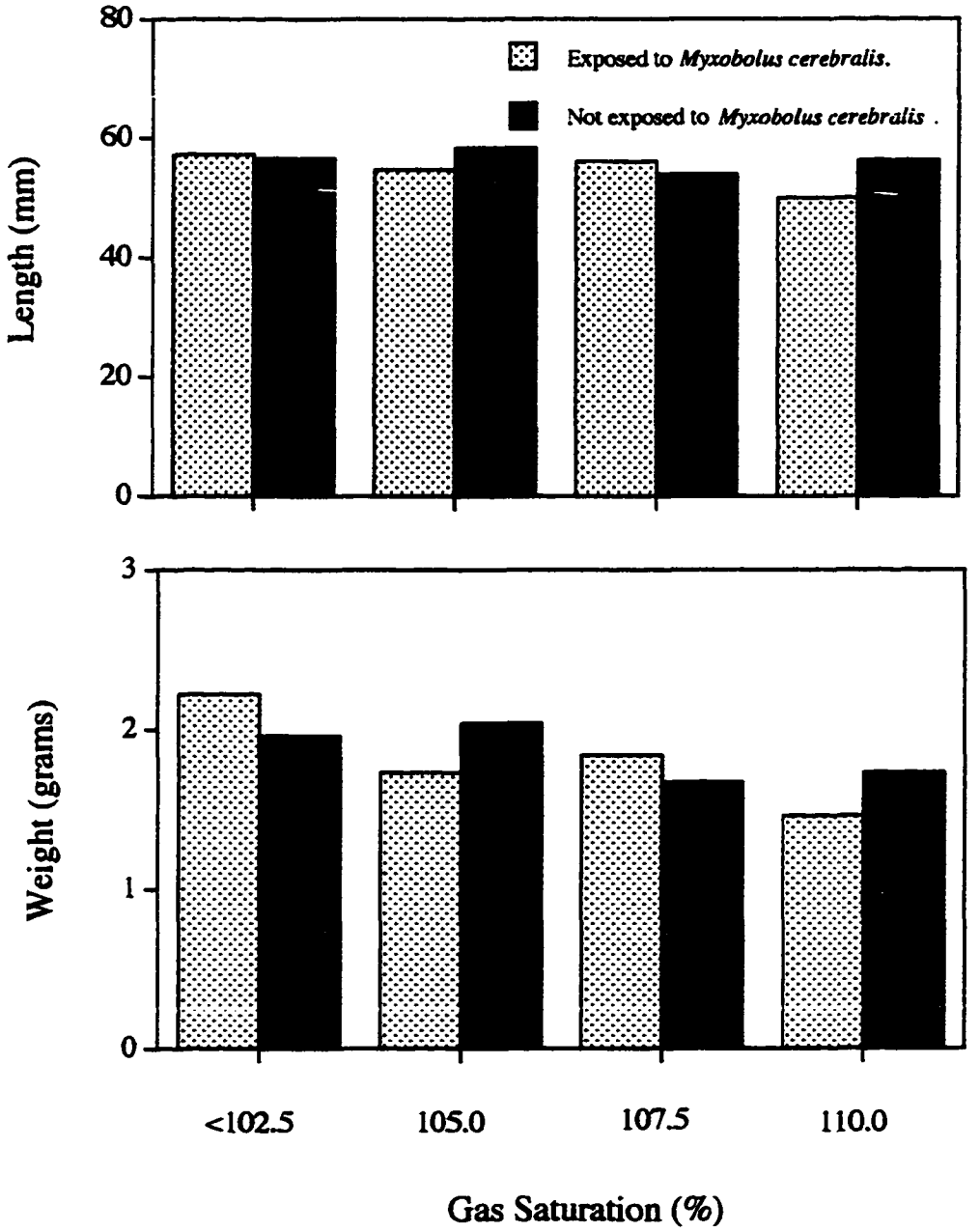

Figure 4.2. - Mean lengths (a) and weights (b) for rainbow trout fingerlings, exposed and not exposed to *M. cerebralis* and held at four different gas saturation levels for 10 weeks.

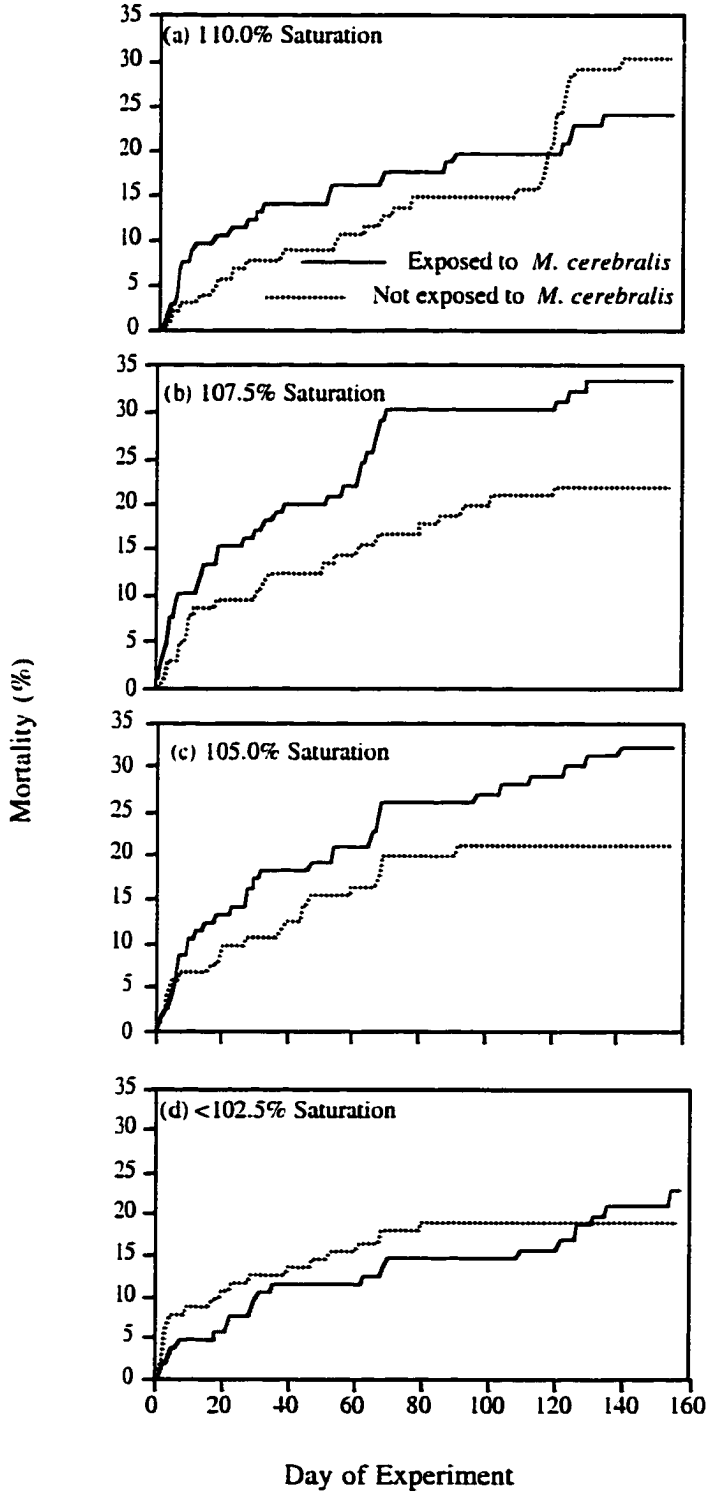

Figure 4.3. - Cumulative mortalities of fingerling rainbow trout exposed and not exposed to *M. cerebralis* and held in four levels of saturated water at 12.5 ° C for 160 days.

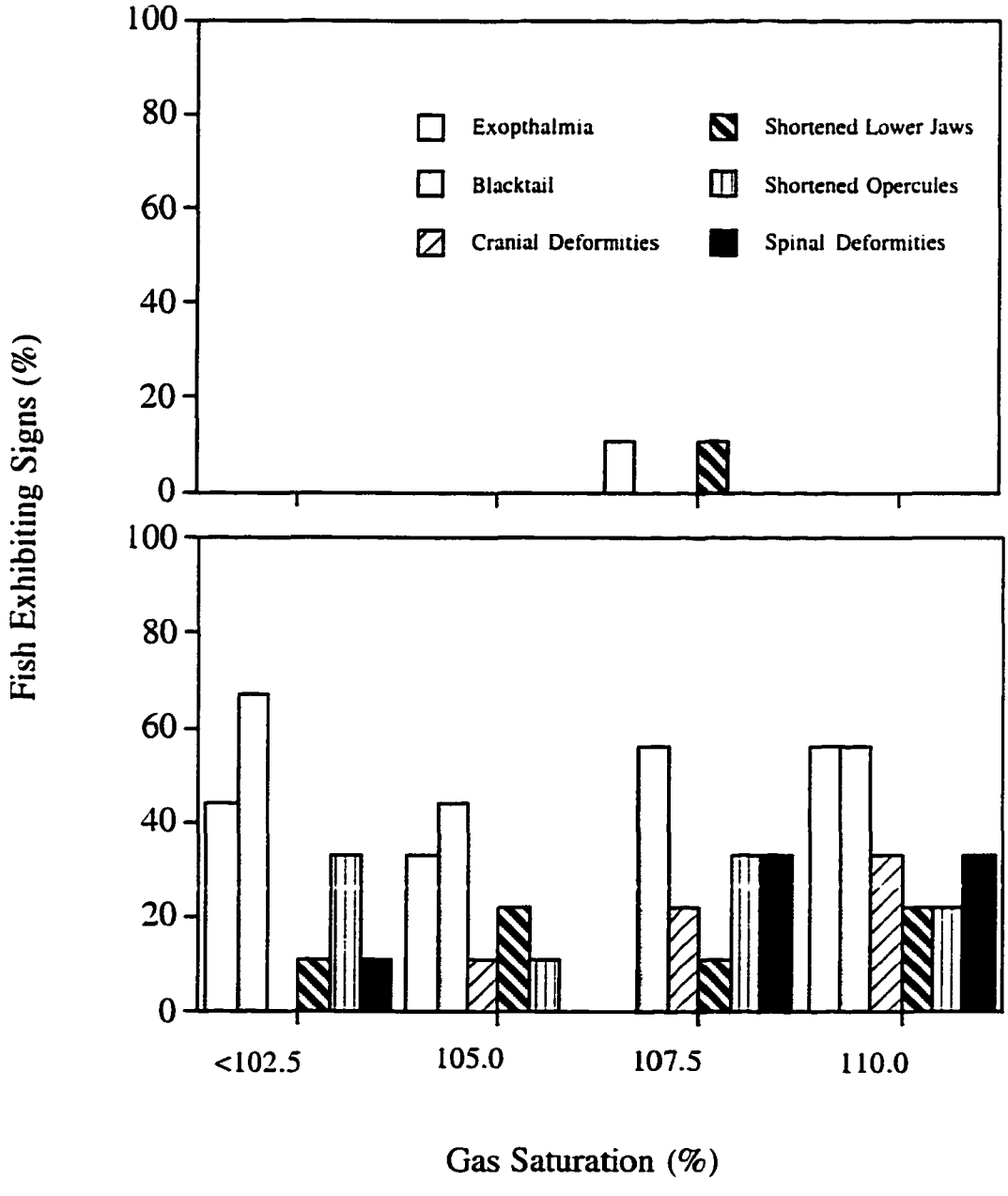

Figure 4.4. - Percentage of fingerling rainbow trout not exposed (a) and exposed (b) to *M. cerebralis* exhibiting signs of disease after 22 weeks at four gas supersaturation levels.

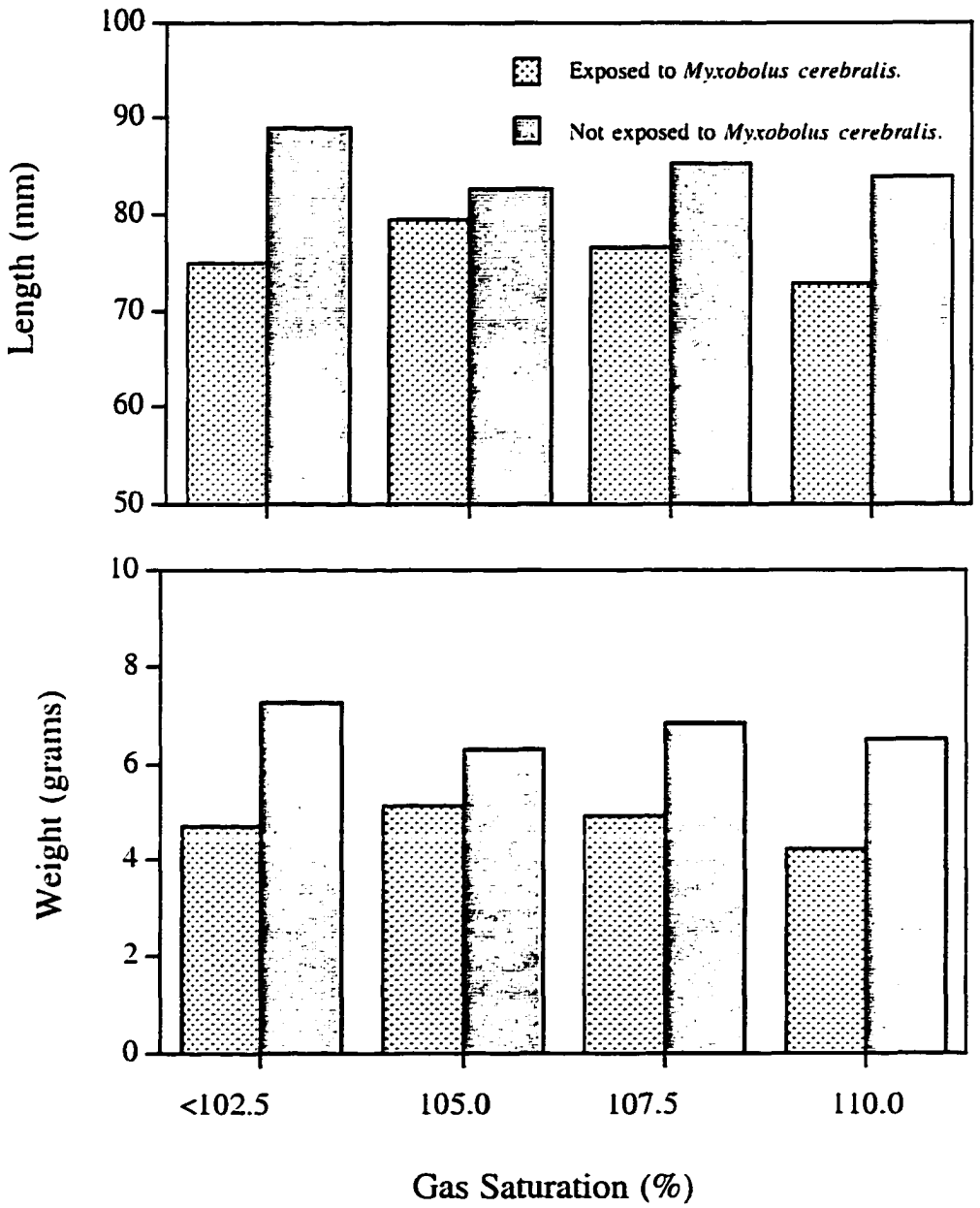

Figure 4.5. - Mean lengths (a) and weights (b) for rainbow trout fingerlings, exposed and not exposed to *M. cerebralis* and held at four different gas saturation levels for 22 weeks.

References

Bouck, G. R. 1976. Supersaturation and fishery observations in selected alpine Oregon streams. Pages 37-40 In *Gas bubble disease*. D. H. Flikeisen and M. J. Schneider, editors. Technical Information Service, U. S. Dept. of Commerce. Springfield, Virginia.

Dawley, E. M., and W. J. Ebel. 1975. Effects of various concentrations of dissolved atmospheric gas on juvenile chinook salmon and steelhead trout. Fishery Bulletin 73:787-796.

Dennison, B. A., and M. J. Marchyshyn. 1973. A device for alleviating supersaturation of gasses in hatchery water supplies. The Progressive Fish-Culturist 35:55-58.

Jensen, J. O. T., J. Schnute, and D. F. Alderice. 1986. Assessing juvenile salmonid response to gas supersaturation using a general multivariate dose-response model. Canadian Journal of Fisheries and Aquatic Science 43:1694-1709.

Jensen, J. O. T. 1988. Combined effects of gas supersaturation and dissolved oxygen levels on steelhead trout (*Salmo gairdneri*) eggs, larvae, and fry. Aquaculture 68:131-139.

Markiw, M. E. 1992. Experimentally induced whirling disease. I. Dose response of fry and adults of rainbow trout exposed to the triactinomyxon stage of *Myxobolus cerebralis*. Journal of Aquatic Animal Health 4: 40-43.

Maule, A. G., M. G. Mesa, K. M. Hans, J. J. Warren, and M. P. Swihart. 1997, Gas bubble trauma monitoring and research of juvenile salmonids. 1995 Annual Report. U. S. Geological Survey. Biological Resources Division, Columbia River Research Laboratory, Cook, Washington.

National Marine Fisheries Service. 1995. Report and Recommendations of the Second Working Group Meeting Panel on Gas Bubble Disease. Northwest Fisheries Science Center. Seattle, Washington.

Nebeker, A. V., A. K. Hauck, F. D. Baker, and S. I. Weitz. 1980. Comparative responses of speckled dace and cutthroat trout to air-supersaturated water. Transactions of the American Fisheries Society 109:760-764.

Nehring, B. R. and K. G. Thompson. 1996. Stream fisheries investigations: Federal Aid in Fish and Wildlife Restoration Job Progress Report, Project F-237R-3. Colorado Division of Wildlife Fish Research Section. Fort Collins, Colorado.

Piper, R. G., and five coauthors. 1982. United States Department of the Interior, Fish and Wildlife Service, Washington, D. C.

Shrimpton, J. M., D. J. Randall and L. E Fidler. 1989. Assessing the effects of positive buoyancy on rainbow trout (*Oncorhynchus mykiss*) held in gas supersaturated water. Canadian Journal of Zoology 68:969-973.

Schiewe, M. H. 1974. Influence of dissolved atmospheric gas on swimming performance of juvenile chinook salmon. Transactions of the American Fisheries Society 103: 717-721

United States Environmental Protection Agency. 1976. Quality criteria for water. EPA-440/9-76-023. United States Government Printing Office, Washington, District of Columbia, USA.

Walker, P. G. and R. B. Nehring. 1995. An investigation to determine the cause(s) of the disappearance of young wild rainbow trout in the upper Colorado River, in Middle Park, Colorado. Colorado Division of Wildlife, Brush, Colorado.

Weitkamp, D. E. and M. Katz. 1980. A review of dissolved gas supersaturation literature. Transactions of the American Fisheries Society 109: 659-702.

CHAPTER 5:

The Effects of Multiple Stressors on the Survival of Fingerling Rainbow Trout Infected with *Myxobolus cerebralis*

Myxobolus cerebralis, the causative agent of salmonid whirling disease, has been implicated in partial or complete year class losses of rainbow trout (*Oncorhynchus mykiss*) in the Upper Colorado, Gunnison, Rio Grande, South Platte, and Cache la Poudre rivers in Colorado, and the Madison River in Montana. The pathogen, until recently, has not been considered a threat to wild salmonid populations. However, most current knowledge regarding *M. cerebralis* is related to fish culture situations and very little is known about this disease in wild fish populations.

The occurrence of year-class losses of rainbow trout has changed the long-held belief of the refractory behavior of wild fish populations to *M. cerebralis* quite drastically. Yet rivers such as the Big Thompson in Colorado that have tested positive for *M. cerebralis* for several years continue to maintain high levels of rainbow trout recruitment. The inconsistent response of wild rainbow trout populations to *M. cerebralis* exposure raises the possibility that other factors may be contributing to year-class losses of rainbow trout in *M. cerebralis*-positive environments.

The presence of an additional stressor or stressors is one explanation for situations where population level effects occur when *M. cerebralis* is present. Three stressors have been identified that may be acting on wild fish populations in the Upper Colorado River in addition to *M. cerebralis* . These include elevated water temperature, bacterial pathogens, and gas supersaturation.

Water temperature is an important physiological element for fish and has been implicated as a stress factor (Wedemeyer 1973). Elevated water temperature has been shown to increase the virulence and maturation rate of *Myxobolus cerebralis*. Halliday (1976) stated that the optimum temperature range for growth of the parasite is between 15 and 17° C. Higher metabolic rate of both the fish and the parasite may contribute to this effect. At higher temperatures (20 - 23° C) the pathogen reportedly begins to lose its

virulence (Schaperclaus 1931; El-Matbouli et al. 1998). The triactinomyxon stage is very short-lived at high temperatures, surviving only two to three days at temperatures exceeding 19° C (Markiw 1992). Water temperatures in the Colorado River appear to be ideal for *M. cerebralis* growth (Figure 5.1). I hypothesized that these optimum temperatures may also result in higher mortality among fingerling trout infected with the parasite, and could help explain the loss of year-classes in some Colorado rivers.

Gas supersaturation is a stressor commonly found in hatchery environments, which can result in deleterious effects to young fish. Gas supersaturations of up to 110% occur in the Colorado (Chapter 2) and Gunnison rivers of Colorado (Nehring and Thompson 1996). Saturations of these levels have been reported to cause a variety of physiological problems for young fish ranging from reduced growth to death (Dennison and Marchyshyn 1973; Shrimpton et al 1989; National Marine Fisheries Service 1995; Schiewe 1974; Bouck 1976). Other studies have indicated that gas saturations up to 110% do not have a serious effect on survival of fish (Dawley and Ebel 1975; Nebeker et al. 1980; Jensen 1988).

Flavobacterium psychrophilum is a common soil and water bacterium that has been known to cause heavy mortality among salmonid fry (Lorenzen and Karas 1992; Lorenzen 1994). Several fish examined during routine fish health evaluations on the Upper Colorado River exhibited signs of disease that may be related to this pathogen (Walker and Nehring 1995). The results of a prior study investigating the effects of low levels of gas saturation on *M. cerebralis* infected rainbow trout may have been affected by the presence of this organism (Chapter 4). I hypothesized that *F. psychrophilum* may exacerbate the effects of *M. cerebralis*, and that its presence may also contribute to the loss of year-classes of rainbow trout in the Colorado River.

The focus of this study was to test all four of the individual factors simultaneously to identify the relative contributions of each to mortality, and determine if the addition of

more than one stress factor increases mortality of fingerling rainbow trout. This information would help us determine if any or all of these factors are contributing to the loss of rainbow trout year-classes in some Colorado rivers.

Methods

Certified disease-free eyed Erwin strain rainbow trout (*Oncorhynchus mykiss*) eggs were incubated in a 38-liter aquarium at 12.5° C with a flow of 3 liters of well water (pH 7.2, CaCO3 450 ppm) per minute. Treatment and control fish were placed in separate aquaria 2 days prior to swim-up as 18-day old sac fry. All sac fry in the treatment group were exposed to an average of 485 triactinomyxons per fish, the infective stage of *M. cerebralis*, for 3 hours. This exposure level was chosen based on previous experiments that resulted in medium to heavy infections of fingerling rainbow trout exposed to similar numbers of triactinomyxons. Twenty *M. cerebralis*-exposed fish were placed in each of 24 aquaria. Fish were distributed five at a time to ensure random allocation of fish to treatment groups. Twenty unexposed fish were placed in each of 24 additional aquaria in a similar manner. Flow rate to each aquarium was set at 0.5 liters per minute well water. Water temperatures were set at 12.5° C for the lower temperature treatment groups and 17° C for the higher temperature treatment groups. The lower temperature was based on average temperatures observed in early summer months in the Upper Colorado River. The higher temperature was chosen based on average late summer temperatures.

Gas saturations used in the experiment were chosen to test the upper bound of gas saturations found in Colorado rivers. Saturations were set at 100% for the nominal saturation groups, and 110% for the elevated saturation (supersaturation) groups. Nominal saturations were achieved by rigorously aerating water in 300-liter headboxes before it was delivered to the aquaria. Supersaturation was achieved by forcing atmospheric gasses

together with water in a VMG Industries In-line Oxygenator at a rate of 18 liters per minute of air and water. This produced saturations of approximately 118%. The water was then aerated in 300-liter headboxes to reduce supersaturation to 110% before it was gravity-fed into the aquaria.

F. psychrophilum positive groups were exposed to bacteria at a dosage of 99.4 x 10^6 colony forming units per aquaria 12 weeks after the beginning of the experiment. The culture used was isolated from Bellvue State Fish Hatchery, and is thought to have been the cause of a particularly serious epizootic occurring at that facility earlier in 1997. To prevent possible infection in treatment groups designated as *F. psychrophilum* negative, Terramycin (oxytetracycline) was added to the food of these fish at a rate of 2.5 grams of active ingredient per 45.35 kg of fish for 10 days, at 1-month intervals. Fish were fed daily using guidelines in Piper et al. (1982).

Mortalities were removed daily from each aquarium over the duration of the experiment. At the end of the experiment, signs of disease were recorded for all fish remaining in each aquarium. Deformities and clinical signs of disease recorded included blacktail, spinal deformities, cranial deformities, deformed mandibles, deformed opercula and exopthalmia. Four-factor analysis-of-variance (ANOVA) tests were conducted to determine if differences in mortality and morbidity existed due to the treatment effects used in the experiment. All 2, 3, and 4-way interactions were tested in the ANOVA. Analyses were conducted with both raw data and transformed data (arcsine-square root). The transformation did not affect interpretation of the results, so *P*-values reported are from the raw data analyses. Regression analysis was used to test if addition of multiple stress factors caused a subsequent increase in mortality and morbidity.

Results

Mortality

Mortality was strongly affected by exposure to factors tested in this study (Table 5.1). Cumulative mortality was higher ($P = 0.0002$) among *M. cerebralis* -exposed fish than among their unexposed counterparts. Average mortality across all treatment groups was 50.0% (SD = 20.6) for exposed fish and 25.8% (SD = 20.0) for unexposed fish. Mortality among fish across all treatment groups at 17.0° C (Mean = 49.79 %, SD = 24.6) was much higher ($P = 0.0002$) than those at 12.5° C (Mean = 26.0 %, SD = 20.3). Treatment groups with combinations of elevated temperature and *M. cerebralis* exposure or *F. psychrophilum* exposure experienced the most noticeable increases in mortality. Treatment groups exposed to *F. psychrophilum* suffered slightly higher mortality (Mean = 41.5 %, SD = 27.9) than those unexposed (Mean = 34.4 %, SD = 22.6) to the bacterium ($P = 0.2249$). A significant interaction effect was observed for *F. psychrophilum* and water temperature ($P = 0.0364$). Mortality among fish in water at 110% saturation (Mean = 38.9 %, SD = 26.5) was not noticeably different ($P = 0.7183$) than those in 100% saturated water (Mean = 36.9 %, SD = 24.7).

Although the only significant interaction effect was *F. psychrophilum* and water temperature, treatment groups with combinations of factors often experienced higher mortality. Regression analysis revealed a significant increase ($P = 0.0020$) in mortality with the addition of stress factors. Lack-of-fit-tests indicated that a linear model was adequate to described the increase of mortality with added stress factors. When no factors were present, average mortality was 11.7 %. When *M. cerebralis* was the only stress factor, mortality was 41.7%. *M. cerebralis* combined with one stress factor resulted in 40.5% mortality. Addition of two stress factors increased mortality to 55.6%. Addition of three stress factors elevated mortality to 70.0 % (Figure 5.2). The relationship of higher

mortality with addition of stress factors clearly illustrates the effect multiple stress factors can have on survival of trout.

Morbidity

Fish exposed to *M. cerebralis* began exhibiting clinical signs of disease within 60 days. By the end of the experiment, 97.7% (SD = 5.6) of surviving *M. cerebralis* exposed fish exhibited at least one sign of disease, compared with 23.3% (SD = 22.9) of those not exposed to *M. cerebralis* ($P = 0.0001$).

Exposure to *F. psychrophilum* had an effect on clinical signs of disease only when other stressors were present. Overall proportions of fish exhibiting signs of disease when exposed to *F. psychrophilum* were 64.8% (SD =38.9), while 56.2% (SD = 43.5) of those not exposed to the bacteria showed signs of disease ($P = 0.0785$). The interaction effect of *M. cerebralis* and *F. psychrophilum* on signs of disease was significant ($P = 0.0556$).

Water temperature ($P = 0.4767$) and gas saturation ($P = 0.4481$) did not have significant effects on morbidity of fish. Nearly the same percentage of fish not exposed to gas supersaturation exhibited signs of disease (Mean = 58.7 %, SD = 41.0), as fish exposed (Mean = 62.3 % SD = 41.9). Signs of disease occurred in 58.8% (SD = 42.8) of fish held at low water temperature, and 62.2% (SD = 40.1) of those held in higher water temperature were affected.

Deformities occurred in 14.8% of fish not exposed to stress factors. Exposure to *M. cerebralis* as a single stress factor resulted in morbidity among 95.1% of fish tested. Because of the high proportion of fish exhibiting signs of disease with exposure to *M. cerebralis*, subsequent addition of stress factors did not increase morbidity in a linear manner as was observed with the mordidity data (Figure 5.3).

Discussion

Addition of stressors increased mortality in varying amounts. The most substantial increase was due to *M. cerebralis* infection. Water temperature also increased mortality quite drastically. *F. psychrophilum* exposure was found to be a serious concern only when other stressors were present. Gas supersaturation had a fairly minor effect on mortality.

A prior study investigating the chronic effects of low levels of gas supersaturation had similar results, in which gas supersaturations of 110% or less had very little effect on the growth or survival of fingerling rainbow trout (Chapter 4). While gas supersaturation does exist in the Colorado and Gunnison Rivers, the effect is minor on rainbow trout year-class losses.

F. psychrophilum did not have the expected effect at 12.5° C. Very similar mortality and morbidity results occurred with infected and uninfected groups at this temperature. Noticeable differences in mortalities were, however, observed at 17° C. This led to the significant interaction effect observed for *F. psychrophilum* and water temperature. *F. psychrophilum* is generally considered to be more virulent at colder water temperatures, and affects fish most dramatically when water temperature is less than 15° C (Holt et al. 1989). My results indicate that this particular strain of the pathogen was more damaging to fish at 17° C than at 12.5° C. Morbidity results show that addition of any additional factor caused an increase in signs of disease among *F. psychrophilum*-exposed fish.

Elevated water temperatures contributed greatly to mortality of fingerling rainbow trout in this study. While the effect was relatively minor with temperature used as the only factor, it was quite noticeable with the addition of other factors. This was especially true when *M. cerebralis*, *F. psychrophilum*, and elevated water temperature were combined. It

is possible that increased metabolic rate of both the fish and pathogens at higher temperatures may have contributed to this effect.

M. cerebralis was the most important contributor to mortality tested in this experiment. Fish in the exposed test groups experienced higher mortality than their unexposed counterparts in every case. These results support the hypothesis that whirling disease is the predominant cause of declines in fingerling rainbow trout survival in some Colorado rivers, with other stressors playing an important but secondary role.

My results support the hypothesis that optimal temperatures for the parasite, along with very high infectivity levels are the most important contributors to losses of rainbow trout in Colorado rivers. In many Colorado rivers, water use dictates flows, which can have an effect on water temperatures. Shallow, silty-bottomed impoundments such as Windy Gap Reservoir on the Colorado River further contribute to the problem by acting as point sources of *M. cerebralis* infection. Alteration of natural rivers may not be a problem for healthy fish populations, but once a pathogen such as *M. cerebralis* is added to the system, the effects of the alterations are realized. According to Snieszko's model of fish disease (Snieszko 1974), a fish must be exposed to a pathogen and physiological stressors in order for disease to occur. In other words, the mere presence of the pathogen is not enough to cause disease. If this theory is accepted, then it is not unusual to see the majority of fish populations incurring no obvious harm from the presence of *M. cerebralis*. The populations within an altered ecosystem such as the Colorado River may not show any deleterious effects in the absence of a serious pathogen, but the arrival of the pathogen can make the other problems more apparent. This experiment has shown that addition of stressors can exacerbate the effects of *M. cerebralis*, and addition of multiple stress factors does result in higher mortality and morbidity among fingerling rainbow trout. While the presence of bacterial pathogens in natural systems is virtually impossible to control, factors

such as point sources of infection from *M. cerebralis* and water temperatures may be manipulated in highly regulated systems such as the Colorado River to reduce their effects on trout populations.

Table 5.1.-Mortality and morbidity of fingerling rainbow trout (n = number of aquaria) exposed to gas supersaturation, *F. psychrophilum*, *M. cerebralis*, and elevated water temperature for six months.

		MORTALITY (%)		MORBIDITY (%)	
Treatment	n	Mean	SD	Mean	SD
Control	3	11.7	10.4	14.8	13.9
Saturation	3	23.3	12.6	4.4	7.7
F. psychrophilum	3	11.7	11.5	14.6	6.0
Temperature	3	23.3	7.6	17.6	4.8
M. cerebralis	3	41.7	7.6	95.1	4.3
F. psychrophilum, Saturation	3	15.0	0.0	43.8	28.6
M. cerebralis, *F. psychrophilum*	3	23.3	5.8	97.6	4.1
Temperature, Saturation	3	30.0	34.6	20.2	12.2
M. cerebralis, Saturation	3	38.3	32.1	100.0	0.0
M. cerebralis, Temperature	3	60.0	22.9	100.0	0.0
F. psychrophilum, Temperature	3	46.7	15.3	29.7	4.3
F. psychrophilum, Temperature, Saturation	3	45.0	26.5	41.0	52.4
M. cerebralis, Saturation, *F. psychrophilum*	3	43.3	36.9	100.0	0.0
M. cerebralis, Temperature, Saturation	3	46.7	15.3	97.4	4.4
M. cerebralis, Temperature, *F. psychrophilum*	3	76.7	7.6	100.0	0.0
M. cerebralis, *F. psychrophilum*, Temperature, Saturation	3	70.0	20.0	91.7	14.4

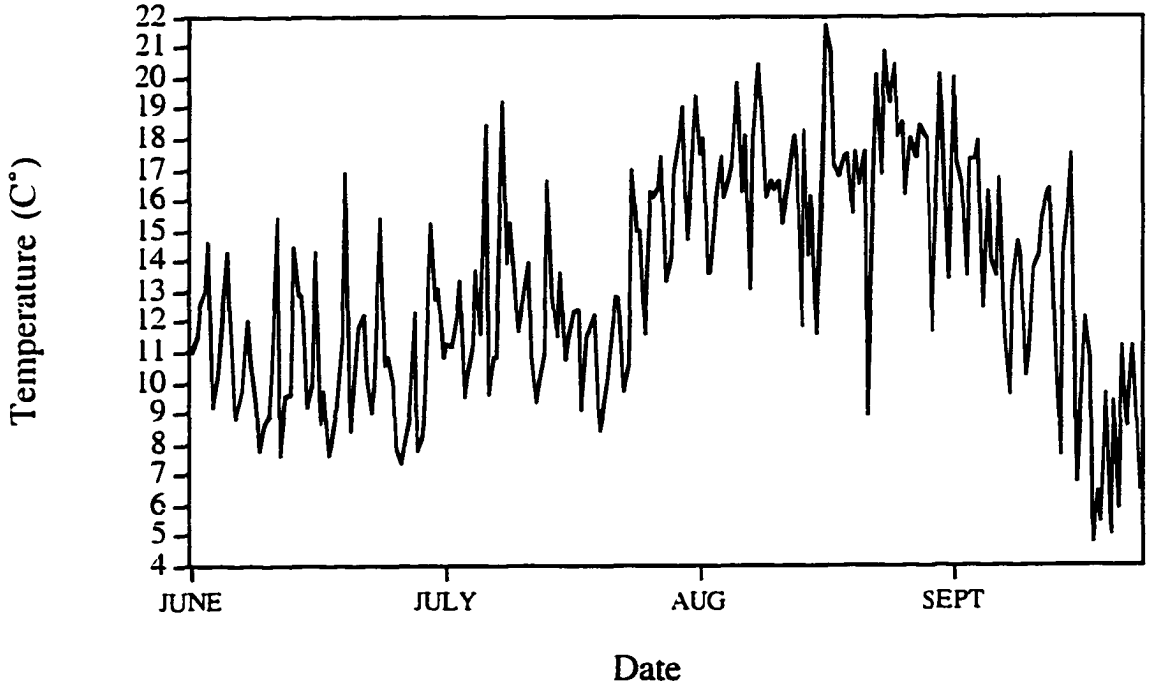

Figure 5.1- Summer water temperatures in the Upper Colorado River sampled from 40 randomly chosen sample sites during 1994.

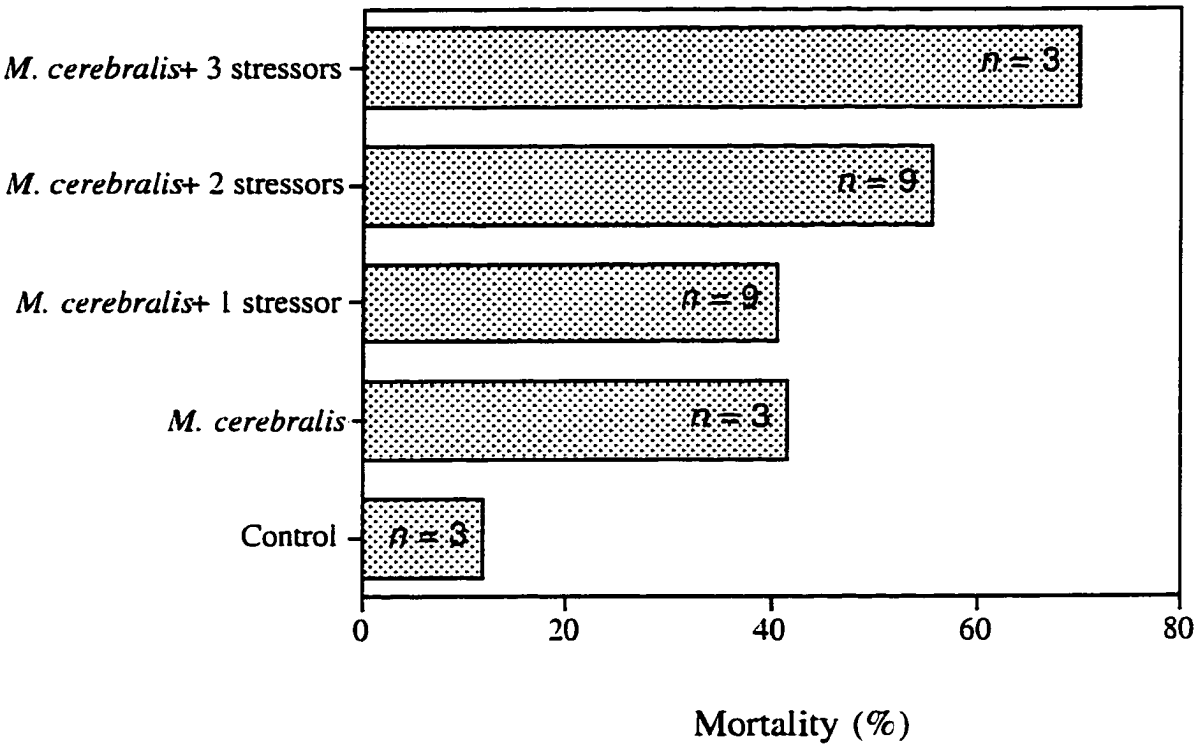

Figure 5.2.- Mortality (%) of fingerling rainbow trout as a function of numbers of stress-disease factors after six months. Sample sizes are equal to number of aquaria.

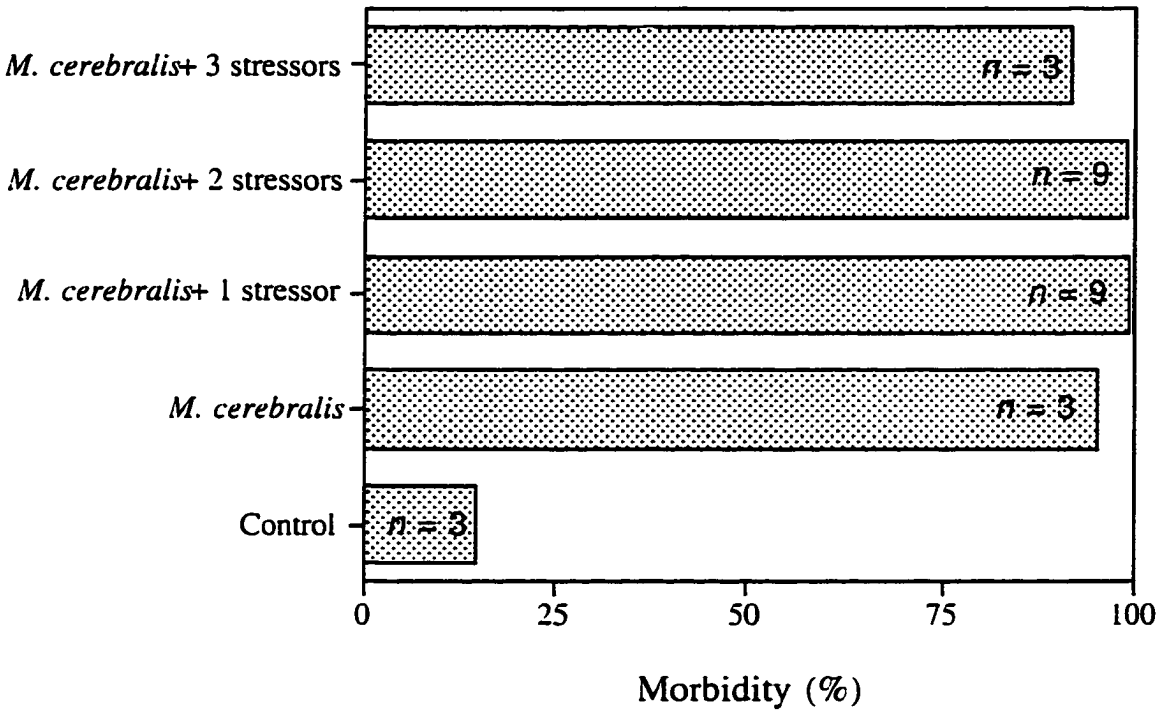

Figure 5.3.- Morbidity (percent of fish exhibiting at least one deformity or sign of disease) of fingerling rainbow trout as a function of numbers of stress-disease factors after six months. Sample sizes are equal to number of aquaria.

References

Bouck, G. R. 1976. Supersaturation and fishery observations in selected alpine Oregon streams. In *Gas Bubble Disease*. D. H. Flikeisen and M. J. Schneider. Technical Information Service, U. S. Dept. of Commerce. Springfield, Virginia.

Dennison, B. A., and M. J. Marchyshyn. 1973. A device for alleviating supersaturation of gasses in hatchery water supplies. The Progressive Fish-Culturist 35:55-58.

El-Matbouli, M., T. S. McDowell, and R.P. Hedrick. 1998. Effect of temperature on the development of the triactinomyxon stage of *Myxobolus cerebralis* in the intestine of *Tubifex tubifex*: Light and electron microscopic observations. Whirling Disease Symposium: Research in Progress. Colorado State University, Fort Collins, Colorado. February 19-21, 1998.

Halliday, M. M. 1976. The biology of *Myxosoma cerebralis*: The causative organism of whirling disease in salmonids. Journal of Fish Biology. 9: 339-357.

Holt, R. A., A. Amandi, J. S. Rohovec, and J. L. Fryer. 1989. Relation of water temperature to bacterial cold-water disease in coho salmon, chinook salmon, and rainbow trout. Journal of Aquatic Animal Health 1:94-101.

Lorenzen, E. 1994. Studies on *Flexibacter psycrophilus* in relation to rainbow trout fry syndrome (RTFS). Ph. D. Thesis, Royal Veterinary and Agricultural University, Copenhagen.

Lorenzen, E. and N. Karas. 1992. Detection of *Flexibacter psychrophilus* by immunofluorescence in fish suffering from fry mortality syndrome: a rapid diagnostic method. Diseases of Aquatic Organisms 13: 231-234.

Markiw, M. E. 1992. Experimentally induced whirling disease. II. Determination of longevity of the infective triactinomyxon stage of *Myxobolus cerebralis* by vital staining. Journal of Aquatic Animal Health. 4: 44-47.

National Marine Fisheries Service. 1995. Report and Recommendations of the Second Working Group Meeting Panel on Gas Bubble Disease. Northwest Fisheries Science Center. Seattle, Washington.

Nehring, R. B., and K. G. Thompson. 1996. Colorado Division of Wildlife Stream Fisheries Investigations. Federal aid in fish and wildlife restoration project F-237R-3 Progress Report, August 1996.

Piper, R. G., and five coauthors. 1982. In *Fish Hatchery Management* pp.208 - 262. United States Department of the Interior, Fish and Wildlife Service, Washington, D. C.

Schaperclause, W. 1931. Die Drehkrankheit in den Forellenzucht und ihre Bekampfung. (Whirling Disease in trout farming and its control). Zeitschrift fur Fischerei. 29:521-567.

Schiewe, M. H. 1974. Influence of dissolved atmospheric gas on swimming performance of juvenile chinook salmon. Transactions of the American Fisheries Society 4: 717-721.

Shrimpton, J. M., D. J. Randall and L. E. Fidler. 1989. Assessing the effects of positive buoyancy on rainbow trout (*Oncorhynchus mykiss*) held in gas supersaturated water. Canadian Journal of Zoology 68:969-973.

Snieszko, S. F. 1974. The effects of environmental stress on outbreaks of infectious diseases of fishes. Journal of Fish Biology 6: 197-208.

Strange, R. J., C. B. Schreck, and J. T. Golden. 1977. Corticoid stress responses to handling and temperature in salmonids. Transactions of the American Fisheries Society 106(3): 213 - 218.

Walker, P. G. and R. B. Nehring. 1995. An investigation to determine the cause(s) of the disappearance of young wild rainbow trout in the upper Colorado River, in Middle Park, Colorado. Colorado Division of Wildlife, Brush, Colorado.

Wedemeyer, G. 1973. Some physiological aspects of sublethal heat stress in the juvenile steelhead trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). Journal of the Fisheries Research Board of Canada 31:179-184.

Chapter 6:

Summary and Overview of Other Possible Contributors to Mortality of *M. cerebralis*-Infected Rainbow Trout

The studies described in the preceding chapters were designed to help clarify the role of gas supersaturation, bacterial infection, water temperature, and *M. cerebralis* infection on year-class losses of rainbow trout in the Upper Colorado River. The factors evaluated in this study were chosen based on preliminary work conducted by Walker and Nehring (1995). The results of their work strongly suggest that gas supersaturation, opportunistic bacteria, *Ichthyophthyrius multifilis* (commonly known as Ich), whirling disease, and other factors are acting in a synergistic manner contributing to mortality of rainbow trout (*Oncorhynchus mykiss*) in the Upper Colorado River (Figure 6.1). The results of the field work and laboratory experiments conducted during our studies have shown that while *M. cerebralis*, bacterial infection, and elevated water temperatures are likely contributing to rainbow trout year-class losses, gas supersaturation is a very minor contributor to rainbow trout mortality.

Gas Supersaturation

During the lengthy field work conducted on the Upper Colorado River, gas supersaturation was identified as a possible cause of mortality among rainbow trout and was found to be associated with Windy Gap Reservoir. High correlation of morbidity among both rainbow and brown trout fingerlings with Windy Gap Reservoir supported the argument that gas supersaturation was playing a role in loss of rainbow trout year-classes. While gas supersaturation was identified in higher than normal levels in the Upper Colorado River, subsequent laboratory experiments revealed no strong effect on growth, morbidity or survival of fish exposed to this stressor. These results indicate that gas supersaturation is not a major factor contributing to year-class losses of rainbow trout in the Upper Colorado River.

Bacterial Infection

In my final laboratory experiment, exposure to *Flavobacterium psychrophilum* increased morbidity and mortality of fingerling rainbow trout when other stressors such as elevated water temperature and *M. cerebralis* exposure were involved. These results were not surprising due to the opportunistic nature of this pathogen. Because opportunistic bacteria such as *F. psychrophilum* are impossible to control in natural systems, manipulating this factor to enhance rainbow trout survival is unlikely. Minimizing other stress factors seems to be the best method for reducing the effects of this and other bacterial pathogens in natural systems.

Water Temperature

Water temperature was found to be a major factor influencing mortality of *M. cerebralis*-infected rainbow trout in this study. Water temperature affects almost every aspect of fish physiology, and has been shown to dictate development and virulence of *M. cerebralis* (Chapter 1). Nehring and Thompson (1996) maintained that water temperatures did not affect survival of rainbow trout young-of-the-year in the Upper Colorado River. Water temperatures reported by Nehring and Thompson differ quite dramatically from those recorded during our study (Figure 6.2). One explanation for this is the fact that their measurements were taken at one location near the lower end of the study area, while my measurements were taken at random locations throughout the study area. The results of my laboratory experiment clearly show that water temperatures are of great importance to *M. cerebralis*-infected rainbow trout survival. This factor cannot be disregarded as a contributor to rainbow trout year-class losses. Manipulation of this factor may help reduce the effects of not only *M. cerebralis*, but of bacterial pathogens and ectoparasites as well.

M. cerebralis Infection

The results of the final laboratory experiment show that with added stressors, mortality among *M. cerebralis* exposed fish increase quite dramatically. These results follow Sniezko's (1974) model of fish disease quite nicely, and indicate that stressful environments can result in greater mortality among *M. cerebralis* infected fish. *M. cerebralis* infection is the most obvious factor causing losses of rainbow trout year-classes in the Upper Colorado River, but is it not acting alone. Other stress factors must be present for this pathogen to cause total mortality of young-of-the-year rainbow trout.

While Sniezko's (1974) model of fish disease explains the onset of disease in laboratory or hatchery situations quite well, it is more difficult to apply this model to natural situations. Conditions in hatcheries are closely monitored and stressors can usually be pinpointed rather easily. Identifying a specific stress factor in a natural system can be problematic because of the wide variety of environmental conditions present. Specific stress factors are likely to vary considerably from river to river. Even within drainages, environmental stress factors contributing to mortality of *M. cerebralis*-infected fish may vary. Mortality of fingerling rainbow trout is nearly 100% only 4 months post-emergence in the Upper Colorado River. After 6 months exposure, mortality in my final lab experiment with all four stress factors was only 70%. This clearly indicates that other factors must be acting on fish in natural situations to result in these losses.

Additional factors affecting survival of *M. cerebralis*-infected juvenile rainbow trout

Intraspecific competition, reduced foraging and predator avoidance behavior, ectoparasite infestation, and point sources of *M. cerebralis* infection are factors that are capable of affecting the survival of *M. cerebralis*-infected rainbow trout in natural

situations. In a laboratory or hatchery, these factors are not an issue, because fish are fed to satiation, and exist in a protected environment. Although they were not tested in a laboratory setting, observations during my field and laboratory studies suggest that these additional factors may be very important in contributing to the loss of *M. cerebralis*-infected-rainbow trout in natural environments. The topic of ectoparasite infestation is discussed in Appendix I. The effects of competition, reduced foraging and predator avoidance behavior and point sources of *M. cerebralis* infection must also be addressed.

Competition

Competition between rainbow and brown trout (*Salmo trutta*) fingerlings could be influencing rainbow trout survival in Colorado rivers. Brown trout are present in every river where year-class losses of rainbow trout have been documented. The densities of age-1 brown trout have increased in sections of the Colorado and Gunnison Rivers while age-1 rainbow trout have declined (Figure 6.2). Brown trout emerge earlier and are on average 10 - 20 mm larger than rainbow trout fingerlings throughout their first summer of growth (Figure 6.3). Brown trout fingerlings have been shown to dominate and force rainbow trout fingerlings into sub-optimal habitat, more specifically into faster moving and deeper water than they occupy in the absence of brown trout (Gatz et al. 1987). Factors thought to be fairly innocuous from a human perspective such as dominance hierarchies have been known to cause stress response and reduced disease resistance in fish. Scott and Curry (1980) found that subordinant swordtails (*Xiphophorus helleri*) had more active inter-renal tissue than the dominant individual in a given hierarchy. Ellis (1981) suggested that resultant elevated cortisol levels could lead to immunosuppression in the subordinant individuals. Inter-renal cell dimensions of pumpkinseeds (*Lepomis gibbosus*) were also greater among subordinant fish (Erickson 1967). Similar results were found among

subordinant rainbow trout (Noakes and Leatherland 1977), and coho salmon (Ejike and Schreck 1980). More direct evidence that intraspecific competition can result in stress-induced immunosupression among fish was found by Barrow (1955). He concluded that only the dominant members of a social hierarchy of carp (*Cyprinus carpio*) produced antibodies to experimental infection with trypanosomes. If this is the case with other species of fish, intraspecific and interspecific competition could be a strong force in determining if fish actually become diseased after exposure to a pathogen. If immune response to *M. cerebralis* in rainbow trout is compromised by the presence of more aggressive and dominant brown trout fingerlings, reduced survival among rainbow trout would be expected, especially if the infected fish are forced into deeper and faster water where maintaining position in the water column would become more difficult.

Compromised feeding behavior

Reduced feeding efficiency of infected rainbow trout was observed in my laboratory experiments, and has been noted by hatchery workers at *M. cerebralis* infected facilities (E. Hughes, Colorado Division of Wildlife, personal communication). Reduced ability to capture prey, especially in a stream environment, could seriously affect the survival of fingerling rainbow trout. Because of the increased number of unsuccessful attacks on prey items, infected fish are required to make more forays into the water column to capture the same amount of prey as uninfected individuals. The result is greater energy expenditure and lower growth rates among infected fish. The survival of these fish would subsequently be reduced due to poor condition, particularly during winter months.

Compromised predator avoidance behavior

Predator avoidance is obviously compromised among *M. cerebralis* infected

salmonids. Whirling episodes caused by excitement during feeding or flight make infected individuals much more vulnerable to predation by both fish-eating birds and other fish. Blacktail also makes infected individuals more susceptible to predation due to their loss of camouflage.

Modified behavior is quite well documented among other species of fish when they become parasitized. For example, threespine stickleback (*Gasterosteus aculeatus*) parasitized by a cestode (*Schistocephalus solidus*) feed nearer to predators and recover from fright response more rapidly than uninfested individuals (Jakobsen et al. 1987, Giles 1983). Crowden and Broom (1980) found that infestation by eye flukes (*Diplostomum spathaceum*) caused dace (*Leuciscus leuciscus*) to swim closer to water surface than uninfested fish, making them more susceptible to avian predators. Similarly, common shiners (*Notropis cormutus*) infected with *Ligula intestinalis* are less gregarious, found in shallower water, and are sluggish compared to uninfected individuals (Dence 1958).

Like *M. cerebralis*, each of the aforementioned parasites requires an alternate host to complete their life cycle. Modified host behavior benefits the parasite by increasing the probability that the host will be eaten and dispersed to new hosts. "Host suicide" is a term used to describe behavior modification of parasitized hosts which results in greater susceptibility of the host to predators (Smith Trail 1980). Host suicide may actually reduce the abundance of parasites by preventing maturation and dispersal of the parasite in some situations. This is the case when rainbow trout young-of-the-year succumb to the effects of whirling disease and other stressors prior to the formation of *M. cerebralis* myxospores. However, if the behavior modification continues after maturation of the parasite, the infected host acts as a source of new infection. Because *M. cerebralis* myxospores are extremely durable, and can survive passage through the digestive tract of predators, rainbow trout exhibiting signs of disease after spore maturation add to the pool of *M.*

cerebralis. Brown trout co-existing with rainbow trout are much less likely to die prior to spore maturation, and further compound the problem by acting as a continuous source of infection. The end result is not only a decline in rainbow trout survival due to increased susceptibility to predation, but an increase in total numbers of *M. cerebralis* spores in the environment available to *T. tubifex*.

Point-sources of M. cerebralis infection

Point-sources of *M. cerebralis* infection may be more important than any of the previously mentioned factors. It is possible that in natural systems, sheer numbers of infective units are the over-riding factor contributing to mortality, and simply overwhelm fish in a naturally stressful environment. In California, the closure of three *M. cerebralis*-positive private aquaculture facilities has resulted in *M. cerebralis* declining to undetectable levels in downstream rainbow trout populations (Modin 1998). These declines were presumably due to the loss of the point sources of re-infection.

Windy Gap Reservoir on the Colorado River has been shown to produce extremely high numbers of triactinomyxons and contains a disproportionate number of infected *T. tubifex* when compared with upstream and downstream locations (J. Zendt, Colorado Cooperative Fish and Wildlife Research Unit, personal communication). Similarly, the Poudre Canyon State Trout Rearing Unit on the Cache la Poudre River has been identified as a source of high levels of *M. cerebralis* infectivity (B. Allen, Colorado Cooperative Fish and Wildlife Research Unit, personal communication). The Roaring Judy State Fish Hatchery located on the East River, a tributary of the Gunnison River, could be acting as a point source of infection on the Gunnison River. A valid argument could be made that a reduction in population-level effects due to *M. cerebralis* may occur in Colorado rivers if known point sources of infection are eliminated.

The topic of *M. cerebralis* and wild rainbow trout year-class losses is a complicated issue. Politics, individual agendas and public sentiment have all influenced management decisions associated with *M. cerebralis* more than biological considerations. On the one hand, the parasite has been deemed a ruthless killer that will most certainly destroy all wild trout populations once it has become established. Others believe that the parasite is no more detrimental to fish populations than a common cold is to human populations. Somewhere between is the reality of the situation, in which the parasite can cause great harm to trout populations given the right circumstances. Arguments that the parasite alone is the cause of complete year-class failures are not valid. When tested without other stress factors, the parasite has consistently caused less than 50% mortality after 6 months in my laboratory experiments. In the Upper Colorado River, virtually 100% mortality occurs in less than 4 months, strongly suggesting that other factors are playing a significant role in rainbow trout year-class losses. Further, rainbow trout population level declines have not been observed in the Big Thompson and Frying Pan Rivers of Colorado, despite the presence of the organism in these drainages. The theory that the parasite is completely innocuous is obviously incorrect. *M. cerebralis* has certainly played a large role in losses of rainbow trout year-classes in the Upper Colorado River, and others.

While completely eradicating the parasite from wild trout populations in Colorado is unlikely, the potential for reducing its effects is good in some situations. Elimination of point-sources of infection and manipulating water temperatures and flows in highly regulated systems appears to be the best strategy for reducing the effects of the parasite on wild rainbow trout populations. Manipulation of other habitat variables may also be appropriate. Management goals should aim toward identifying and removing point sources of infection and minimizing environmental stressors in systems heavily impacted by *M. cerebralis*.

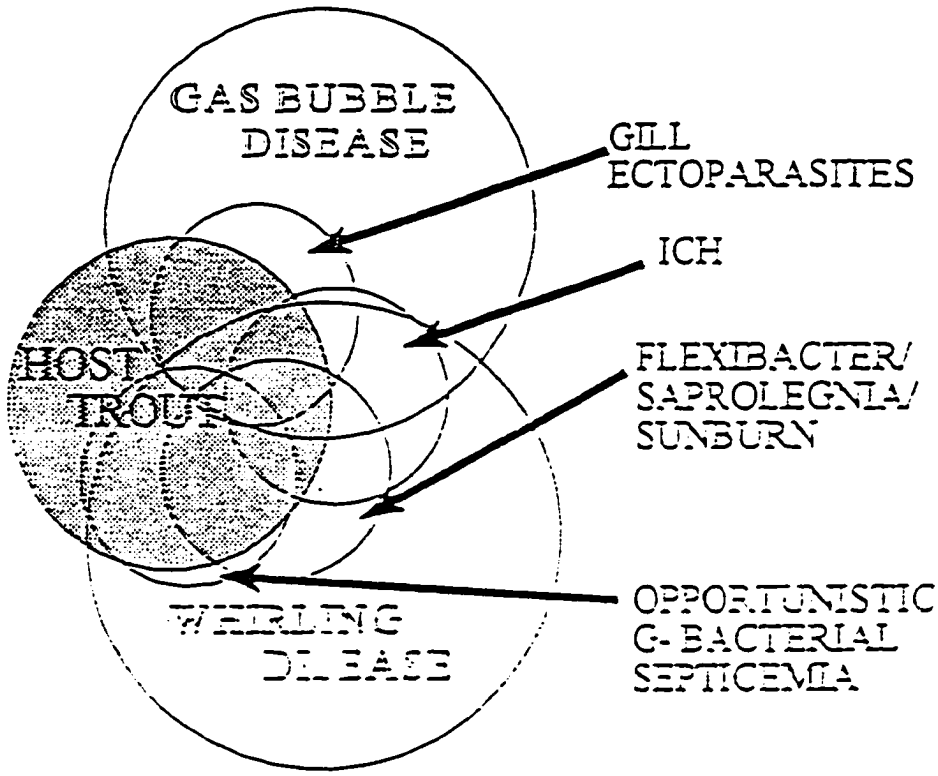

Figure 6.1. Conceptual model of stressors influencing survival of rainbow trout young-of-the-year in the Upper Colorado River (from Walker and Nehring 1995).

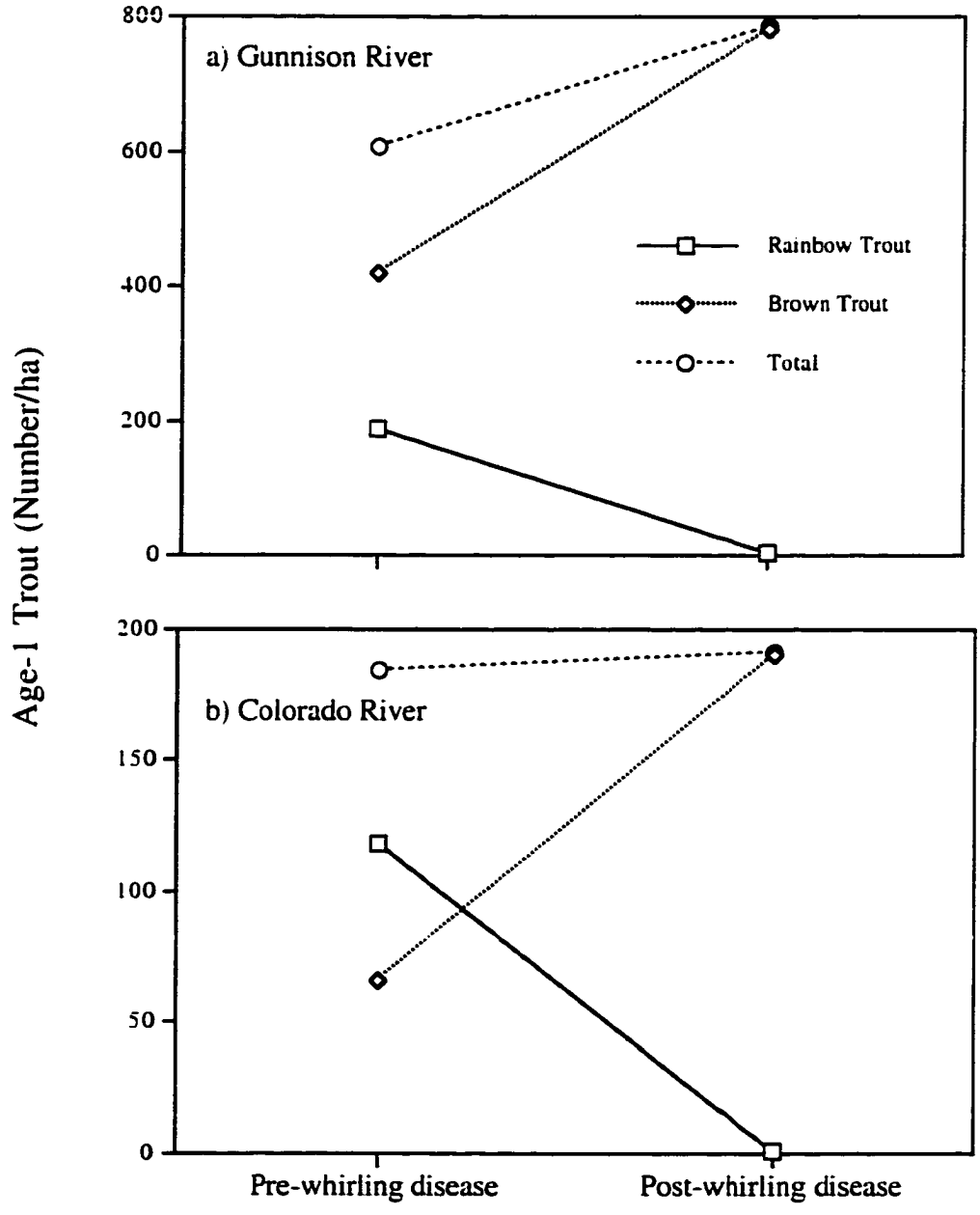

Figure 6.2 Density (number/ha) of age-1 rainbow and brown trout in the Gunnison River (Black Canyon) and Upper Colorado River during pre-whirling disease and post-whirling disease years.

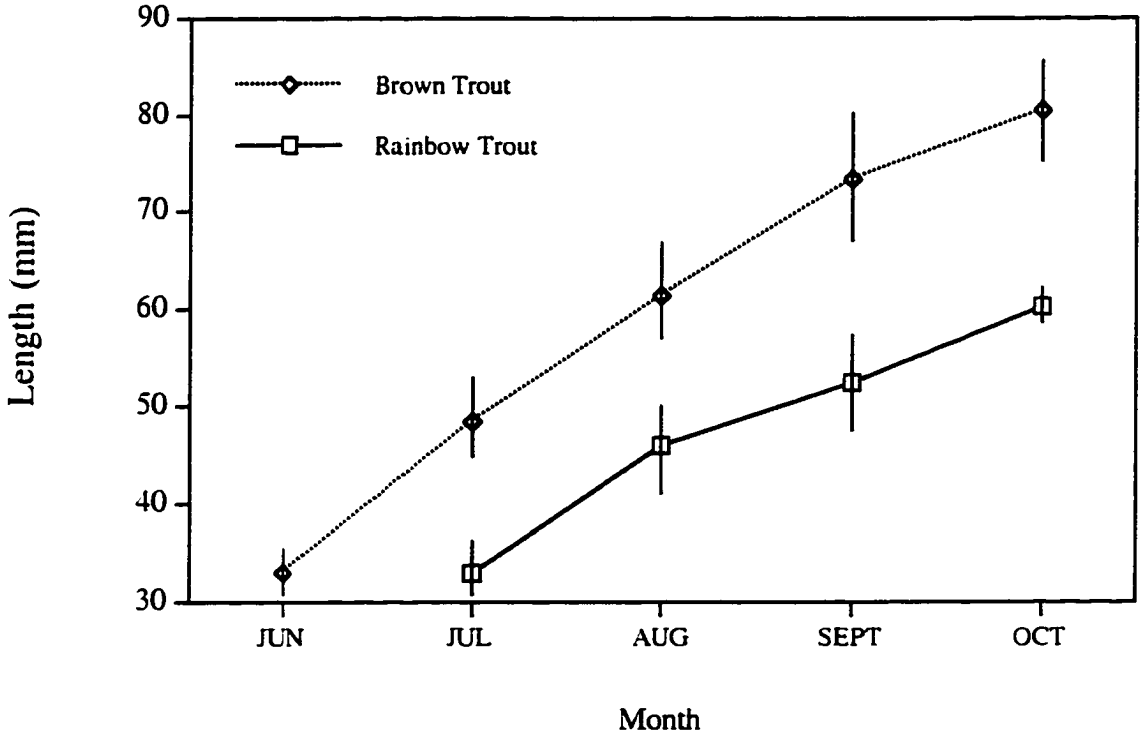

Figure 6.3 Mean lengths and standard deviations of rainbow and brown trout young-of-the-year during 1995 in the Upper Colorado River.

References

Barrow, J. H. 1955. Social behavior in freshwater fish and its effect on resistance to trypanosomes. Proceedings of the National Academy of Science U.S.A. 41:676-679.

Crowden, A. E. and D. M. Broom. 1980. Effects of the eye-fluke *Diplostomum spathaceum* on the behavior of dace (*Leuciscus leuciscus*). Animal Behavior 28:287-294.

Dence, W. A. 1958. Studies on *Ligula*-infected common shiners (*Notropis cormutus frontalis* Agassiz) in the Adirondacks. Journal of Parasitology 44:334-338.

Ejike, C. and Schreck, C. B. 1980. Stress and social hierarchy rank in coho salmon. Transactions of the American Fisheries Society 109:423-426.

Ellis, A. E. 1981. Stress and the modulation of defense mechanisms in fish. In *Stress and Fish* . A. D. Pickering [ed.], pp. 147 - 169. London and New York: Academic Press.

Erickson, J. G., 1967. Social hierarchy, territoriality, and stress reactions in sunfish. Physiological Zoology 40:40-48.

Giles, N. 1983. Behavioral effects of the parasite *Schistocephalus solidus* (Cestoda) on an intermediate host, the three-spined stickleback, *Gasterosteus aculeatus* L.

Jakobsen, P. J., G. H. Johnson, and P. Larsson. 1988. Effects of predation risk and parasitism on the feeding ecology, habitat use, and abundance of lacustrine threespine stickleback (*Gasterosteus aculeatus*). Canadian Journal of Fisheries and Aquatic Sciences 45:426-431.

Modin, J. 1998. Whirling disease in California: A review of its history, distribution, and impacts, 1965-1997. Journal of Aquatic Animal Health 10:132-142.

Nehring, R. B., and K. G. Thompson. 1996. Colorado Division of Wildlife Stream Fisheries Investigations. Federal aid in fish and wildlife restoration project F-237R-3 Progress Report, August 1996.

Noakes, D. L. and Leatherland, J. F. 1977. Social dominance and interrenal cell activity in rainbow trout, *Salmo gairdneri* (Pisces, Salmonidae). Environmental Biology of Fish 2:131-136.

Scott, D. B. C., and C. E. Curry. 1980. Social hierarchy in relation to adrenocortical activity in *Xiphophorus helleri* Heckel. Journal of Fish Biology 16:265-277.

Smith Trail, D. R. 1980. Behavioral interactions between parasites and hosts: Host suicide and the evolution of complex life cycles. The American Naturalist 116(1):77-91.

Snieszko, S. K. 1974. The effects of environmental stress on outbreaks of infectious diseases of fishes. Journal of Fish Biology 6:197-208.

Walker, P. G. and R. B. Nehring. 1995. An investigation to determine the cause(s) of the disappearance of young wild rainbow trout in the upper Colorado River, in Middle Park, Colorado. Colorado Division of Wildlife, Brush, Colorado.

APPENDIX:

Gill Ectoparasites of Young-of-the-year Rainbow and Brown Trout in the Upper Colorado River

Health of wild and feral fish populations in the Rocky Mountain west has recently become more important due to the decline or complete loss of year classes of rainbow trout (*Oncorhynchus mykiss*) in rivers such as the Madison River in Montana, and the Upper Colorado River in Colorado. While these disappearances have been attributed to whirling disease (caused by the myxozoan parasite *Myxobolus cerebralis*) other factors may be contributing to the declines in these fish populations. Preliminary sampling during fall of 1994 and 1995 in the Upper Colorado River revealed that several genera of gill ectoparasites were present among fish in the drainage. Evaluations of freshwater protozoan fish ectoparasites in wild salmonid populations have been largely overlooked in the past. My objective was to identify genera of gill ectoparasites, quantify the relative number of parasites on juvenile rainbow and brown trout (*Salmo trutta*), and identify seasonal or species-specific trends that could contribute to the loss of rainbow trout year-classes in the drainage.

Methods

The study area consisted of 40 kilometers of the Upper Colorado River starting at Granby Reservoir, and extending downstream just past Parshall, Colorado. This reach has been the focus of several years of study on the disappearance of year classes of rainbow trout. Brown trout abundance in the river has not been noticeably affected. Preliminary sampling indicated that rainbow trout fingerlings may be more severely affected than brown trout fingerlings by gill ectoparasites in the drainage (Walker and Nehring 1995). Young-of-the-year rainbow and brown trout were captured and examined at 2 to 4 week intervals from June through October, 1996, and from June through September 1997. On each sampling occasion, 2 sampling points were randomly chosen from 40 possible locations in the study area. Fish were captured using a backpack electroshocker set at 150

volts pulsed D.C. current (50% pulse width, 100 Hz). Fish were euthanized with an overdose of tricaine methanesulphonate (MS-222) immediately prior to gill examination. All four gill arches were removed from the right side of each fish, mounted on microscope slides, and examined under 400x for presence or absence of protozoans. Protozoans were quantified by recording the total number by genera on 10 randomly chosen gill filaments per arch (40 total filaments per fish). The average number of protozoans per filament was calculated by dividing the total number of protozoans by the number of filaments examined. Relative abundance of each protozoan genera was classified as light, medium, heavy, or none, based on:

None: No protozoans

Light: average of less than or equal to 0.1 protozoans per filament ($n \leq 4$ individuals)

Medium: average of less than 1, but greater than 0.1 protozoans per filament ($4 < n < 40$ individuals).

Heavy: average of equal to or greater than 1 protozoan per filament ($n > 40$ individuals).

Sixty-eight rainbow trout and 86 brown trout young-of-the-year were captured and examined in this manner during the 1996 field season. During 1997, 44 rainbow trout and 108 brown trout were captured and examined.

Logistic regression analysis was used to test the null hypotheses that prevalence of infestations were independent of sampling month, year and species of fish. Month and year were treated as continuous variables, while species of fish was treated as a class variable. Prevalence of infestation by any ectoparasite was used as the response variable.

Results and Discussion

Nine genera of ectoparasites were observed in the drainage over the course of the study. These included *Apiosoma*, *Ambiphrya*, *Chilodonella*, *Epistylis*, *Ichthyobodo*, *Trichodina*, *Trichodinella*, *Tripartiella*, and an unidentified cochliopodid gill amoeba. Numbers of genera observed varied widely throughout the study area (Figure A.1). The greatest richness of ectoparasites in 1996 occurred near Hot Sulphur Springs, Colorado, where six of eight genera observed that year were found. The lowest richness was observed in the furthest upstream sampling locations, particularly above the Willow Creek confluence of the Colorado River, where no ectoparasites were observed. During 1997, ectoparasite prevalence and richness was lower, with only seven genera of ectoparasites observed.

A temporal effect on numbers of genera was observed during both years of sampling (Figure A.2). During 1996, only two genera were identified on fish sampled in June, followed by four in July, six in August, four in September, and three in October. The same pattern held in 1997, with one genus observed in June, three in July, five in August, and three in September.

Numbers of infested fish varied widely over the course of both sampling seasons, but trends appearing in the raw data indicated that prevalence of infestations were related to seasonal effects, with higher prevalence occurring in August and September (Figure A.3). Apparent differences in infestation between fish species were observed in 1996, with 31.4% of all brown trout (n = 86) and 55.9% of all rainbow trout (n = 68) infested with at least one genus of ectoparasite. In 1997, the prevalence of infestations on brown trout (18.6 %, n = 108) was much closer to the prevalence of infestations on rainbow trout (29.5%, n = 44).

When rainbow and brown trout data collected in 1996 were separated by species

(Figure A1.3), rainbow trout appeared to have a higher prevalence of infestations than brown trout in August and September. In 1997, prevalence of infestations were higher on rainbow trout than brown trout in August, but not in September.

A significant month-year-species interaction ($P = 0.0295$) was revealed, reflecting the changes in infestation prevalence among months, years, and species of salmonid. Because of the strong interaction effects observed, individual effects were difficult to interpret. As a result, Fisher's exact tests were used to test for differences in infestation prevalence between fish species during each month and year of sampling. The results indicated that ectoparasite infestations occurred on rainbow trout more frequently than brown trout during August of 1996 ($P < 0.0001$). Species differences were not significant for all other months of sampling.

Solitary Ectocommensal Ciliate Infestations

Two genera of solitary ectocommensal ciliates were identified among the sampled fish. *Ambiphrya* was observed in a single brown trout sampled 2 kilometers downstream of the Williams Fork River confluence of the Colorado River in June, 1996. It was not observed among fish sampled in 1997.

Apiosoma was observed throughout the drainage in both 1996 and 1997, appearing as a light infestation in 5.2%, medium infestation in 5.8%, and as a heavy infestation in 1.9% of the fish sampled in 1996. In 1997, this commensal was identified as a light infestation in 1.9%, medium infestation in 1.2% and as a heavy infestation in 1.2% of the fish sampled.

Colonial Ectocommensal Ciliate Infestations

Epistylis infestations were observed in the middle sections of the study area, below

Windy Gap Reservoir, and at Hot Sulphur Springs in 1996. Infestations were categorized as light in four individuals. The organism occurred only on rainbow trout in August and September, and in every case *Apiosoma* was also present on the infested fish. During 1997, *Epistylis* was observed as light and medium infestations on two rainbow trout sampled 5 kilometers above the Williams Fork Confluence in August. These fish, like rainbow trout sampled in 1996, were also infested with *Apiosoma*.

Chilodonella

In June 1996, *Chilodonella* was observed on two individual rainbow trout in two separate locations; directly below Windy Gap Reservoir, and at Hot Sulphur Springs. The infestation at Windy Gap Reservoir was light, and the infestation at Hot Sulphur Springs was rated as medium. The ectoparasite was observed as a light infestation on one individual brown trout above Windy Gap Reservoir, and as a medium infestation on one individual brown trout 5 kilometers above the Williams Fork River confluence in 1997. Although very few fish were found to be infested during both years of sampling, this ectoparasite is known to cause mass mortality in wild populations (Langdon et al. 1985). Fish dying as a result of infestation by this parasite are unlikely to be sampled.

Ichthyobodo

Ichthyobodo necator occurred as a light infestation in 6.5% of the fish sampled in 1996. This ectoparasite was widely distributed throughout the drainage, and observed from the end of July through October in both rainbow and brown trout. During 1997 only one light infestation by this ectoparasite occurred in a single brown trout sampled in August. Ichthyobodiasis, caused by *Ichthyobodo necator*, has been shown to cause up to 25% mortality among salmonid fry (Robertson 1979) and is considered a very important

pathogen in fish culture.

Gill Amoeba Infestations

Infestations by an unidentified cochliopodid gill amoeba were medium in 3.9% and heavy in 1.9% of the individuals sampled in 1996. Amoeba infestations were observed only in two locations in the drainage (at Hot Sulphur Springs and above the Williams Fork confluence) in July and August. However, preliminary sampling in the late fall of 1994 and 1995 identified the amoeba in locations above Windy Gap Reservoir, and below the Williams Fork confluence. Gill amoebas were associated only with brown trout in 1996, but they were present in both species in 1994 and 1995. No gill amoebas were found among fish sampled in 1997.

Paramoeba sp. was reported by Munday et al. (1988) to cause severe gill disease among saltwater reared Atlantic salmon *Salmo salar* and rainbow trout in Tasmania. Kent et al. (1988) reported infestations by amoebas *Paramoeba pemaquidensis* on saltwater reared coho salmon *Oncorhynchus kisutch* in Washington and California. Occurrence of amoeba-like cells associated with nodular gill disease in freshwater reared rainbow trout were reported by Daoust and Ferguson (1985). *Thecamoeba hoffmani* has been described from fingerling hatchery-reared freshwater salmonids in Washington, Oregon, and Michigan (Sawyer et al. 1974). This protozoan is described as a free-living species that only infests fish gills under sub-optimal water quality conditions. It is possible that the amoebae observed in the Colorado River are the same or a similar species. To my knowledge this is the first diagnosis of a gill amoeba in a wild population of salmonids in the United States.

Trichodina and Tripartiella

Trichodina was observed on both rainbow and brown trout throughout the drainage. *Trichodina* was the most common gill ectoparasite, occurring as a light infestation in 9.7%, medium infestation in 4.5%, and heavy infestation in 4.5% of the fish sampled in 1996. Light infestations were found among 11.1%, and medium infestations among 1.9% of the fish sampled in 1997. On the basis of gross morphology, *Trichodina* was the only genus for which more than one species was observed. Two or more *Trichodina* species were sometimes observed from the same sampling site and occasionally on the same fish.

Tripartiella was observed near Hot Sulphur Springs on both rainbow and brown trout in 1996. It was diagnosed as a light infestation in 2.6% of the fish sampled, and as a heavy infestation on one fish. This ectoparasite was found as a light infestation among 3.1% of the fish sampled in 1997. The majority of these infestations were found downstream of the Williams Fork River confluence.

Trichodinella, an ectoparasite not observed in 1996, was identified in two locations during 1997. One heavy and two light infestations were found near Hot Sulphur Springs. Four individuals were found with light infestations downstream of the Williams Fork confluence.

Incidental Fish Sampling

Several species of non-salmonids were incidentally captured in the study area, including white sucker (*Catostomus commersoni*), longnose dace (*Rhinichthys cataractae*), speckled dace (*Rhinichthys osculus*), fathead minnow (*Pimephales promelas*), and mottled sculpin (*Cottus bairdi*). Ectoparasites were found in the majority (64%) of these fish. Although the sample size was quite small ($n = 14$), infestations among these fish appear to

be much more frequent and severe than among rainbow and brown trout fingerlings. It is possible that these fish are reservoirs of infestation for young-of the year salmonids.

Conclusions

Ectoparasites found among fish in this study are commonly associated with poor water quality, high organic loads, or crowded conditions when found in fish culture operations. The differences in genera composition and temporal variation of ectoparasites in the study area indicate that infestations by these organisms in wild fish populations is a dynamic process. Environmental conditions, opportunities for transmission, and the health of potential hosts all play a role in this process. Water temperatures in the Upper Colorado River are greatest in August and September, corresponding closely with ectoparasite abundance. Low flows during this time of year may exacerbate the problem by reducing water velocities and forcing fish into closer proximity to each other.

This study indicates that gill ectoparasites are relatively common among young-of-the-year trout in the Upper Colorado River. While gill ectoparasites may contribute to the loss of some individual fish, they do not appear to be abundant enough to explain the total collapse of rainbow trout year-classes observed in this reach of the Colorado River. They may, however, contribute to the pool of environmental stressors faced by *M. cerebralis*-infected rainbow trout. Examinations of wild and feral fish in more pristine habitats, and establishment of baseline infestation rates in other drainages are needed to better define the dynamics of ectoparasites in wild and feral populations, and their potential role in the survival of salmonids.

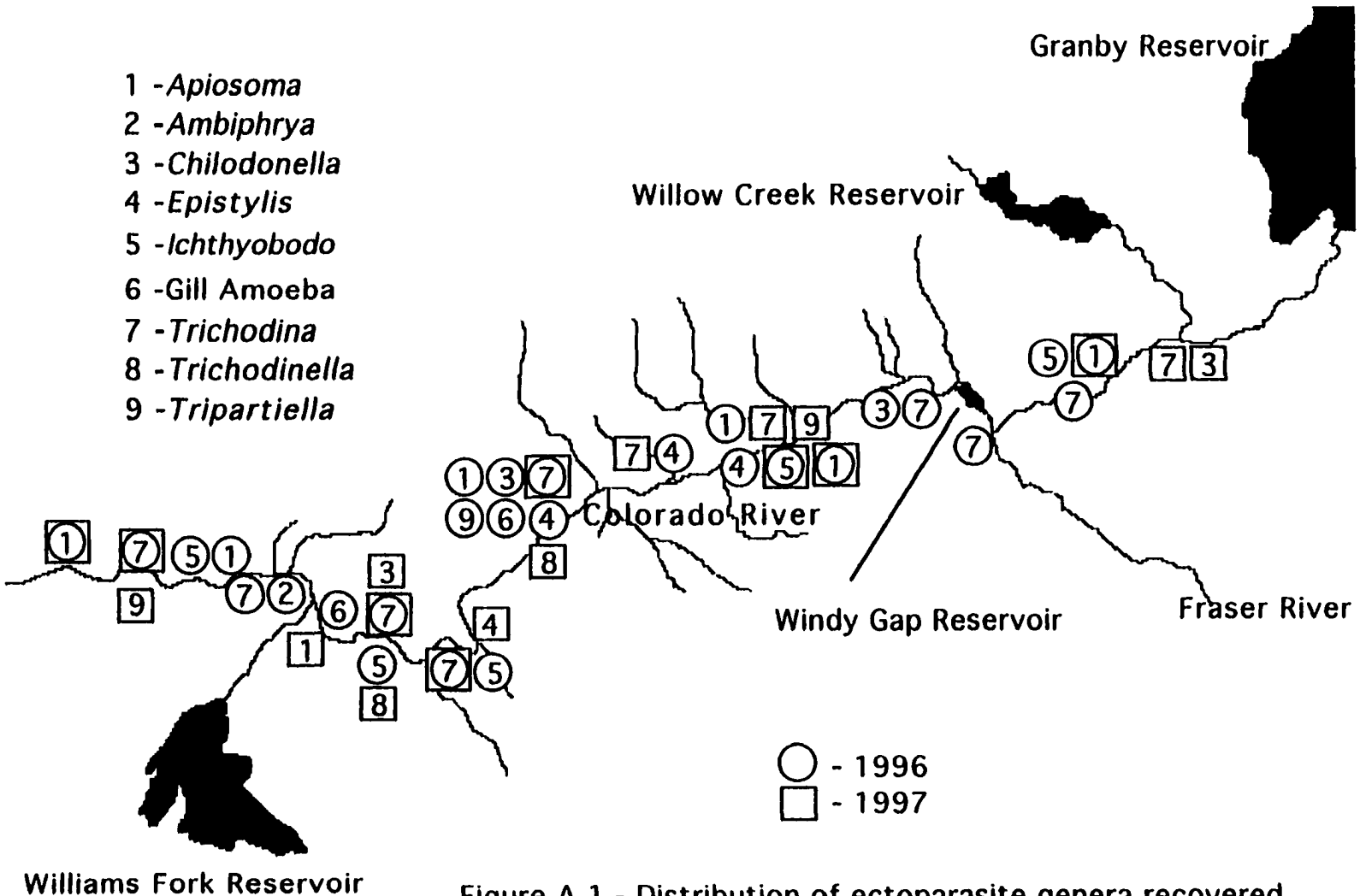

Figure A.1.- Distribution of ectoparasite genera recovered from young-of-the-year rainnbow and brown trout in the Upper Colorado River during 1996 and 1997.

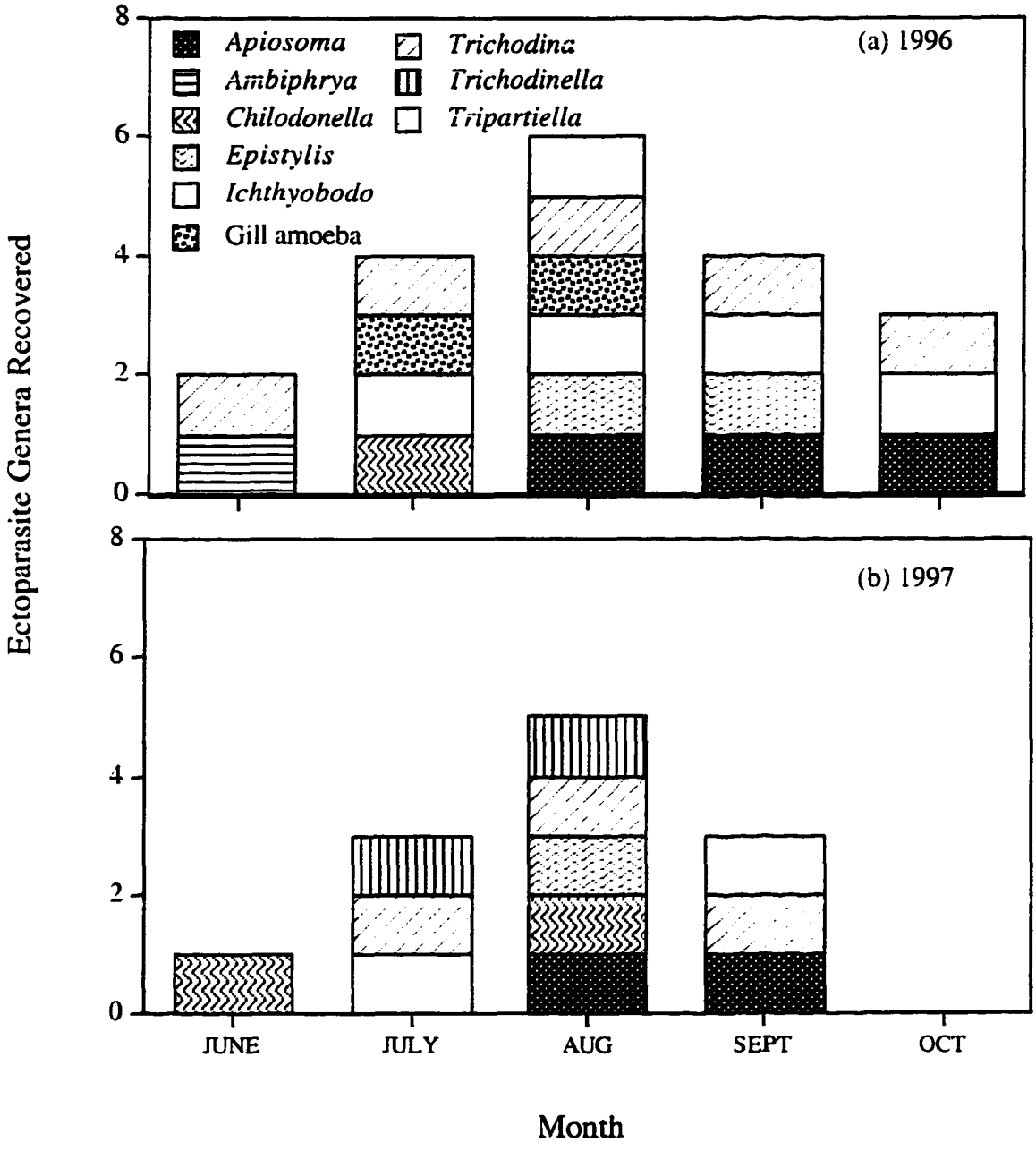

Figure A.2.-Ectoparasite genera recovered from young-of-the-year rainbow and brown trout in the Upper Colorado River during 1996 and 1997.

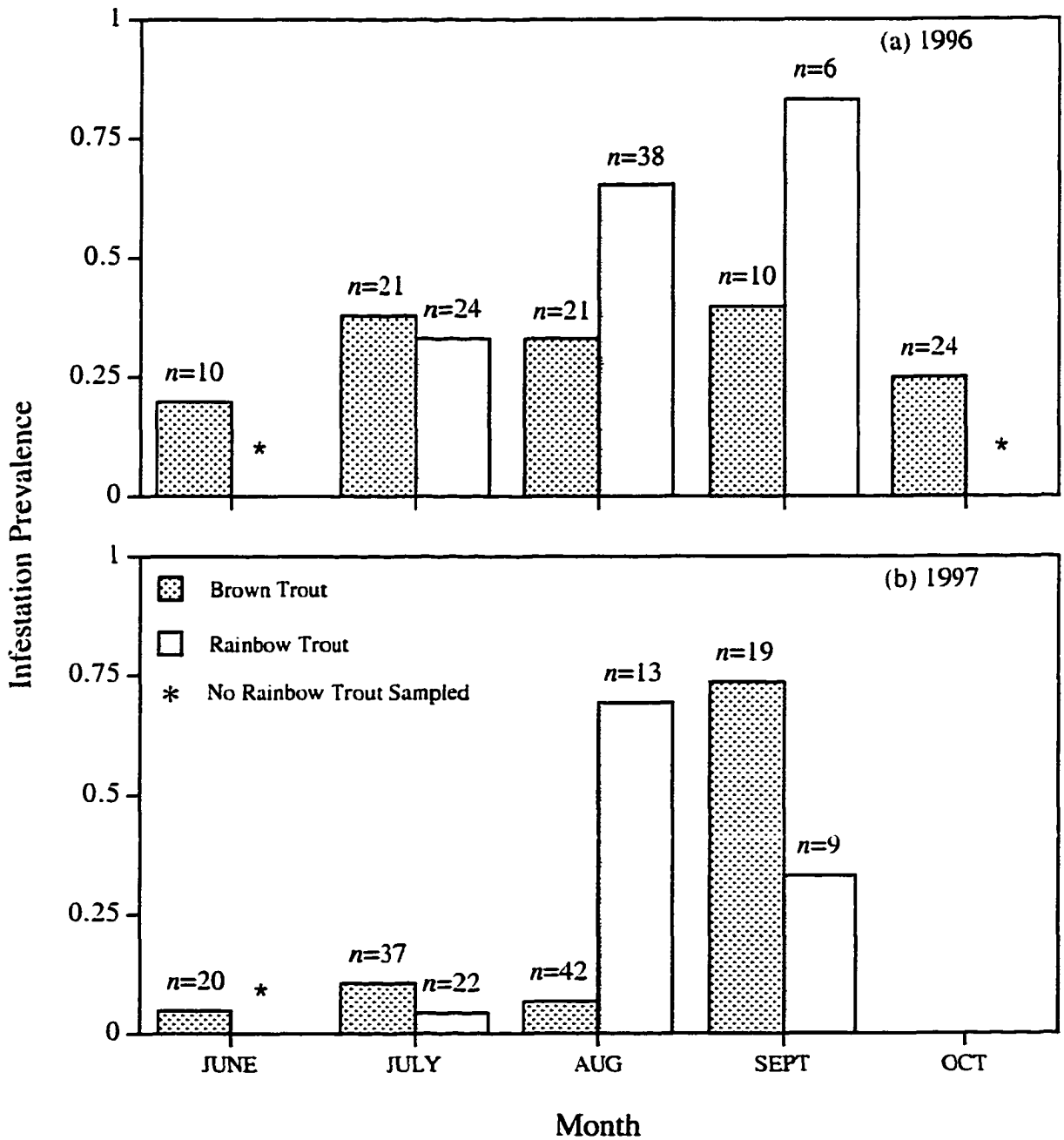

Figure A.3.-Ectoparasite prevalence on young-of-the-year rainbow and brown trout in the Upper Colorado River during 1996 and 1997.

References

Daoust, P.-Y, and H. W. Ferguson. 1985. Nodular gill disease: a unique form of proliferative gill disease in rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Diseases 8:511-522.

Kent, M. L, T. K. Sawyer, and R. P. Hedrick. 1988. *Paramoeba pemaquidensis* (Sarcomastigophora: Paramoebidae) infestation of the gills of coho salmon *Oncorhynchus kisutch* reared in sea-water. Diseases of Aquatic Organisms 5:163-169.

Langdon, J. S., N. Gudkovs, J. D. Humphrey, and E. C. Saxon. 1985. Death in Australian freshwater fishes associated with *Chilodonella hexasticha* infection. Australian Veterinary Journal 62:409-413.

Munday, B. L., C. Foster, F. R., and R. J. G Lester. 1988. Paramoebic gill infection of Atlantic salmon (*Salmo salar*) and rainbow trout (*Salmo gairdneri*). *3rd International Colloquium on Pathology in Marine Aquaculture, 2-6 October 1988, Gloucester Point, Virginia.* Abstracts, pp. 53-54.

Robertson, D. A. 1979. Host-parasite interactions between *Ichthyobodo necator* (Henneguy, 1883) and farmed salmonids. Journal of Fish Diseases 2:481-491.

Sawyer, T. K., Gnath, J. G., and J. F. Conrad. 1974. *Thecamoeba hoffmani* sp. N. (Amoebida: Thecamoebidae) from gills of fingerling salmonid fish. The Journal of Parasitology 60(4):677-682.

Walker, P. G. and R. B. Nehring. 1995. An investigation to determine the cause(s) of the disappearance of young wild rainbow trout in the upper Colorado River, in Middle Park, Colorado. Colorado Division of Wildlife, Brush, Colorado.