



Interactions among oligochaetes and a myxozoan parasite, *Myxobolus cerebralis*
by Leah Candace Steinbach

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Biological Sciences

Montana State University

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Abstract:

Myxobolus cerebralis, the causative agent of salmonid whirling disease, has caused the decline of two populations of wild rainbow trout (*Oncorhynchus mykiss*) on the Madison River MT and the Colorado River CO. The life cycle of *Myxobolus cerebralis* alternates between two hosts: various salmonid species and one aquatic oligochaete species, *Tubifex tubifex*. The objectives of this study were to determine the threshold myxospore dose needed to achieve complete infection within *Tubifex tubifex* and the influence that interactions among oligochaetes and *M. cerebralis* had on oligochaete fitness and the success of *M. cerebralis*. I examined the threshold myxospore dose of two *T. tubifex* strains experimentally and using nine myxospore doses. I determined that a dose of 50 myxospores per individual *T. tubifex* achieved complete infection in the susceptible strain of *T. tubifex*. Interactions were examined in two response surface competition design experiments using two strains of *T. tubifex*, two species of oligochaetes and various doses of myxospores. The interactions among strains of resistant and susceptible *T. tubifex* were density dependent. The infection prevalence and the proliferation of the parasite (triacinomyxon production) within treatments containing resistant and susceptible individuals were comparable to treatments containing only susceptible individuals. The interactions among susceptible *T. tubifex* and *Limnodrilus hoffmeisteri* and the infection prevalence of *T. tubifex* were density dependent. *Tubifex tubifex* had greater infection prevalence in treatments with *L. hoffmeisteri*, but had variable triacinomyxon production. In both interaction experiments, susceptible *T. tubifex* had greater adult growth when interacting with resistant *T. tubifex* or *L. hoffmeisteri* and when exposed to myxospores. This suggests that susceptible *T. tubifex* may have benefited from a low dose of myxospores. The results of these experimental interactions must be now be contrasted with interactions in natural systems. Further, the next task is to characterize oligochaete communities by strain of *T. tubifex* and species of oligochaete, which has the potential to increase our understanding of host-parasite dynamics.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Myxobolus cerebralis, the causative agent of salmonid whirling disease, has caused the decline of two populations of wild rainbow trout (*Oncorhynchus mykiss*) on the Madison River MT and the Colorado River CO. The life cycle of *Myxobolus cerebralis* alternates between two hosts: various salmonid species and one aquatic oligochaete species, *Tubifex tubifex*. The objectives of this study were to determine the threshold myxospore dose needed to achieve complete infection within *Tubifex tubifex* and the influence that interactions among oligochaetes and *M. cerebralis* had on oligochaete fitness and the success of *M. cerebralis*. I examined the threshold myxospore dose of two *T. tubifex* strains experimentally and using nine myxospore doses. I determined that a dose of 50 myxospores per individual *T. tubifex* achieved complete infection in the susceptible strain of *T. tubifex*. Interactions were examined in two response surface competition design experiments using two strains of *T. tubifex*, two species of oligochaetes and various doses of myxospores. The interactions among strains of resistant and susceptible *T. tubifex* were density dependent. The infection prevalence and the proliferation of the parasite (triacinomyxon production) within treatments containing resistant and susceptible individuals were comparable to treatments containing only susceptible individuals. The interactions among susceptible *T. tubifex* and *Limnodrilus hoffmeisteri* and the infection prevalence of *T. tubifex* were density dependent. *Tubifex tubifex* had greater infection prevalence in treatments with *L. hoffmeisteri*, but had variable triacinomyxon production. In both interaction experiments, susceptible *T. tubifex* had greater adult growth when interacting with resistant *T. tubifex* or *L. hoffmeisteri* and when exposed to myxospores. This suggests that susceptible *T. tubifex* may have benefited from a low dose of myxospores. The results of these experimental interactions must now be contrasted with interactions in natural systems. Further, the next task is to characterize oligochaete communities by strain of *T. tubifex* and species of oligochaete, which has the potential to increase our understanding of host-parasite dynamics.

CHAPTER 1

INTRODUCTION

Myxobolus cerebralis (Myxozoa: Myxosporea/ Actinosporea), the cause of salmonid whirling disease (El-Matbouli et al. 1992), was discovered in 1893 in Germany (Nickum 1996). Initially *M. cerebralis* was a serious pathogen of hatchery trout, however modifications in hatchery practices reduced its damaging effects. Throughout the 1980's, it was widely accepted that the parasite was not a threat to wild trout populations (Nickum 1996). However, recent evidence suggest that *M. cerebralis* has caused declines of wild trout (*Oncorhynchus mykiss*) populations in the Intermountain West (USA) (Nehring and Walker 1996, Vincent 1996).

The life cycle of *M. cerebralis* involves two obligatory hosts: various salmonid species and an aquatic oligochaete, *Tubifex tubifex* (Oligochaeta: Tubificidae)(Wolf and Markiw 1984). Because the ecology of the salmonid and *T. tubifex* hosts are linked by *M. cerebralis*, investigations of both hosts are required to fully understand interaction dynamics among all three species (Kerans and Zale 2002, Gilbert and Granath 2003). Studies examining the interactions between salmonids and *M. cerebralis* have revealed important facets of the whirling disease problem. For example, infection in salmonids varies with species, age and environmental variables (Halliday 1973, Markiw 1992, Hedrick et al. 1998, Zendt and Bergersen 2000, Ryce et al. 2001, Krueger 2002, MacConnell and Vincent 2002, Ryce 2003). Research on *M. cerebralis* has focused on

the life cycle, pathology, phylogeny, and conditions that are favorable for the parasite (El-Matbouli et al. 1992, Siddall et al. 1995, El-Matbouli and Hoffmann 1989, El-Matbouli et al. 1999a, Kent et al. 2001). Research on the effects of *M. cerebralis* on *T. tubifex* has revealed infections are persistent, cause reduced growth and reproduction, and strains of *T. tubifex* vary in their susceptibility to infection (Gilbert and Granath 2001, Stevens et al. 2001, Kerans et al. submitted).

Comparatively little research has been conducted on the interactions among oligochaetes and *M. cerebralis*, although such interactions may influence salmonid disease ecology in natural populations. The associations between two species are often affected by the presence of a third species, which may ultimately influence community structure (Price et al. 1986, Miller and Kerfoot 1987, Wootton 1994). Oligochaete interactions that may influence whirling disease ecology include interactions between and among strains of *T. tubifex*, and interactions between *T. tubifex* and other oligochaetes such as *Limnodrilus hoffmeisteri* (Stevens et al. 2001, Kerans et al. submitted). Little is known about the characteristics of aquatic communities that may be related to catastrophic infection in salmonids. It seems reasonable that the probability of infection of a salmonid will depend on a variety of abiotic and biotic factors, including interactions among hosts. If oligochaete assemblages and thus, interactions among oligochaetes differ appreciably among streams with high and low salmonid whirling disease severity, such interactions may influence salmonid infection. By characterizing the interactions among oligochaetes, I hope to make a connection between the mechanisms of infection among salmonid populations exposed to *M. cerebralis*. Our understanding of interactions

and their role in the success of *M. cerebralis* may improve the difficult task of predicting whirling disease incidents and understanding their influence on whirling disease ecology.

The life cycle of *Myxobolus cerebralis*

Originally, *M. cerebralis* was thought to infect only salmonid hosts; however, further research proved that *T. tubifex* was also an obligatory host (Markiw and Wolf 1983, Wolf et al. 1986, El-Matbouli and Hoffmann 1989). *Myxobolus cerebralis* has two distinct spore stages: the triactinomyxon (TAM) and the myxospore. Genetic analyses confirmed that the alternate stages of the parasite belong to a single species (Andree et al. 1997) and revealed little genetic variation between *M. cerebralis* from Europe and North America (Andree et al. 1999). The life cycle of *M. cerebralis* (Fig. 1.1) begins as *T. tubifex* ingests the myxospore stage while consuming sediments of aquatic substrate. Once the myxospore is ingested and inside the lumen of the intestine, it attaches itself to the intestinal mucosa. There, the germ cell moves between cells of the intestinal epithelium and reproduces asexually and sexually. After approximately 70-100 days, depending on *T. tubifex* strain and temperature (El-Matbouli and Hoffmann 1989, Stevens et al. 2001, Kerans et al. submitted, B. L. Kerans and R. I. Stevens, Montana State University, unpublished data), the oligochaete releases a buoyant stage of the parasite into the water, the TAM. The TAM floats in the water and, upon contact with the epidermis of a salmonid; the sporoplasms of the TAM are injected. The parasite migrates through the epidermis to the nervous tissue and finally to the cartilaginous

skeletal system where it produces myxospores (El-Matbouli et al. 1995). The fish may also become infected if it consumes infected oligochaetes (Markiw 1986).

As the infection progresses in the fish, cartilaginous regions are consumed, loss of function ensues and survivability decreases (Hoffman et al. 1962, Hedrick et al. 1998). Clinical signs of the disease include blackened areas of the flesh and skin, deformed skeletal structure and characteristic tail chasing “whirling” behavior (Hoffman et al. 1962). Once the fish dies, the decaying carcass releases *M. cerebralis* myxospores into the sediment where *T. tubifex* may ingest myxospores.

Tubifex tubifex and *Myxobolus cerebralis*

Tubifex tubifex is a widespread and environmentally tolerant oligochaete that is found in most aquatic habitats (Brinkhurst and Jamison 1971, Chapman et al. 1982). In monocultures, the growth and reproduction of *T. tubifex* is density dependent suggesting intraspecific interactions influence population size (Kosiorek 1974, Bonacina et al. 1989). Growth rates, reproduction and environmental versatility vary among *T. tubifex* from different global regions, suggesting that the species is comprised of multiple genetic strains (Poddubnaya 1980, Milbrink 1983). Molecular analysis of populations of *T. tubifex* has demonstrated genetic differences among individuals from various regions in North America and Europe (Anlauf 1994, 1997, Sturmbauer et al. 1999, Erséus et al. 2000, Beauchamp et al. 2001, Beauchamp et al. 2002, Kerans et al. submitted). Studies

of local populations of *T. tubifex* on the upper Colorado River, Colorado suggest *T. tubifex* are comprised of multiple strains (Beauchamp et al. 2002).

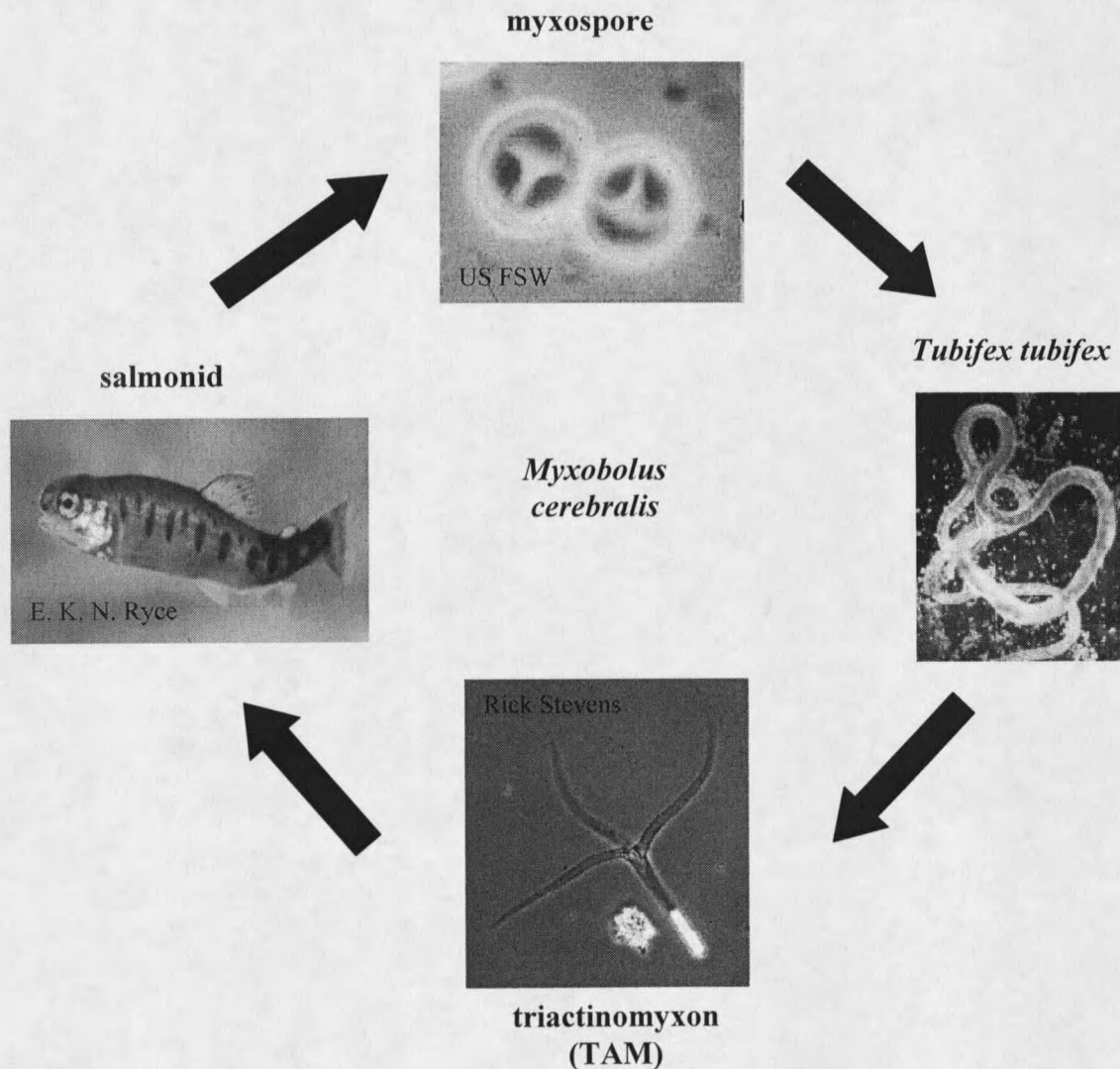


Figure 1.1 – The life cycle of the parasite *Myxobolus cerebralis*. The parasite has two distinct transmission stages, the myxospore and the triactinomyxon, which are infective to the obligatory hosts *Tubifex tubifex* and many salmonid species, respectively.

The interactions between individual strains of *T. tubifex* and *M. cerebralis* have been examined. The effects of *M. cerebralis* on *T. tubifex* are negative and the strength of the effect is positively related to TAM production (Kerans et al. submitted). Susceptible strains of *T. tubifex* that produce high numbers of TAMs have lower fitness than uninfected susceptible individuals. Moderately susceptible *T. tubifex* that produce few TAMs have lower fitness than uninfected individuals (Stevens et al. 2001, Kerans et al. submitted). Some strains of *T. tubifex* do not produce TAMs and have not been infected (Beauchamp et al. 2002). However, the fitness of resistant *T. tubifex* challenged with myxospores is unknown. The decrease in fitness among susceptible *T. tubifex* may be the result of increased mortality or decreased reproductive output due to parasitism. *Tubifex tubifex* exhibit low mortality if exposed to low doses of myxospores, and mortality increases only with very high myxospore doses (Gilbert and Granath 2001, Stevens et al. 2001). Furthermore, Kerans et al. (submitted) suggest that reduced reproductive output of infected *T. tubifex* is the cause of fitness reduction.

The effects of *T. tubifex* on *M. cerebralis* are primarily positive and appear directly related to the susceptibility of *T. tubifex*. *Myxobolus cerebralis* is successfully transmitted by susceptible and moderately susceptible *T. tubifex* that produce TAMs, whereas, the transmission success of *M. cerebralis* decreases with resistant *T. tubifex* that do not produce TAMs. Several factors may be responsible for the fact that resistant *T. tubifex* do not produce TAMs: myxospores may not be able to invade gut tissue, myxospores may invade but are destroyed by immune response or oligochaetes may have the capability of deactivating myxospores (M. El-Matbouli, University of Munich,

personal communication). Therefore, the interactions among resistant and susceptible *T. tubifex* could reduce infection among susceptible individuals because resistant individuals may deactivate myxospores or reduce the overall TAM production because they consume myxospores. Plausibly, communities of tubificids containing strains of *T. tubifex* that vary in susceptibility could influence the success of *M. cerebralis* and therefore infection in salmonids. It is unknown how mixed communities of resistant and susceptible *T. tubifex* can affect the success of *M. cerebralis* or how *M. cerebralis* affects the success of particular strains of *T. tubifex*. Consequently, it is important to genetically catalogue and understand oligochaete communities throughout areas where *M. cerebralis* has been detected (Beauchamp et al. 2002, Kerans et al. submitted).

Interactions among strains of *T. tubifex* exposed to *M. cerebralis* are likely to differ from those not exposed to myxospores. I expect infected *T. tubifex* to have reduced growth and reproduction compared to uninfected *T. tubifex*, consistent with findings of Stevens et al. 2001. I would expect the competitive ability of susceptible *T. tubifex*, when growing in combination with resistant *T. tubifex*, to be lower when susceptible oligochaetes were infected than when not infected. Further, if resistant *T. tubifex* can deactivate myxospores then its presence could reduce the infection prevalence among susceptible *T. tubifex*. Therefore, comparing and contrasting interactions of various strains of *T. tubifex* and their subsequent production of *M. cerebralis* may provide us with information of how infection levels can vary among *T. tubifex* within streams and potentially how this relates to salmonid infection. Experimental inter-strain interactions must then be compared to interactions in natural systems. Understanding oligochaete

interactions has the potential to improve our abilities to predict potential whirling disease outbreaks and disease severity in salmonids, and further characterize host-parasite interactions among varying host strains.

Tubifex tubifex, *Limnodrilus hoffmeisteri* and
Myxobolus cerebralis

Many studies of tubificid ecology, conducted throughout the past century (e.g., Kennedy 1966, Chapman et al. 1982, Bonacina et al. 1989), have examined life history, tubificid response to environmental pollutants, population dynamics and interactions among tubificid species. *Tubifex tubifex* and *Limnodrilus hoffmeisteri* (Oligochaeta: Tubificidae), two oligochaetes that typically inhabit stream communities, have similar distributions, physical attributes, and functional roles but different ecological requirements (Brinkhurst and Jamieson 1971, Block et al. 1982, Mermillod-Blondin et al. 2001). For example, both oligochaetes are deposit feeders but each prefers different sediment sizes and depths within the substrate (Mermillod-Blondin et al. 2001). In monocultures, individual growth and reproduction of *T. tubifex* and *Limnodrilus* sp. are density dependent suggesting intraspecific competition influences oligochaete populations (Kosiorek 1974, Poddubnaya 1980, Bonacina et al. 1989).

Interactions between *L. hoffmeisteri* and *T. tubifex* are apparent in mixed cultures, where their respiration rates are lower and population growth rates increase compared to monoculture each species (Brinkhurst 1974, Milbrink 1987), suggesting a facultative

mutualism with each species providing the other with beneficial food resources (Milbrink 1987). However in other experiments, *L. hoffmeisteri* depressed the growth of *T. tubifex* when *M. cerebralis* was not present suggesting that nutrients or space may have been limiting factors for these mixed oligochaete cultures (B.L Kerans and J.C. Lemmon, Montana State University, unpublished data). Conversely, when *M. cerebralis* was present, *T. tubifex* grew much better in the presence of *L. hoffmeisteri* than in pure culture, suggesting facultative mutualism (B.L Kerans and J.C. Lemmon, Montana State University, unpublished data). The complex and variable interactions between *T. tubifex* and *L. hoffmeisteri* warrant further investigation, particularly in their influence on *M. cerebralis* success.

The occurrence of both positive and negative interactions between *T. tubifex* and *L. hoffmeisteri* suggest that the interactions can vary with biotic factors. Shifts between positive and negative interactions along environmental gradients have been documented among plants and marine invertebrates (e.g. Callaway and Walker 1997, Dudgeon and Petraitis 2001). Further, species interactions comprised of both competitive and facultative components can vary spatially and temporally (Bruno et al. 2003), depending on environmental conditions, indirect interactions, and the density and diversity of species (Callaway and Walker 1997). This suggests the importance of observing interactions under various conditions simultaneously to properly assess the types of interactions that may be occurring.

If the primary interaction between *L. hoffmeisteri* and *M. cerebralis* is mutualistic, I predict that the interactions among uninfected *T. tubifex* and *L. hoffmeisteri* to result in

enhanced growth of both species compared to growth in monocultures. As competitors, uninfected *T. tubifex* and *L. hoffmeisteri* would have depressed growth compared to monocultures. As mutualists, infected *T. tubifex* compared to uninfected *T. tubifex* would have reduced growth and provide less benefit to *L. hoffmeisteri*. Additionally, the growth of infected *T. tubifex* would be less than *L. hoffmeisteri* (Stevens et al 2001), due to decreased competitive ability of *T. tubifex*. Competitive interactions will result in lower growth of *T. tubifex*, than mutualist interactions.

Because *L. hoffmeisteri* cannot be infected by *M. cerebralis* (Wolf et al. 1986, Kerans et al. submitted), it is possible that *L. hoffmeisteri* deactivates myxospores by digestion as it feeds. The feeding activity of *L. hoffmeisteri* may reduce myxospore abundance and alter the prevalence of infection in *T. tubifex* populations. Therefore, I would expect the growth of *T. tubifex* grown with *L. hoffmeisteri* to be less depressed compared to monoculture because *L. hoffmeisteri* has deactivated myxospores and hence minimized the negative effects of *M. cerebralis* infection upon *T. tubifex*. Thus, the type of oligochaete interaction and the ability of *L. hoffmeisteri* to deactivate myxospores will influence the success of *M. cerebralis*. For example, the success of *M. cerebralis* will be greatest in a community where *T. tubifex* is present and *L. hoffmeisteri* does not deactivate myxospores.

The objectives of my work were to:

1. Examine the interactions among strains of *Tubifex tubifex* and *Myxobolus cerebralis*.

2. Examine the interactions among *Tubifex tubifex*, *Limnodrilus hoffmeisteri* and *Myxobolus cerebralis*.
3. Determine the threshold dose of myxospores to achieve complete infection among *Tubifex tubifex*.

The purpose of objectives one and two were to determine the relative importance of interactions on the success of *T. tubifex* and *M. cerebralis* transmission. Each is addressed in a separate chapter. Objective three determined the doses of myxospores I used in the other objectives and is addressed in Chapter 3. The final chapter, Conclusions, integrates the various sections, provides suggestions for applying this work in management situations and possible future research. This research will hopefully reveal how oligochaete interactions influence *M. cerebralis* success and potentially provide information for predicting future *M. cerebralis* infections based on oligochaete communities.

CHAPTER 2

OBJECTIVE 1: INTERACTIONS AMONG TWO STRAINS OF TUBIFEX TUBIFEX
AND MYXOBOLUS CEREBRALISIntroduction

The dynamics of host-parasite interactions may influence host population growth and host evolution (Anderson and May 1979, May and Anderson 1979, May and Anderson 1990). The virulence of a parasite depends on the relative susceptibility of a specific host, parasite phenotype, host density and competition among parasites within hosts (Ewald 1983, Bull 1994). Moreover, the evolution of host susceptibility and parasite success depends on the variability of virulence among host-parasite combinations (Vandame et al. 2000). Further, the variation among host susceptibility and parasite virulence may affect the outcome of interactions among hosts (Ebert et al. 1998, Yan et al. 1998).

Parasite-mediated competition among hosts was first noted in Park's (1948) classical experiment with two species of tribolium beetle and a protozoan parasite, showing that a parasite could reverse the outcome of competition between two beetle species. Later research supports the importance of parasite-mediated interactions among competitors (e.g., Settle and Wilson 1990, Grosholz 1992, Schall 1992, Yan et al. 1998).

Models of parasite-mediated competition predict that in the presence of a parasite, hosts with low susceptibility will be superior competitors to hosts with high susceptibility (Yan 1996). However, interactions among hosts depend on the environment and host life history parameters, such as geographic range, population density, growth rate and body size (Price et al. 1988, Begon et al. 1992, Bruno et al. 2003). For example, when a parasite common to two host species has a differential impact upon host fitness, the host species with a wide geographic range will displace host species with a narrow range because of population size and associated immune response (Price et al. 1988). The geographic-range hypothesis assumes that host species with a large geographic range will have a higher effective population size than the host species with a small geographic range. Consequently, the host with the larger geographic range and population size can maintain parasitic populations and stimulate rapid immune response (Price et al. 1988).

I examined intra-strain and inter-strain variation in the response of *Tubifex tubifex* to parasitism by *Myxobolus cerebralis*. *Myxobolus cerebralis* (Myxozoa: Myxosporaea /Actinosporaea) causes whirling disease in salmonids (El-Matbouli et al. 1992) and alternates between two obligate hosts: various salmonid species and one known aquatic oligochaete species, *Tubifex tubifex* (Oligochaeta: Tubificidae) (Markiw and Wolf 1983, Wolf and Markiw 1984). At least two populations of wild rainbow trout (*Oncorhynchus mykiss*) within the Intermountain West (USA) have declined as a result of *M. cerebralis* (Nehring and Walker 1996, Vincent 1996). *Myxobolus cerebralis* from various locations in Europe and North America exhibit very little genetic variation (Andree et al. 1999).

Myxobolus cerebralis has a complex life cycle with two spore stages, the myxospore and the triactinomyxon (TAM). *Tubifex tubifex* ingests the myxospore stage while consuming sediments. Parasite reproduction occurs between epithelial cells of the intestine of *T. tubifex*. After approximately 70-100 days, depending on *T. tubifex* strain and temperature (El-Matbouli and Hoffmann 1989, El-Matbouli et al. 1999a, Stevens et al. 2001, Kerans and Stevens in preparation), the buoyant stage of the parasite, the triactinomyxon (TAM), is released into the water column. The TAM floats in the water and when a salmonid is contacted, sporoplasms of the TAM are injected into the epidermis. The degradation of the cartilaginous skeletal system occurs as myxospores are produced (El-Matbouli et al. 1995). Myxospores are released into the sediment after infected salmonids die and their carcasses decompose.

Tubifex tubifex is a widespread and environmentally tolerant oligochaete that is found in most aquatic habitats (Brinkhurst and Jamison 1971, Chapman et al. 1982). In monocultures, the growth and reproduction of *T. tubifex* is density dependent suggesting intraspecific interactions influence populations (Kosiorek 1974, Bonacina et al. 1989). However, populations of *T. tubifex* are distinguished by genetic variation (Sturmbauer et al. 1999, Beauchamp 2001). Strains of *T. tubifex* differ in reproduction, growth rates and environmental versatility (Poddubnaya 1980, Milbrink 1983). Further, molecular analysis has demonstrated genetic differences among individual *T. tubifex* from North America and Europe (Anlauf 1994, 1997, Sturmbauer et al. 1999, Erséus et al. 2000, Beauchamp et al. 2001, Beauchamp et al. 2002, Kerans et al. submitted) and studies of

local populations of *T. tubifex* in the Colorado River CO showed that *T. tubifex* populations are comprised of multiple strains (Beauchamp et al. 2002).

The effects of *M. cerebralis* on *T. tubifex* are negative and the strength of the effects is related to TAM production (Stevens et al. 2001, Kerans et al. submitted). Some strains of *T. tubifex* do not produce TAMs and cannot be infected (Beauchamp et al. 2002). Further, the fitness of resistant *T. tubifex* challenged with myxospores is unknown. The decrease in fitness among susceptible *T. tubifex* may be the result of increased mortality or decreased reproductive output. *Tubifex tubifex* exhibit low mortality if exposed to low doses of myxospores, and mortality increases only with very high myxospore doses (Gilbert and Granath 2001, Stevens et al. 2001). Furthermore, Kerans et al. (submitted) suggest that reduced reproductive output is the main cause of fitness reduction.

The effects of *T. tubifex* on *M. cerebralis* are primarily positive, depending on the susceptibility of *T. tubifex*. *Myxobolus cerebralis* is successfully transmitted by susceptible and moderately susceptible *T. tubifex* that produce TAMs, whereas the transmission success of *M. cerebralis* decreases with resistant *T. tubifex* that do not produce TAMs. Resistant *T. tubifex* that do not produce TAMs suggest immunity to myxospore invasion or a capability to deactivate myxospore prior to invasion (M. El-Matbouli, University of Munich, personal communication). Therefore, the interactions among resistant and susceptible *T. tubifex* could reduce infection among susceptible individuals because resistant individuals deactivate myxospores or reduce the overall TAM production because they consume myxospores. Plausibly, tubificid communities

that vary in susceptibility could influence the success of *M. cerebralis* and therefore infection in salmonids. It is unknown how mixed communities of resistant and susceptible *T. tubifex* affect the success of *M. cerebralis* or how *M. cerebralis* affects the success of particular strains of *T. tubifex*. Consequently, it is important to genetically catalogue and understand oligochaete communities throughout areas where *M. cerebralis* has been detected (Beauchamp et al. 2002, Kerans et al. submitted).

I conducted a laboratory experiment that examined the interactions among susceptible and resistant *T. tubifex* and *M. cerebralis*. The major objectives of this research were to determine: 1) whether the interactions between strains of *T. tubifex* differed in strength or direction when they were exposed and not exposed to *M. cerebralis*, 2) whether resistant *T. tubifex* influenced the transmission of *M. cerebralis* by susceptible *T. tubifex*, and 3) whether resistant *T. tubifex* can be infected when exposed to high doses of myxospores.

I predict susceptible and resistant *T. tubifex* would exhibit intra-strain and inter-strain density dependent competition. Further, the addition of *M. cerebralis* would not affect intra-strain interactions. However, I predict parasitized, susceptible *T. tubifex* to have reduced growth and reproduction compared to unparasitized, susceptible *T. tubifex* or resistant *T. tubifex*. With the addition of resistant *T. tubifex*, susceptible *T. tubifex* would have reduced infection compared to susceptible *T. tubifex* in monoculture due to deactivation of myxospores by resistant *T. tubifex*. Further, I predict resistant *T. tubifex* would become infected when exposed to extremely high doses of myxospores. Finally, I predict the success of *M. cerebralis* to be greater in treatments with only susceptible *T.*

tubifex compared to treatments with both resistant and susceptible *T. tubifex* because of greater production of TAMs by susceptible individuals.

Methods

Experimental procedures

I used two strains of *T. tubifex*: strain A that had produced large numbers of TAMs in previous experiments (Stevens et al. 2001, Kerans et al. submitted) and strain B that had been reported as not susceptible to *M. cerebralis* infection (Beauchamp et al. 2002), both from disease-free, pure laboratory cultures. Strain A was collected from the Mt. Whitney California State fish hatchery settling ponds, where *M. cerebralis* has been enzootic since 1984 (Modin 1998). Strain B was obtained from bays of Lakes Erie and Ontario where *M. cerebralis* was first documented in the 1950's in wild salmonids (Reynoldson et al. 1996, R. Nelson, United States Fish and Wildlife Service, La Crosse Wisconsin, personal communication). These cultures have been maintained in our laboratory since 1997 (see Stevens et al. 2001, Kerans et al. submitted, for detailed culture propagation methods).

Myxobolus cerebralis myxospores were extracted from infected rainbow trout using continuous centrifuge methods (O'Grodnick, 1975). This process results in an emulsion containing myxospores. Trout originated at a Montana State fish hatchery, Ennis MT and were infected in our laboratory with TAMs from the United States Fish

and Wildlife Service, Fish Health Laboratory, Bozeman MT. Using 1 μ l of extracted myxospore emulsion, I determined the abundance of myxospores by counting all viable myxospores on a hemacytometer with a compound microscope (400X magnification). The total number of myxospores in the emulsion was extrapolated from the mean number of myxospores from four hemacytometer counts and was used to create the required experimental doses. The emulsion was evenly mixed with a stirring plate as myxospores were removed with an automated pipette and administered to oligochaetes.

The laboratory experiment examined the intra-strain and inter-strain interactions between strain A and strain B *T. tubifex* when myxospores were present and absent. Fifty *T. tubifex* A, 100 *T. tubifex* A, 50 *T. tubifex* A + 50 *T. tubifex* B, 50 *T. tubifex* B and 100 *T. tubifex* B were exposed to 0 (3 replicates) or 2500 (4 replicates) myxospores per container, in a response surface competition design (Goldberg and Scheiner 2001). Oligochaete densities were within the natural range found in streams (6,000 to 13,000 individuals/m²) (Lazim and Learner 1986). Experimental densities of 50 and 100 individuals per container translate to natural densities of 6180 and 12,360 individuals/m². I chose the dose of 2500 myxospores per container because it is below the threshold of 100 % infection (See Chapter 3, Results), which allowed me to observe interactions between *T. tubifex* when myxospores were abundant enough to cause high infection prevalence, but not so abundant that the effects of the interactions would be obscured due to excess myxospores. A response surface design allowed me to partition and evaluate intra-strain and inter-strain interaction effects. For example by comparing measures of fitness of each strain between densities of 50 and 100, I can examine intraspecific

interactions. Alternatively, by comparing measures of fitness between densities of 100 strain A and 50 strain A + 50 strain B, I can examine inter-strain interactions.

Additionally, I prepared one treatment with three replicates of strain B at a density of 100 that were exposed to 50,000 myxospores per container to infect resistant strain B *T. tubifex* and to examine how high doses affect the fitness of this strain.

At the beginning of the experiment, oligochaetes were removed from mass cultures and were placed in plastic containers for 24 hours without substrate to equalize hunger levels. I then wet weighed *T. tubifex* in groups of 50 to the nearest 0.0001 gram. The appropriate numbers of oligochaetes were placed in plastic containers ($9 \times 9 \times 5$ cm) with 40 ml of sand, 1 ml of organic matter, and 200 ml of dechlorinated tap water and inoculated with myxospores. Treatments without myxospores were inoculated with a similar quantity of emulsion from parasite-free rainbow trout. Oligochaetes were exposed to myxospores for 5 days in accordance with previous myxospore exposure procedures (see Chapter 3). Thereafter, all oligochaetes were removed from each container and placed in a new container with fresh substrate and water.

I maintained containers, supplied with a constant flow of air, in incubators at 15°C with a 12:12 h L:D photoperiod. Oligochaetes were fed 0.10 to 0.12 grams of dried *Spirulina* weekly, which is sufficient for growth and reproduction (J. C. Lemmon, Montana State University, personal communication). I replaced approximately 95 % of the water from each container with fresh dechlorinated water weekly.

Prior to expected TAM production, on day 65 post-exposure to myxospores, I began to scan for TAMs in each replicate. The water from each container was poured

separately through a 20 μ l sieve that retained TAMs. Triactinomyxons were back flushed and concentrated in 10 ml of water. I scanned water from two, 55 μ l aliquots with a phase contrast microscope (100X magnification) every three days until TAMs were found. Once TAMs were detected, I began counting TAMs weekly. I did TAM counts on all replicates until day 108 post-exposure. After day 108 post-exposure, I counted TAMs from two replicates for each myxospore positive treatment and all three replicates for each treatment that were not exposed to myxospores (see below). Average TAM production per replicate was recorded as the mean number of TAMs counted from two slides each week, which was extrapolated to determine the quantity of TAMs contained in the TAM concentrate. Total TAM production was calculated by summing the average TAM production per replicate per week.

On day 108 post-exposure, two replicates from each treatment that were exposed to myxospores were randomly chosen to determine the prevalence of infected *T. tubifex*. Adult oligochaetes were removed from plastic containers and placed individually in 12-well plates, with 4 ml capacity. After two days, to allow TAMs to accumulate in the wells, I scanned plates with a dissecting microscope (10X magnification) and recorded individuals as positive if TAMs were visible in the well. I scanned plates two times within a 10-day period, each time replacing the wells with fresh dechlorinated water. The prevalence of infected *T. tubifex* was calculated for each replicate by dividing the number of individuals producing TAMs by the total number of *T. tubifex* observed. I measured three response variables for each strain of *T. tubifex* to assess TAM production and

infection prevalence of *M. cerebralis*. These response variables were based on equation (2.1).

$$\begin{aligned} \text{Total TAM production / days observed} = \\ & [\text{susceptible strain A (infection prevalence)(initial density)(total TAMs produced /} \\ & \{\text{number of infected individuals*days observed}\}) + \text{resistant strain B (infection} \quad (2.1) \\ & \text{prevalence) (initial density)(total TAMs produced / \{number of infected} \\ & \text{individuals*days observed}\})] \end{aligned}$$

Total TAM production per day was calculated by dividing the total TAMs produced per replicate by the days observed (hereafter referred to as "TAM production"). Total TAMs produced per infected individual per day was calculated by dividing the total TAMs produced by the number of infected individuals and the days observed (hereafter referred to as "TAMs per individual"). Prevalence of infection was calculated by dividing the total number of individual adults producing TAMs by the number of adults observed. Triactinomyxon response variables from the 50 strain A + 50 strain B treatments were calculated based on a density of 50 because the molecular assay showed that only *T. tubifex* strain A produced TAMs (See Results).

At the termination of a replicate (either day 108 or day 135 post-exposure), all adults and progeny were weighed to the nearest 0.0001 gram. Further, because strains of *T. tubifex* are physically identical, all adults from 50 A *T. tubifex* + 50 B *T. tubifex* treatments were analyzed with molecular markers to identify the strain of each individual (see Molecular Analyses). Further, fifty or thirty randomly chosen progeny from each

replicate were analyzed using molecular markers to determine which strain of progeny were produced.

The effects of host-parasite interactions are often associated with reduced growth and reproductive success (Forbes 1993). I measured three responses to assess the effects of interactions among strains of *T. tubifex* and *M. cerebralis* on the fitness of the oligochaetes: 1) total biomass production per initial individual per day ($((\text{final adult weight} - \text{initial adult weight}) + \text{final progeny weight}) / \text{initial density of adults} \times \text{days of growth}$) (hereafter referred to as “total biomass”), 2) adult growth per initial individual per day ($((\text{final adult weight} / \text{final density}) - (\text{initial adult weight} / \text{initial density of adults})) / \text{days of growth}$) (hereafter referred to as “adult growth”), and 3) progeny biomass produced by an individual adult per day ($\text{final progeny weight} / \text{initial density of adults} \times \text{days of growth}$) (hereafter referred to as “progeny biomass”). I separated total biomass into response variables of adult growth and progeny biomass to determine how each factor influenced total biomass. Each set of response variables was measured for each strain of *T. tubifex* separately. Because significant mortality typically occurs only when myxospore doses are high (Stevens et al. 2001), I did not examine oligochaete mortality as a response variable, and mortality during this experiment was 2 % (± 1 % SD). I calculated mortality by subtracting the abundance of oligochaetes at the start from the abundance of oligochaetes at the end in each replicate and averaged among all replicates. Oligochaete strain-density combinations will hereafter be referred to as “oligochaete combination”.

Molecular Analyses

To identify the strain of each *T. tubifex* within 50 strain A + 50 strain B treatments, I used a maximum of three randomly amplified polymorphic DNA (RAPD) primers that provided ample banding differences between the strains. Genomic DNA was extracted from whole adult and progeny *T. tubifex* using Nucleospin® Multi-96 tissue kits as per BD Biosciences Clontech protocol (PT3629-1) and was frozen until needed. I assessed the quantity and quality of DNA using gel electrophoresis with 5.0 µl of sample DNA on 1.5 % agarose gels with 0.5 X Tris-acetate (TAE) buffer. Two types of DNA ladders were used to compare DNA samples: 0.5 µl each of 1Kb and hyperladder I Bioline™. Ethidium bromide was used to stain gels and visualize the DNA bands with ultra-violet light. All gels were documented using Lab Works® program with a UVP EPI-Chemi dark room.

Only one RAPD primer was necessary to compare and assess the genetic composition from polymerase chain reaction (PCR) amplified adult *T. tubifex* DNA. The RAPD primer consists of the following base pairs; 5'-GGG CCG TTT A-3' (see Rasmussen et al. 1998 for details of RAPD primer development). The PCR reaction mixture contained the following standard reagents: Sigma™ 1X buffer without MgCl₂, 2.5 mM MgCl₂, 200 µM dNTP's, 20 picomoles of RAPD primer, Research Genetics Inc. Rediload™, 1.0 unit of Sigma™ Taq DNA polymerase and either 0.5 or 1.0 µl of sample DNA. All PCR amplification followed the subsequent procedure on a MJ Research, Inc. PT-100 thermocycler (Watertown, MA): 1) 93°C for 3 minutes, 2) 93°C for 15 seconds,

3) decrease by 47°C degrees at a rate of 0.4°C per second, 4) 46°C for 1 minute 30 seconds, 5) increase by 26°C at a rate of 0.4°C per second, 6) 72°C for 1 minute 30 seconds, 7) repeat steps 2 through 6, 34 times, 8) hold at 15°C. Following PCR amplification, products were visualized by electrophoresis on 2% 0.5 X TAE agarose gels.

Each strain of *T. tubifex* from 50 strain A *T. tubifex* + 50 strain B *T. tubifex* treatments was distinguished by different banding patterns from PCR products. All individuals were allocated to a strain and the proportion of each strain was calculated by dividing the number of each strain identified by PCR by the total number of *T. tubifex* identified. The proportion of each strain could then be applied to biomass calculations.

To determine the strain of each progeny, I analyzed individual progeny using three RAPD primers with the following sequences; #18: 5'-GGG CCG TTT A-3', #2: 5'-CCT GGG CTT G-3', and #54: 5'-GTC CCA GAG C-3'. Three primers were used to reduce the possibility of not recognizing a hybrid progeny. Considering the number of banding pattern differences (at least two between each strain among each primer), I had a 1 in 8 ($\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$) chance of not recognizing a hybrid progeny. I calculated the relative proportion of each strain by dividing the identified strain number by the total number of progeny amplified which was then applied to calculate the total number of progeny produced per strain.

Statistical analyses

Because replicates used for infection prevalence had a shorter growth period, biomass response variables were tested for differences between the days of growth, (108 or 135 days) and among oligochaete combinations using two-factor analyses of variance (PROC GLM, SAS Institute, 2001). Total biomass, adult growth or progeny biomass production did not differ between days of growth, oligochaete combinations or with the interaction between the two factors (Appendix A). Therefore, data were combined and each biomass variable was tested for significant differences among oligochaete combination and myxospore dose using two-factor analyses of variance (ANOVA, SAS Institute, 2001). The treatment of 100 strain B *T. tubifex* with 50,000 myxospores was not used in the calculation of the biomass response variables of strain B because their inclusion created imbalance in the ANOVA analyses model. Residuals were examined for normality and variances stabilized with log or square root transformations, where necessary. To examine the fitness of strain B at high doses, I compared total biomass, adult growth and progeny biomass of 100 strain B exposed and not exposed to myxospores using a t-test for significant differences between myxospore doses of 0 and 50,000 (SAS Institute, 2001).

Because two replicates from each treatment were removed from the daily calculation of TAM production on day 108, total TAM production was initially tested for differences in the number of days observed producing TAMs (end day of 108 or 135) and among oligochaete combinations using two-factor ANOVA (SAS Institute, 2001).

Triactinomyxon production did not differ among oligochaete combinations, days observed nor with the interaction between the two factors (Appendix A). Therefore, all replicates were combined for analyses. Triactinomyxon production, TAMs produced per individual and prevalence of infected *T. tubifex* were tested for significant differences among oligochaete combinations using one-way ANOVAs (SAS Institute, 2001). The response variables were examined for normality and did not require transformation. Tukey's Honestly Significant Differences tests (Tukey's HSD) were used in all analyses to determine differences among treatment levels when main effects were significant ($P < 0.05$).

Results

The total biomass of strain A was highest at densities of 50 individuals and decreased as densities increased to 100 with the addition of either strain of *T. tubifex*. (Table 2.1, Tukey's HSD, $P < 0.05$, Fig 2.1a). Total biomass did not differ between myxospore doses, nor with the interaction between oligochaete combination and myxospore dose (Table 2.1).

The adult growth of strain A was highest at densities of 50 alone or when in combination with 50 strain B, and lowest in 100 strain A treatments (Table 2.1, Tukey's HSD, $P < 0.05$, Fig 2.1b). Growth of adults was greatest when exposed to myxospores (Table 2.1, Fig 2.1b). The interaction between oligochaete combination and myxospore dose was not significant.

Table 2.1 - Analysis of variance results for the effects of oligochaete combination and myxospore dose on biomass response variables. All biomass response variables are expressed as a per capita daily rate.

Response variable	Oligochaete strain* (†)	Source of variation	ss §	F	df	P
Total biomass	A	dose	0.0009	0.24	1	0.62
		oligochaete combination	0.2569	32.64	2	<0.0001
		interaction	0.0165	2.10	2	0.15
		error	0.0590		15	
	B	dose	0.0002	0.10	1	0.75
		oligochaete combination	0.1824	34.65	2	<0.0001
		interaction	0.0044	0.84	2	0.45
		error	0.0394		15	
Adult growth	A (sqrt)	dose	2.16E-06	7.46	1	0.01
		oligochaete combination	2.65E-06	4.57	2	0.02
		interaction	8.13E-06	1.40	2	0.27
		error	4.36E-06		15	
	B (ln)	dose	1.47E-09	0.19	1	0.67
		oligochaete combination	4.59E-08	2.89	2	0.08
		interaction	8.64E-09	0.54	2	0.59
		error	1.19E-07		15	
Progeny biomass	A	dose	0.0044	1.51	1	0.23
		oligochaete combination	0.1885	32.04	2	<0.0001
		interaction	0.0120	2.04	2	0.16
		error	0.0441		15	
	B	dose	0.0053	1.62	1	0.22
		oligochaete combination	0.1502	22.71	2	<0.0001
		interaction	0.0027	0.42	2	0.66
		error	0.0496		15	

* A represents strain A *T. tubifex*, B represents strain B *T. tubifex*

(†)=the transformation: (sqrt)= square root, (ln)=log

§ = sums of squares

Strain A *T. tubifex* produced greater biomass of progeny at densities of 50 individuals, and as densities increased to 100 with the addition of either strain, the progeny biomass decreased (Table 2.1, Tukey's HSD, $P < 0.05$, Fig 2.1c). Progeny biomass did not differ between myxospore doses, nor with the interaction between the two factors (Table 2.1, Fig. 2.1c).

The total biomass of strain B was highest at densities of 50 and lowest at densities of 100 individuals of strain B or in combination with strain A (Table 2.1, Tukey's HSD, $P < 0.05$, Fig 2.1d). Total biomass did not differ between myxospore doses, nor with the interaction between the two factors (Table 2.1, Fig 2.1d). However, when comparing the total biomass between 0 and 50,000 myxospore doses, the total biomass of strain B was greatest when exposed to 0 myxospores (Mean $\pm$ 1 SE: 0.2316 mg / individual*day $\pm$ 0.015, $n = 3$) and lower at a dose of 50,000 myxospores (Mean $\pm$ 1 SE: 0.1377 mg /individual*day $\pm$ 0.011, $t_4 = 4.95$, $P = 0.0078$, $n = 3$).

The adult growth of strain B did not differ between oligochaete combinations, myxospore doses, nor with the interaction between the two factors (Table 2.1, Fig 2.1e). Furthermore, when comparing the adult growth between 50,000 and 0 myxospores, the adult growth of strain B did not differ ($t_4 = 1.24$, $P = 0.2817$, $n = 3$).

Strain B *T. tubifex* produced greater biomass of progeny at densities of 50 individuals and as densities increased to 100 with the addition of either strain the progeny biomass decreased (Table 2.1, Tukey's HSD, $P < 0.05$, Fig 2.1f). Progeny biomass did not differ between myxospore doses, nor with the interaction between the two factors (Table 2.1). However, strain B produced greater progeny biomass when exposed to 0

myxospores (Mean $\pm$ 1 SE: 0.2057 mg / individual*day $\pm$ 0.014, n = 3) than when exposed to 50,000 myxospores (Mean $\pm$ 1 SE: 0.1189 mg / individual*day $\pm$ 0.006, t_4 = 5.43, P = 0.0056, n = 3).

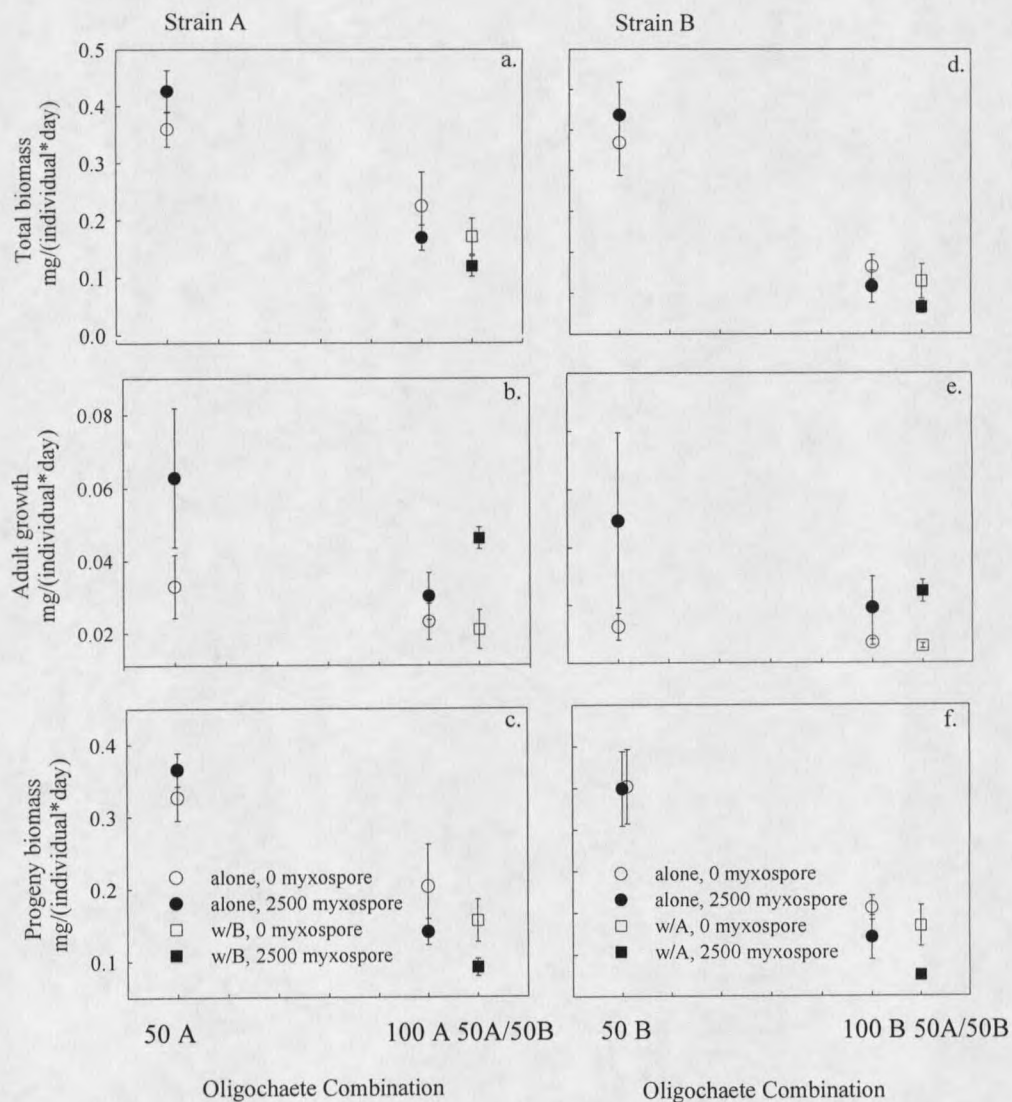


Figure 2.1 - The effects of *Tubifex tubifex* strain, density and relative abundance of the two strains and *Myxobolus cerebralis* infection on biomass variables. Each column illustrates the particular variable for the target strain listed, a) total biomass, b) adult growth, c) progeny biomass, d) total biomass, e) adult growth, and f) progeny biomass. Error bars are one standard error of the mean.

I determined that individuals producing TAMs in the 50 strain A + 50 strain B treatments were all strain A *T. tubifex* using a randomly amplified polymorphic DNA assay. Total TAM production (Table 2.2, ANOVA $F_{2,3} = 1.08$, $P = 0.3795$), TAMs produced per individual (Table 2.2, ANOVA $F_{2,3} = 0.55$, $P = 0.5968$) and prevalence of infected strain A individuals (Table 2.2, ANOVA $F_{2,3} = 1.01$, $P = 0.4621$) did not vary with oligochaete combination. No TAMs were found in any of the control treatments.

Table 2.2 - Total triactinomyxons produced per day, infection prevalence and the triactinomyxons produced per infected individual per day. In the 50 strain A + 50 strain B treatment, triactinomyxon production and infection prevalence were calculated based on the fact that only the 50 susceptible *Tubifex tubifex* (strain A) produced TAMs. Strain B did not produce TAMs.

Oligochaete combination	Total TAMs per day (± 1 SE)	Infection prevalence (± 1 SE)	TAMs produced per infected individual per day (± 1 SE)
50 strain A <i>T. tubifex</i>	17586.73 (± 4536.66)	0.41 (± 0.10)	819.79 (± 148.59)
100 strain A <i>T. tubifex</i>	16261.41 (± 3180.77)	0.26 (± 0.07)	637.07 (± 121.21)
50 strain A/50strain B	10935.34 (± 1921.5)	0.31 (± 0.04)	682.77 (± 113.18)

The range of total TAM production per replicate for each oligochaete combination is as follows: 50 strain A, 382,515.04 – 1,271,757.36; 100 strain A, 366,454.40 – 1,531,636.02; and 50 strain A + 50 strain B, 220,454.50 – 967,136.29 (Appendix B).

A total of 239 progeny were assayed from oligochaete combinations containing strain A and strain B *T. tubifex* and revealed 2 individuals from the same replicate shared

bands of both strain A and strain B *T. tubifex* (Fig. 2.2). Strain A and strain B *T. tubifex* produced similar numbers of progeny regardless of the oligochaete combination (Appendix C).

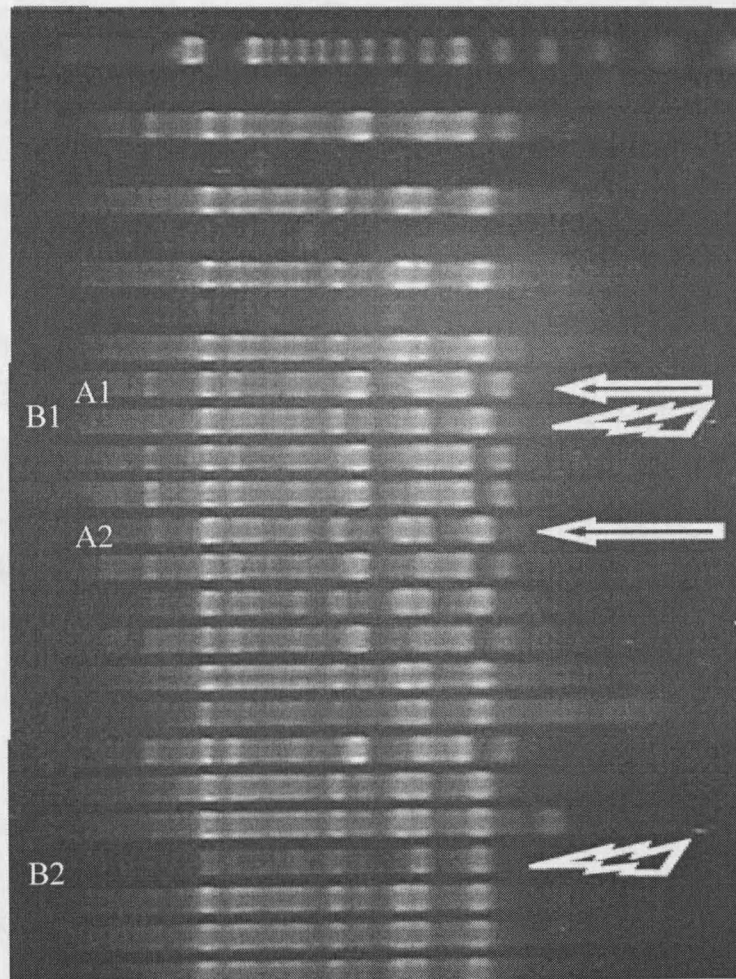


Figure 2.2 - Polymerase Chain Reaction agarose gel of *Tubifex tubifex* progeny using randomly amplified polymorphic DNA primer number 2. The progeny were from a replicate exposed to myxospores and containing both strain A and strain B *T. tubifex*. A1) This row marked with an arrow contains known strain A *T. tubifex* for identification. A2) This row marked with an arrow contains known strain B *T. tubifex* for identification. B1) and B2) The rows marked with lightning bolts contain possible hybrid progeny of strain A and strain B *T. tubifex*.

Discussion

Both strains of *T. tubifex* exhibited density dependent intra-strain interactions, which is consistent with previous studies (Kosiorek 1974, Bonacina et al. 1989). Strain A *T. tubifex* exhibited strong intra-strain effects indicated by a decrease in total biomass, adult growth and progeny biomass production as initial density increased. Thus, the intra-strain density dependent effect on strain A total biomass resulted from reductions in both adult growth and reproduction. Strain B *T. tubifex* also exhibited strong intra-strain effects suggested by a decrease in total biomass and progeny biomass as initial density increased. However, I detected no difference in adult growth of strain B *T. tubifex* between density treatments, perhaps as result of the high variance of 50 strain B treatments. Thus, the intra-strain density dependent effect on strain B total biomass resulted primarily from a reduction in reproduction.

The effects of inter-strain interactions between strain A and B *T. tubifex* were primarily negative. The total and progeny biomass produced by an individual strain A *T. tubifex* was lowest in the 100 strain A and 50 strain A + 50 strain B treatments. Thus, strain A or strain B *T. tubifex* have similar effects on the total and progeny biomasses produced by strain A *T. tubifex*. Adult growth of strain A *T. tubifex* was similar in the 50 strain A and 50 strain A + 50 strain B treatments and lower in the 100 strain A treatment. Thus, the overall effect is that strain B does not affect strain A adult growth. This

suggests that the inter-strain density dependent effects on total biomass of strain A occurred because strain B depressed reproduction of strain A.

The total and progeny biomass produced by an individual strain B *T. tubifex* were highest at densities of 50 individuals and lowest in 100 strain B and 50 strain A + 50 strain B treatments. Thus, strain A or strain B *T. tubifex* have similar effects on progeny biomass produced by strain B *T. tubifex*. Adult growth of strain B *T. tubifex* was similar for all treatments and was unaffected by increasing density with addition of strain B or strain A individuals. This suggests that inter-strain density dependent effects on the total biomass production of strain B occurred because strain A depressed the reproduction of strain B *T. tubifex*.

Adult growth of strain A *T. tubifex* was the only biomass variable that differed among myxospore doses of 0 and 2500. Infected strain A adults grew more than those not infected. This result is contrary to a previous experiment where infected *T. tubifex* exposed to the same myxospore dose of 2500 grew less than uninfected *T. tubifex* (Stevens et al. 2001). Two key differences between my study and Stevens et al. (2001) may contribute to the different results. First, Stevens et al. (2001) exposed oligochaetes to myxospores for the duration of the experiment. An extended exposure period to myxospores could have allowed oligochaetes to continue to ingest myxospores thus, having a continuing negative effect on its biomass. Second, the results of Stevens et al. (2001) were reported as the total biomass of adults and progeny of each treatment at the beginning and end of the experiment, as opposed to per capita*day adult growth as I reported.

An explanation for the higher growth of exposed strain A adults at this particular dose may be parasite manipulation by *M. cerebralis*. By allocating more energy for growth, strain A individuals may have been manipulated to have larger gut capacity; hence greater TAM production potential. Although progeny biomass production did not differ statistically among myxospore doses, there was a trend of decreasing progeny biomass production as myxospore dose increased suggesting that fewer resources may be allocated to reproductive output due to parasite manipulation. Host manipulations by parasites to increase parasite success have been detected in numerous host-parasite interactions (Moore 1984, Poulin 1994, Thompson and Kavaliers 1994), and include altering host growth to benefit parasite production (Théron et al. 1992).

The response of strain B to extremely high doses of myxospores revealed that total biomass and progeny biomasses were higher at a dose of 0 myxospores, and lower at 50,000 myxospores. Adult growth was not affected by myxospore dose. Thus, strain B *T. tubifex* experiences a fitness cost when exposed to a high abundance of myxospores. This cost could be the result of a debilitating by-product of infection or a damage limitation strategy. Fecundity reduction as a by-product of infection can be a result of host impaired feeding or direct competition between host and parasite for host nutrients (Hurd 2001). Further, damage limitation strategies, such as immune response to tissue damage by infection, can direct resources into tissue repair thereby reducing host fecundity (Hurd 2001). The reduced reproductive output of resistant *T. tubifex*, consistent with other previous host responses to parasitism, may be a response of

increased immunity (Minchella 1985, Bayne 1991, Forbes 1993, Sheldon and Verhulst 1996, Stevens et al. 2001).

Parasite production and infection prevalence did not differ statistically among oligochaete combinations suggesting resistant strain B individuals did not affect the success of *M. cerebralis* within strain A individuals. The non-significant trend of deactivation of myxospores by strain B *T. tubifex* in this research warrants further consideration. A higher dose of myxospores and more replicates per treatment may clarify the relationship between infection prevalence and oligochaete combination.

By examining two levels of susceptibility, two relative densities of *T. tubifex* and high myxospore exposure of strain B, I was able to show the effects of density dependent competition and reduced fitness of resistant *T. tubifex* at high myxospore doses. The intra-strain progeny biomass production of exposed susceptible and resistant *T. tubifex* were similar and did not decrease with myxospore exposure. However, in previous work by Stevens et al. (2001), susceptible *T. tubifex* exposed to a dose of 50,000 myxospores had lower total biomass than unexposed individuals. Further, the total biomass and progeny biomass of strain B individuals was low at 50,000 myxospores, suggesting that resistant strain B individuals incur a cost for resistance. The relative costs associated with resistance and infection should be measured. Additionally, although previous model predictions state parasitized, susceptible individuals will bear a disadvantage in competition with resistant individuals (Yan 1996), I was unable to detect this disadvantage. These results may reflect the similarities of physiological characteristics among these two *T. tubifex* strains, such as niche specialization or particle preference that

create density dependent competition. However, because the strains differed in their ability to propagate the parasite, the results support the idea that the *T. tubifex*-*M. cerebralis* relationship differs among *T. tubifex* strains.

Host response to a parasite may be characterized in several ways. A compatible host is one in which the parasite develops and an ensuing cost to the host is incurred, whereas for incompatible hosts, parasites do not develop so there is no cost to the host (Vandame et al. 2000). Compatible hosts have varying degrees of susceptibility with varying levels of parasite development. Resistance by a compatible host prevents parasite development and typically involves an additional cost to the host (Vandame et al. 2000). In general, *T. tubifex* are compatible with *M. cerebralis*. Specifically, strain A *T. tubifex* are susceptible to infection, however it is not clear if strain B *T. tubifex* are less susceptible or resistant to infection, because both responses incur a cost to the host.

Host susceptibility varies widely in other host-parasite interactions (Nayar et al. 1992, Ebert 1998, Vandame et al. 2000) and is often coupled with parasite evolution (Ewald 1983, Minchella and Scott 1991). Additionally, parasites may be capable of influencing the evolution of genetic variation for resistance among hosts (Ebert 1998), leading to geographic patterns of adaptation where parasites are most virulent within local host populations and less virulent in novel hosts (Ebert 1994). This suggests that hosts with high susceptibility are local host populations that may have evolved with *M. cerebralis*, whereas resistant or less susceptible individuals are the novel host. The occurrence of *M. cerebralis* in North America is recent, first documented in 1956 (Hedrick et al. 1998). Because *M. cerebralis* shows minimal genetic variation among

isolates (Andree et al. 1999), there is little evidence that co-evolution has occurred.

Examining the local adaptation of *M. cerebralis* and *T. tubifex* strains will determine the occurrence of such co-evolutionary relationships (Kerans et al. submitted).

The discovery of progeny that shared bands of both resistant and susceptible *T. tubifex* suggests that gene flow among *T. tubifex* strains may occur at least in the laboratory. However, if the resulting progeny are rare in occurrence due to isolation or reinforcement (Freeman and Herron 2001), then these progeny suggest speciation among *T. tubifex* strains may be possible. The occurrence of prezygotic or postzygotic isolation of *T. tubifex* "hybrids" and their reproductive success must be determined to predict gene flow among strains. If *T. tubifex* "hybrids" survive and reproduce successfully, then their ability to propagate *M. cerebralis* must be determined to predict any effects upon host-parasite interactions.

I have shown that intraspecific and inter-strain interactions between *T. tubifex* were density dependent, and the success of the parasite was not reduced by the presence of resistant individuals. Although, resistant individuals do not change infection in susceptible *T. tubifex*, resistant *T. tubifex* still do not produce TAMs. Thus, if the relative frequencies of susceptibility and resistance are known in a specific stream, this could be used to predict salmonid disease severity. For example, if the frequency of resistant individuals was high, we might expect low disease severity in salmonids. However, extrapolating controlled experimental laboratory results to a dynamic natural system may be challenging.

Future research should clarify the effects of density dependence on strain B individuals (at higher and lower densities), determine the infection potential of “hybrid” progeny when challenged with myxospores, genetically characterize *T. tubifex* strains in the two severely whirling disease affected rivers (Madison River MT and Colorado River CO) and finally, determine the historic dispersal of *T. tubifex* strains. These aspects will contribute to whirling disease management decisions and improve our understanding of host-parasite dynamics.

My research recognizes the importance of examining host competitive outcomes in host-host-parasite relationships and further characterizes relationships among strains of *T. tubifex*. Genetic variation of *T. tubifex* within whirling disease-affected streams is likely influential in the life cycle of this widespread parasite and my results support the recent paradigm in ecology of community genetics that stresses the importance of examining host genetics in host-host-parasite interactions (Whitham et al. 2003).

CHAPTER 3

OBJECTIVES 2 AND 3: INTERACTIONS AMONG TWO TUBIFICID SPECIES AND
A MYXOZOAN PARASITE AND THRESHOLD INFECTION DOSEIntroduction

Associations between two species, which are often affected by the presence of a third species (Price et al. 1986, Miller and Kerfoot 1987), may influence community structure (Wootton 1994). However, predicting the outcome of these interactions can be difficult because the strength and the direction of the interactions often depend on the environment, life history and population structure of species (Thompson 1988, Power et al. 1996). Although researchers have investigated multiple species predator-prey and competitive interactions (Paine 1966, Schmitt 1987, Kneib 1988, Kohler 1992, Martinez and Medel 2002), studies involving host-parasite interactions are limited (Settle and Wilson 1990, Grosholz 1992, Yan et al. 1998, Bonsall and Hassell 2000).

I examined the associations between two aquatic oligochaete species, *Tubifex tubifex* (Oligochaeta: Tubificidae) and *Limnodrilus hoffmeisteri* (Oligochaeta: Tubificidae) and a myxozoan parasite, *Myxobolus cerebralis* (Myxozoa: Myxosporaea/Actinosporaea). *Myxobolus cerebralis* causes whirling disease in salmonids (El-Matbouli et al. 1992) and has caused the declines of at least two populations of wild rainbow trout

(*Oncorhynchus mykiss*) within the Intermountain West (USA) (Nehring and Walker 1996, Vincent 1996). The life cycle of the parasite alternates between two hosts: various salmonid species and one known oligochaete species, *Tubifex tubifex* (Markiw and Wolf 1983, Wolf and Markiw 1984). *Myxobolus cerebralis* has a complex life cycle with two spore stages, the myxospore and the triactinomyxon (TAM). *Tubifex tubifex* ingests the myxospore stage while consuming sediments. Parasite reproduction occurs between the epithelial cells of the intestine. After approximately 70-100 days, depending on *T. tubifex* strain and temperature (El-Matbouli and Hoffmann 1989, El-Matbouli et al. 1999a, Stevens et al. 2001, Kerans and Stevens in preparation), the buoyant stage of the parasite, the triactinomyxon (TAM), is released into the water. The TAM floats in the water and when a salmonid is contacted, sporoplasms of the TAM are injected into the epidermis. The degradation of the cartilaginous skeletal system occurs as myxospores are produced (El-Matbouli et al. 1995). Myxospores are released into aquatic sediment after infected salmonids die and their carcasses decompose.

Benthic assemblages of oligochaetes, which often contain seven or more species (Brinkhurst and Jamieson 1971), may be influenced by intraspecific and interspecific interactions. *Tubifex tubifex* and *Limnodrilus hoffmeisteri*, two oligochaetes that typically inhabit stream communities, have similar distributions, physical attributes and functional roles as deposit feeders but different ecological requirements such as particle size and O₂ concentration preferences (Brinkhurst and Jamieson 1971, Mermillod-Blondin et al. 2001). In monocultures, individual growth and reproduction of *T. tubifex* and *Limnodrilus* sp. are density dependent, suggesting intraspecific competition influences

populations (Kosiorek 1974, Poddubnaya 1980, Bonacina et al. 1989). However, when *T. tubifex* and *L. hoffmeisteri* were held in mixed species cultures their respiration rates were lower and population growth rates higher compared to each species in monocultures (Brinkhurst 1974, Milbrink 1987). The authors suggest the interaction between *T. tubifex* and *L. hoffmeisteri* could be a facultative mutualism caused by species providing each other with beneficial food resources (Brinkhurst 1974, Milbrink 1987).

It is unknown how *M. cerebralis* may influence the interactions between *T. tubifex* and *L. hoffmeisteri*. *Myxobolus cerebralis* infections are persistent (Gilbert and Granath 2001) and cause reduced growth and reproduction of *T. tubifex* (Stevens et al. 2001). Conversely, *L. hoffmeisteri* cannot be infected by *M. cerebralis* and its population growth is unaffected by myxospore exposure (Wolf et al. 1986, Kerans et al. submitted). Anecdotal evidence suggests that *L. hoffmeisteri* can consume and deactivate myxospores, which could make myxospores unavailable to *T. tubifex* (M. El-Matbouli, University of Munich, personal communication), reducing infection potential of *T. tubifex*. Therefore, an examination of the interactions among susceptible *T. tubifex*, *L. hoffmeisteri* and *M. cerebralis* will help determine the effects of mixed species interactions on host and parasite success and contribute to our understanding of the varying effects of whirling disease on salmonids.

I conducted two laboratory experiments: the first examined how myxospore availability influenced infection in *T. tubifex* and the second examined the interactions among *T. tubifex*, *L. hoffmeisteri* and *M. cerebralis*. The main objectives were to determine: 1) the threshold dose of myxospores required to produce 100% infection

within *T. tubifex*, 2) whether the interactions between *L. hoffmeisteri* and parasitized and unparasitized *T. tubifex* were similar, and 3) whether *L. hoffmeisteri* influenced the transmission of *M. cerebralis* by *T. tubifex*.

I expected *T. tubifex* and *L. hoffmeisteri* would exhibit intraspecific density dependent competition when alone. When together, I expected their interspecific interactions to reflect the facultative mutualism found in previous experiments, hence enhanced growth and reproduction of each species versus that found in monoculture. Further, I expected that the addition of *M. cerebralis* would not affect intraspecific interactions. However, I expected parasitized *T. tubifex* to have reduced growth and reproduction compared to unparasitized *T. tubifex* and, with the addition of *L. hoffmeisteri*, *T. tubifex* would have reduced infection due to deactivation of myxospores by *L. hoffmeisteri*.

Methods

Several general methods apply to both laboratory experiments that I performed. I used three types of oligochaetes taken from disease-free, pure laboratory cultures, established and maintained since 1997 (see Stevens et al. 2001, Kerans et al. submitted, for details of culture propagation): *T. tubifex* strain A that produced large numbers of TAMs, *T. tubifex* strain B that produced few TAMs, and *L. hoffmeisteri* that produced no TAMs (Stevens et al. 2001, Kerans et al. submitted). Strain A was collected from the Mt. Whitney California State fish hatchery settling ponds where *M. cerebralis* was detected

locally in 1984 (Modin 1998). Strain B was collected from a pond on a tributary to the Gallatin River, Montana, where *M. cerebralis* had not been detected at collection time (B. L. Kerans, Montana State University, personal communication). *Limnodrilus hoffmeisteri* originated from the Madison River, Montana and a pond on the campus of Montana State University. Each strain of *T. tubifex* can be distinguished genetically with molecular markers (Kerans et al. submitted).

Oligochaetes were removed from mass cultures and placed in plastic containers for 24 hours without substrate to equalize hunger levels, then moved to plastic containers ($9 \times 9 \times 5$ cm) with 40 ml of sand, 1 ml of organic matter and 200 ml of dechlorinated tap water. I maintained containers, supplied with a constant flow of air, in 15° C incubators on a 12:12 h L:D photoperiod. Oligochaetes were fed 0.10 to 0.12 grams of dried *Spirulina* weekly, which was sufficient for growth and reproduction (J. C. Lemmon, Montana State University, personal communication). I replaced about 95 % of the water from each container with fresh dechlorinated water weekly. I used oligochaete densities that are common in natural systems (6,000 – 13,000 individuals/m²) (Lazim and Learner 1986). Experimental densities of 50 and 100 individuals per container translate to natural densities of 6180 and 12,360 individuals/m².

I extracted myxospores from infected rainbow trout (*Oncorhynchus mykiss*) using a continuous plankton centrifuge (O'Grodnick 1975). Trout originated at the Montana State fish hatchery in Ennis MT and were infected in our laboratory using TAMs that originated from *T. tubifex* cultures of the United States Fish and Wildlife Service, Fish Health Laboratory, Bozeman MT. Using 1 µl of extracted myxospore emulsion, I

determined the abundance of myxospores by counting all viable myxospores on a hemacytometer with a compound microscope (400X magnification). The total number of myxospores in the emulsion was extrapolated from the mean number of myxospores from 4 hemacytometer counts and was used to create the required experimental doses. The myxospore containing emulsion or myxospore-free emulsion (derived from parasite-free trout) was added to the containers of oligochaetes. Oligochaetes were exposed to myxospores for 5 days. The 5-day exposure time was based on the average feeding rate of oligochaetes (about $0.0018 \text{ ml dry sediment} \cdot \text{individual}^{-1} \cdot \text{day}^{-1}$ at 15°C) (McCall and Fisher, 1980) and was designed to capture a range of myxospore encounter rates (0.03 through $30.0 \text{ myxospores} \cdot \text{individual}^{-1} \cdot \text{day}^{-1}$) resulting in a range of infection levels. I assumed that all myxospores would be consumed by oligochaetes during this 5-day period based on the feeding rate. After 5 days, all oligochaetes were removed from each container and placed in a new container with fresh substrate and water.

Experiment 1: threshold infection dose

This experiment was designed to determine the threshold number of myxospores needed for 100 percent infection in *T. tubifex*. I exposed two replicates of 75 oligochaetes of strains A and B to doses of 0, 1, 5, 10, 50, 100, 500, 1,000, 5,000 myxospores per oligochaete in a factorial design.

On day 89 post-exposure to myxospores, I began to scan for TAMs. The water from each container was poured separately through a $20 \mu\text{l}$ sieve that retained TAMs.

Triactinomyxons were back flushed and concentrated in 10 ml of water. I scanned water from two, 55 µl aliquots with a phase contrast microscope (100X magnification).

I assumed that by day 90 post-exposure all infected oligochaetes would be producing TAMs. On day 90 post-exposure, I took 72 worms from each container and placed them in individual 12-well plates with 4 ml well capacity to determine the prevalence of infected oligochaetes. A day later (after TAMs could accumulate in the wells), I began to scan each well for the presence or absence of TAMs. I scanned each well, using a dissecting microscope (10X magnification), alternate days, until day 96 post-exposure and recorded wells as positive if TAMs were visible. I calculated the prevalence of infection as the number of oligochaetes producing TAMs divided by the total number of oligochaetes observed. I analyzed the prevalence of infection as a function of myxospore dose using linear logistic regression (SAS Institute, 2001). Because the threshold dose was lower than expected (see Results), I removed the highest myxospore doses from the model to increase its linearity.

Experiment 2: interactions between oligochaetes

This experiment examined intraspecific and interspecific interactions between *T. tubifex* (strain A) and *L. hoffmeisteri* when oligochaetes were exposed and not exposed to myxospores. I used strain A because it produces many TAMs, thus enhancing my ability to detect the effects of the interactions among the three species. I exposed three replicates of 50 *T. tubifex*, 100 *T. tubifex*, 50 *T. tubifex* + 50 *L. hoffmeisteri*, 50 *L. hoffmeisteri* and

100 *L. hoffmeisteri* to three doses of *M. cerebralis* myxospores (0,10 and 100 per oligochaete) in a response surface competition design (Goldberg and Scheiner 2001). I chose these myxospore doses because they bracket the infection threshold found in Experiment 1 (see Fig. 3.1), and by doing so, I could observe the effects of interactions among and within species when the prevalence of infection of *T. tubifex* was at two levels. Additionally, a response surface design allowed me to partition and evaluate intraspecific and interspecific interactions among oligochaetes. For example, comparing response variables between densities of 50 and 100 within a species examines intraspecific interactions and comparing responses between treatments of 100 *T. tubifex* and 100 *L. hoffmeisteri* and 50 *T. tubifex* + 50 *L. hoffmeisteri* examines interspecific interactions.

Starved oligochaetes were wet weighed in groups of 50 to the nearest 0.0001 gram and appropriate numbers of *T. tubifex* and *L. hoffmeisteri* were placed in prepared containers and inoculated with myxospores. On day 65 post-exposure to myxospores, I removed and weighed all original adults and also separated individuals within 50 *T. tubifex* + 50 *L. hoffmeisteri* treatments by species according to Kathman and Brinkhurst (1998) using a dissecting microscope. Also at this time, I preserved all progeny in the original sediment with Kahle's solution (Pennak 1978). I counted and weighed progeny at a later date.

Once separated, I randomly chose 24 *T. tubifex* from each replicate to reduce the total number to manageable levels and observed them for infection prevalence, as in Experiment 1, and TAM production. *Tubifex tubifex* were placed in individual 12-well

plates with water. I extracted all water from each well every other day, replacing it with fresh dechlorinated water. I randomly selected one oligochaete from each replicate to observe individual TAM production for 20 days, thus a total of 3 individuals were observed for each treatments combination. To calculate average TAM production per oligochaete per day, I counted TAMs in two, 55 μ l aliquots of water using a phase contrast microscope every three days. Adults from control and *L. hoffmeisteri* treatments were returned to plastic containers after being weighed. I maintained all controls and *L. hoffmeisteri* treatments in incubators and confirmed the absence of TAM production by scanning water from the experimental containers as described previously in Experiment 1.

The effects of host-parasite interactions are often associated with reduced host growth and reproductive success (Forbes 1993). I measured three responses to assess the effects of the interactions among oligochaetes and *M. cerebralis* on oligochaete fitness: 1) total biomass production per initial individual ($((\text{final biomass of adults} - \text{initial biomass of adults}) + \text{progeny weight}) / \text{initial density of adults}$) (hereafter referred to as "total biomass"), 2) growth rate of adult oligochaetes per initial individual ($(\text{final biomass of adults} - \text{initial biomass of adults}) / \text{initial density of adults}$) (hereafter referred to as "adult growth") and 3) progeny biomass produced by an individual adult ($\text{final progeny biomass} / \text{initial density of adults}$) (hereafter referred to as "progeny biomass"). I separated total biomass into two contributing response variables of adult growth and progeny biomass to determine how each factor influenced total biomass. I did not examine oligochaete mortality as a response variable because significant mortality typically occurs only when

myxospore doses are extremely high (Stevens et al. 2001). Oligochaete mortality during the experiment was 7 % ($\pm 1\%$ SD). Oligochaete species-density combination will be further referred to as “oligochaete combination.”

I tested total biomass, adult growth, and progeny biomass of each oligochaete species for significant differences among oligochaete combinations and myxospore dose using two-factor analyses of variance (ANOVA, SAS Institute, 2001). I examined the residuals for normality and stabilized the variances with log or square root transformations, where necessary. I also tested the prevalence of infected *T. tubifex* and average TAM production for significant differences among oligochaete combinations and myxospore dose using two-factor ANOVA, which required square root and log transformations, respectively, to stabilize the variances. Significant main effects of ANOVA's were followed by Tukey-Kramer Honestly Significant Difference test (Tukey's HSD) to determine which levels of main effects differed from each other.

Results

Experiment 1: threshold infection dose

I observed 1152 *T. tubifex* for the prevalence of infection for two weeks. The prevalence of infection of strain A reached 100% at a myxospore dose of 50 per oligochaete, with a dramatic rise between doses of 5 and 50 myxospores per oligochaete (Fig. 3.1, Appendix D). The linear logistic regression was not significant when all points

were included ($F_{1,13} = 1.96$, $P = 0.1851$). However, regression was significant ($F_{1,9} = 14.27$, $P = 0.004$) when infection prevalence results of doses at or below 100 myxospores were used. I detected no TAMs produced by strain B (Appendix D).

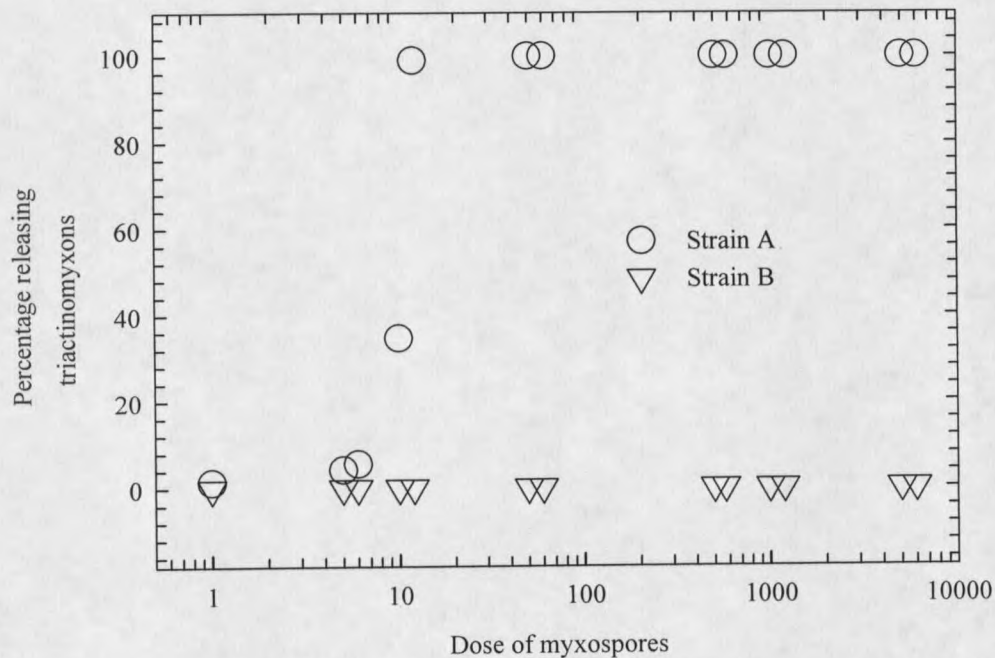


Figure 3.1 - The relationship between myxospore abundance and the percentage of *Tubifex tubifex* releasing triactinomyxons.

Experiment 2: interactions between oligochaetes

The total biomass of *T. tubifex* was highest at densities of 50 individuals and lowest at densities of 100 individuals of *T. tubifex* or 50 *T. tubifex* in combination with 50 *L. hoffmeisteri* (Table 3.1, Tukey's HSD, $P < 0.05$, Fig. 3.2a). Total biomass did not differ between myxospore doses, or with the interaction between oligochaete combination and myxospore dose (Table 3.1, Fig. 3.2a).

Growth of adult *T. tubifex* was highest at densities of 50 individuals alone or in combination with 50 *L. hoffmeisteri* and lowest in 100 *T. tubifex* treatments (Table 3.1, Tukey's HSD, $P < 0.05$, Fig. 3.2b). Growth of adults was greater in the 10 myxospore treatment than in the no myxospore treatment (Table 3.1, Tukey's HSD, $P < 0.05$). All other myxospore dose comparisons were not significant. The interaction between oligochaete combination and myxospore dose was not significant (Table 3.1).

Tubifex tubifex produced greater biomass of progeny at densities of 50 individuals alone, but as densities increased to 100 with the addition of *T. tubifex* or *L. hoffmeisteri* progeny biomass decreased (Table 3.1, Tukey's HSD, $P < 0.05$, Fig. 3.2c). Progeny biomass did not differ among myxospore doses, or with the interaction between oligochaete combination and myxospore dose (Table 3.1).

The total biomass of *L. hoffmeisteri* was highest at densities of 50 individuals and decreased as densities increased to 100 with the addition of *L. hoffmeisteri* or *T. tubifex* (Table 3.1, Tukey's HSD, $P < 0.05$, Fig. 3.2d). Total biomass did not differ among myxospore doses, nor with the interaction between oligochaete combination and myxospore dose (Table 3.1, Fig. 3.2d).

Growth of adult *L. hoffmeisteri* did not vary among oligochaete combinations (Table 3.1, Fig. 3.2e). However, growth of adults was highest in the 100 myxospore treatments and lowest in the no myxospore treatments (Table 3.1, Tukey's HSD, $P < 0.05$, Fig. 3.2e). The interaction between oligochaete combination and myxospore dose was not significant (Table 3.1).

Table 3.1 - Analysis of variance results on the effects of oligochaete combination and myxospore dose for biomass response variables. All biomass response variables are expressed as a per individual rate.

Response		Oligochaete		ss §	F	df	P
variable	species* (†)	Source of variation					
Total biomass	Tt	dose		73.68	1.54	2	0.24
		oligochaete combination		735.18	15.38	2	0.0001
		interaction		166.80	1.75	4	0.18
		error		430.08		18	
	Lh (sqrt)	dose		0.02	1.00	2	0.38
		oligochaete combination		0.29	11.50	2	0.0006
		interaction		0.11	2.14	4	0.11
		error		0.23		18	
Adult growth	Tt	dose		56.22	5.07	2	0.01
		oligochaete combination		129.61	11.69	2	0.0006
		interaction		32.04	1.45	4	0.25
		error		99.76		18	
	Lh	dose		15.10	4.49	2	0.02
		oligochaete combination		3.90	1.16	2	0.33
		interaction		2.94	0.44	4	0.77
		error		30.25		18	
Progeny biomass	Tt (sqrt)	dose		0.05	2.36	2	0.12
		oligochaete combination		0.37	18.31	2	0.0001
		interaction		0.06	1.50	4	0.24
		error		0.18		18	
	Lh (sqrt)	dose		0.04	2.07	2	0.15
		oligochaete combination		0.27	12.27	2	0.0004
		interaction		0.09	2.09	4	0.1242
		error		0.19		18	

* Tt represents *T. tubifex* treatments and Lh represents *L. hoffmeisteri* treatments.

(†) = The transformation (sqrt)=square root and (ln)=log.

§ ss = sums of squares.

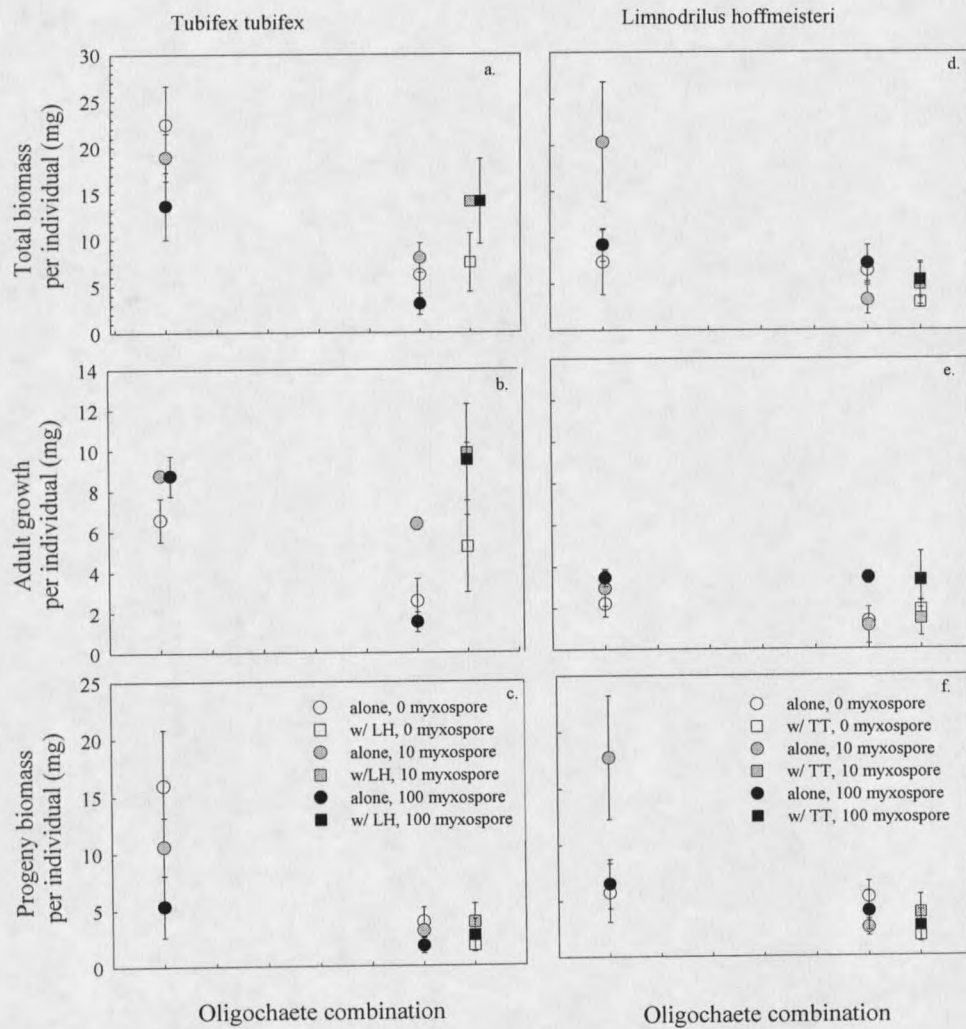


Figure 3.2 - The effects of oligochaete species and density and *M. cerebralis* infection on biomass variables. Each column depicts the particular variable for the target species listed, a) per capita biomass, b) per capita adult growth, c) progeny biomass produced per capita, d) per capita biomass, e) per capita adult growth, f) progeny biomass produced per capita. Error bars are one standard error of the mean. If overlap of individual biomass values occurred on the graph, points were offset.

Limnodrilus hoffmeisteri produced greater biomass of progeny at densities of 50 individuals, and as densities increased to 100 with the addition of *T. tubifex* of *L. hoffmeisteri* progeny biomass decreased (Table 3.1, Tukey's HSD, $P < 0.05$, Fig. 3.2f). Progeny biomass did not vary among myxospore doses or with the interaction between oligochaete combination and myxospore dose (Table 3.1).

I observed 648 individual *T. tubifex* for infection prevalence (24 individual *T. tubifex* from each replicate containing *T. tubifex*). The infection prevalence was lowest for 100 *T. tubifex* treatment, and highest in 50 *T. tubifex*, and 50 *T. tubifex* + 50 *L. hoffmeisteri* treatments (ANOVA: $F_{2,12} = 7.50$, $P = 0.007$, Tukey's HSD, $P < 0.05$, Fig. 3.3).

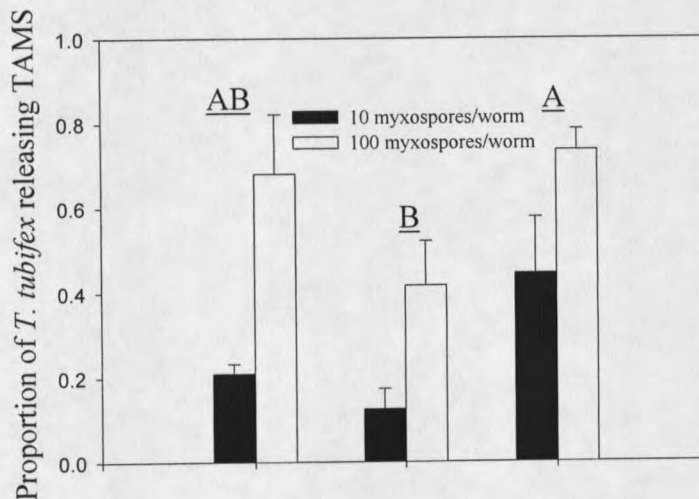


Figure 3.3 - The effect of oligochaete density and composition on the proportion of *Tubifex tubifex* releasing TAMs (N=3). Error bars represent one standard error of the mean. Bars that share the same letter do not differ significantly ($P < 0.05$) based on Tukey's HSD test.

Further, the prevalence of infection increased as myxospore dose increased (ANOVA: $F_{1,12} = 25.85$, $P = 0.0003$, Tukey's HSD, $P < 0.05$, Fig. 3.3). The interaction between oligochaete combination and myxospore dose was not significant (ANOVA: $F_{2,12} = 0.65$, $P = 0.53$).

I observed 18 individual *T. tubifex* for TAM production (one individual from each replicate exposed to myxospores)(Fig. 3.4). Production of TAMs by an individual did not differ with oligochaete combination (ANOVA: $F_{1,12} = 0.71$, $P = 0.414$), myxospore dose (ANOVA: $F_{2,12} = 0.69$, $P = 0.518$), or the interaction between the two (ANOVA: $F_{2,12} = 1.74$, $P = 0.217$). Treatments of 50 and 100 *L. hoffmeisteri* and negative controls did not produce TAMs. The triactinomyxons produced by an individual *T. tubifex* for each oligochaete combination range from 618.19 – 23,490.88 for 50 *T. tubifex*; 509.09 – 4,327.26 for 100 *T. tubifex*; and 1,127.27 – 6,436.35 for 50 *T. tubifex* with 50 *L. hoffmeisteri* (Appendix E).

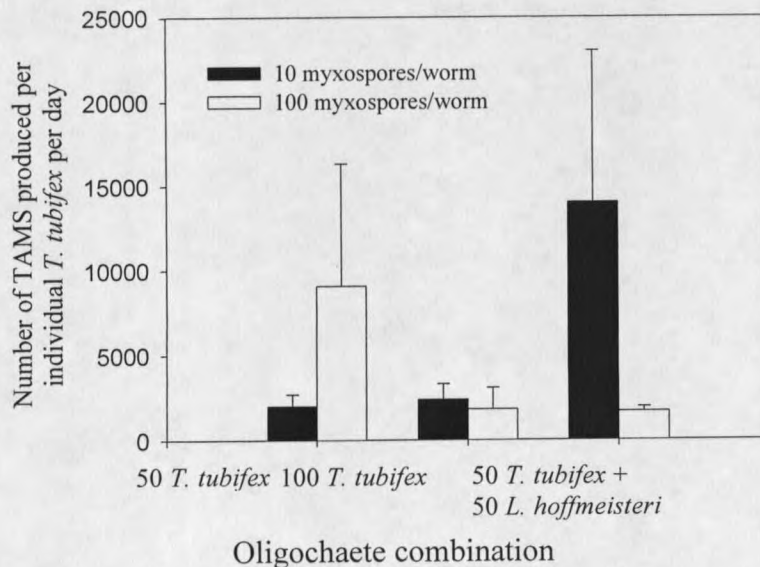


Figure 3.4 - The average TAM production of individual *Tubifex tubifex* over a 20-day period (N=3). The error bars represent one standard error of the mean.

Discussion

The results from Experiment 1 demonstrated that the prevalence of infection in *T. tubifex* depends on the abundance of myxospores and the strain of *T. tubifex*. Complete infection of experimental *T. tubifex* strain A populations occurs at relatively low myxospore abundance. Thus, relatively few myxospores appear to be needed to achieve infection in susceptible *T. tubifex*.

The variable infection among strains of *T. tubifex* is consistent with previous findings by Stevens et al. (2001) and Kerans et al. (submitted). Strain A *T. tubifex* have consistently produced TAMs (Stevens et al. 2001, Kerans et al. submitted, this study). Strain B *T. tubifex* have produced TAMs in a previous experiment; however TAM production was variable and low (Kerans et al. submitted). Beauchamp et al. (2002) have illustrated strains of *T. tubifex* with differential susceptibilities coexist in natural communities. Thus, it is possible that the distribution and differences in the abundances of *T. tubifex* strains may contribute to the disparity in salmonid disease severity seen in the Intermountain West.

Density dependence was the most striking result of the intraspecific interactions of both *T. tubifex* and *L. hoffmeisteri*. Previous work supports the observed density dependence (Kosiorek 1974, Poddubnaya 1980, Bonacina et al. 1989). *Tubifex tubifex* exhibited strong intraspecific effects on other *T. tubifex* indicated by a decrease in total biomass, adult growth and progeny biomass production as initial density increased.

Limnodrilus hoffmeisteri also exhibited intraspecific effects indicated by a decrease in total and progeny biomass production as initial density increased. However, adult growth of *L. hoffmeisteri* was low and density dependent effects were not detectable. Thus, the intraspecific density dependent effect on total biomass of *T. tubifex* resulted from the reduction in both adult growth and reproduction. However, the intraspecific density dependent effect on total biomass of *L. hoffmeisteri* resulted from a reduction in reproduction.

It is possible that *L. hoffmeisteri* was primarily allocating energy to reproduction and simply maintaining its adult condition, hence the observed low adult growth. Oligochaetes, including *L. hoffmeisteri*, are known to go through periods of energy intensive cocoon production, which are related to temporal cycles, and the density of individuals (Poddubnaya 1980, Bonomi and Di Cola 1980).

The interspecific interactions between *T. tubifex* and *L. hoffmeisteri* were mostly negative. Total and progeny biomass produced by an individual *T. tubifex* were highest at densities of 50 individuals and lowest in 100 *T. tubifex* and 50 *T. tubifex* + 50 *L. hoffmeisteri* treatments. This suggests that *L. hoffmeisteri* and *T. tubifex* have similar effects on progeny production by *T. tubifex*. Adult growth of *T. tubifex* was similar in 50 *T. tubifex* and 50 *T. tubifex* + 50 *L. hoffmeisteri* treatments and lower in the 100 *T. tubifex* treatment. This suggests that *L. hoffmeisteri* does not affect *T. tubifex* growth. Thus, the interspecific density dependent effects of *L. hoffmeisteri* on the total biomass production of *T. tubifex* occurred because *L. hoffmeisteri* depressed reproduction of *T. tubifex*.

The total biomass produced per individual *L. hoffmeisteri* was highest at densities of 50 individuals and lowest in 100 *L. hoffmeisteri* and 50 *T. tubifex* + 50 *L. hoffmeisteri* treatments, suggesting that at densities of 100, the addition of *T. tubifex* or *L. hoffmeisteri* have a similar effect on *L. hoffmeisteri*. Adult growth of *L. hoffmeisteri* was similar for all treatments and was unaffected by increasing density with the addition of *L. hoffmeisteri* or *T. tubifex*. Progeny biomass produced by an individual *L. hoffmeisteri* was highest at densities of 50, intermediate at densities of 100 and lowest in 50 *T. tubifex* + 50 *L. hoffmeisteri* treatments. Thus, the interspecific effects of *T. tubifex* on the total biomass production of *L. hoffmeisteri* occurred because *T. tubifex* depressed the reproduction of *L. hoffmeisteri*.

Under hypothesized facultative mutualists conditions, I expected *T. tubifex* and *L. hoffmeisteri* to positively influence adult growth and progeny biomass production of each other because of the reciprocal provision of beneficial resources (Brinkhurst 1974, Milbrink 1987). I detected increased adult growth of *T. tubifex* when *L. hoffmeisteri* replaced *T. tubifex* in the 100 density treatment. When *T. tubifex* replaced *L. hoffmeisteri* in 100 density treatments, there was no change in the adult growth of *L. hoffmeisteri*. On the other hand, both species depressed progeny production of each other. My results suggest that the interactions among these oligochaetes were not all facultative mutualism. In fact, the adult growth of *T. tubifex* appears to be facultative, whereas the depressed progeny production of both *T. tubifex* and *L. hoffmeisteri* are a result of competition.

There are several reasons that my results may differ from other results that suggest mutualism. Previous experiments (Brinkhurst et al. 1972, Brinkhurst 1974,

Milbrink 1993) used primarily low densities of individuals and different measures of interactions (including respiration rate and selective feeding). To determine the interspecific effects on growth, the previous experiments used abundances between 1 individual held in 5 ml of sediment and 18 individuals held in 400 ml of sediment (Brinkhurst et al. 1972, Milbrink 1993), which are lower than my experimental or natural densities of oligochaetes and also differed from the 40 ml of sediment in which my oligochaetes were held (Lazim and Learner 1986). Experiments by Brinkhurst et al. (1972) and Milbrink (1993) measured growth with low abundances, and therefore the experiment was not designed to test for density dependence. Further, determining the selective feeding of species-specific bacteria by tubificids may provide evidence of how oligochaete species partition resources, but the effects of oligochaete density upon physiology and interactions should be considered.

The contrast between my results and previous oligochaete interaction experiments suggest the dynamic nature of interactions in general and differences in methodology. Species interactions can be comprised of competitive and facultative components that vary spatially and temporally (Bruno et al. 2003). My results suggest that the adult growth of *T. tubifex* was affected by facultative mutualism, whereas the progeny production of both *T. tubifex* and *L. hoffmeisteri* was affected by competition. Factors that may influence these interactions include environmental conditions, indirect interactions, and the density and diversity of species (Callaway and Walker 1997, Marler et al. 1999, Dudgeon and Petraitis 2001). Therefore considering the effects of

interactions can be attributed to diverse factors comprised of positive and negative components; my results seem to be reasonable under my laboratory conditions.

Adult growth of *T. tubifex* and *L. hoffmeisteri* was the only biomass variable affected by myxospore exposure. Adult growth of *T. tubifex* was highest when exposed to 10 myxospores per individual, intermediate with 100 myxospores per individual and lowest with no myxospores. These results seem counterintuitive based on the results of Stevens et al. (2001), which showed a decrease in biomass at higher doses of myxospores. However, Stevens et al. (2001) reported total biomass as the difference between adult and progeny biomass at the beginning and end of the experiment, which differs from my reporting of per capita adult growth to illustrate interaction effects.

Adult growth of *L. hoffmeisteri* was greatest when exposed to 100 myxospores, intermediate with 10 myxospores per individual and lowest with no myxospores. One possible explanation for these growth responses may be beneficial bacteria associated with the myxospore emulsion, not present with the myxospore-free emulsion, enhanced the growth of both oligochaetes. The highest growth of *T. tubifex* at low doses may be the result of both beneficial bacteria and infection low enough that still permits growth. Similarly, the intermediate growth of *T. tubifex* at high doses may be the result of beneficial bacteria, but an infection level high enough that it begins to reduce growth. With no myxospores, *T. tubifex* did not benefit from the bacteria associated with myxospore emulsion, hence the lowest growth. It may be valuable to test the bacteria content of emulsions in the future and to determine the benefits of different bacteria.

Another possible explanation for these growth responses of oligochaetes when exposed to low doses of myxospores may be hormesis. Hormesis is recognized as a stimulation of response (i.e. greater growth of adults) that occurs at low stress levels and an inhibition of response that occurs at high stress levels (i.e. reduced growth of adults) (Calabrese and Baldwin 2003). The model of hormesis suggests that the balance between energetic and metabolic costs of resisting environmental stresses result in a continua of fitness with maximum fitness intermediate to the extremes (Parsons 2001). Therefore, oligochaetes exposed to low doses of myxospores may be responding to the stress of infection and exhibiting greater adult growth than those oligochaetes not exposed to myxospores.

At low and high myxospore doses, the prevalence of infected *T. tubifex* in the 50 *T. tubifex* + 50 *L. hoffmeisteri* treatment was higher than the 100 *T. tubifex* treatment. Furthermore, at a low myxospore dose, the prevalence of infected *T. tubifex* in the 50 *T. tubifex* + 50 *L. hoffmeisteri* treatment was two times that of 50 *T. tubifex* treatment, and at a high myxospore dose was similar to 50 *T. tubifex* alone, which may be reasonable considering 50 *T. tubifex* + 50 *L. hoffmeisteri* treatments received more myxospores per container. Since myxospore abundances were similar in 100 *T. tubifex* and 50 *T. tubifex* + 50 *L. hoffmeisteri* treatments, I would expect results similar to 100 *T. tubifex* treatments. A possible explanation for the higher prevalence than expected may be selective feeding techniques. *Tubifex tubifex* and *L. hoffmeisteri* preferentially consume particles previously digested by opposite oligochaete species containing different bacteria (McMurtry et al. 1983). If *T. tubifex* was able to select particles from *L. hoffmeisteri* that

contained active myxospores and increase the number of myxospores that it consumed, this could explain the enhanced infection by *T. tubifex*.

Furthermore, my work does not support the previous hypothesis that *L. hoffmeisteri* deactivates myxospores (El-Matbouli, University of Munich, personal communication). If deactivation had occurred, I would expect infection prevalence of *T. tubifex* in 100 *T. tubifex* and 50 *T. tubifex* + 50 *L. hoffmeisteri* treatments to be similar, due to density dependent competition and the average feeding rate of random particles.

The production of TAMs per individual did not differ among oligochaete combination or with myxospore dose. However, the number of triactinomyxons produced is consistent with previous trials of similar myxospore doses and oligochaete densities (Gilbert and Granath 2001, Stevens et al. 2001, Kerans et al. submitted). The production of TAMs per individual was determined from a small sample size (three individuals per replicate), and therefore the variances were large. In future experiments, a larger sample size should produce a more precise TAM production value.

I have shown that intraspecific and interspecific interactions between oligochaetes were density dependent, and the prevalence of infection to be both density dependent and myxospore dose dependent. Therefore, I suggest that two species interactions will have a positive effect on the success of *M. cerebralis* and increase the incidents of whirling disease among salmonids. Communities composed of primarily *T. tubifex* will have less of a positive effect due to lower infection rates and high rates of intraspecific competition.

However, laboratory interactions may differ from interactions in a natural environment, and correlating experimental interactions to disease severity in wild salmonids must consider a variety of influential factors. Several abiotic and biotic factors may influence myxospore and triactinomyxon abundance: genetic differences among *T. tubifex* populations (Beauchamp et al. 2001, 2002, Kerans et al. submitted), the interactions between *T. tubifex* and other oligochaetes, salmonid host susceptibility (Markiw 1992, Hedrick et al. 1998, Ryce et al. 2001), life history and ecology of *M. cerebralis* (El-Matbouli et al. 1999a, Kent et al. 2001, Kerans and Zale 2002) and environmental characteristics and watershed uses which relate to tubificid success (Zendt and Bergersen 2000, Krueger 2002). Further, only two wild rainbow trout population declines have been attributed to *M. cerebralis* (Nehring and Walker 1996, Vincent 1996), whereas other regional trout populations have remained stable despite the widespread presence of myxospores (Lorez et al. 1989, Modin 1998). Further, infection prevalence of oligochaetes in natural systems is typically low, suggesting that few myxospores are present (Rognlie and Knapp 1998, Zendt and Bergersen 2000). Therefore, a combination of these factors likely results in the observed *M. cerebralis* manifestation within *T. tubifex* and salmonids in natural communities.

Future experiments to determine the lowest myxospore dose to achieve any infection in mixed communities and its resulting infection prevalence would contribute to the risk assessment of whirling disease prevalence. Also, knowledge of oligochaete composition and densities in whirling disease-affected streams would not only aid in whirling disease risk assessment, but also provide further insight into host – parasite

interactions and oligochaete host susceptibility upon *M. cerebralis* infection. The experiments presented in this study underline the importance of understanding natural oligochaete communities, indirect interactions and host – parasite dynamics.

CHAPTER 4

CONCLUSIONS

I demonstrated in Chapter 2 that the fitness of strains of *T. tubifex* and the success of *M. cerebralis* varied with oligochaete combination and myxospore dose. Progeny biomass production of *T. tubifex* was always density dependent regardless of the strain. Adult growth of susceptible *T. tubifex* was density dependent in monoculture. However adult growth of 50 susceptible *T. tubifex* with 50 resistant *T. tubifex* was similar to 50 susceptible alone; therefore resistant *T. tubifex* did not affect the growth of susceptible *T. tubifex*. Further, adult growth of resistant *T. tubifex* was not affected by increasing density by adding either strain of *T. tubifex*. Variation in parasite production among strains of *T. tubifex* suggests that the strains differ in susceptibility to *M. cerebralis*. Progeny biomass production of resistant *T. tubifex* exposed to very high doses of myxospores was the only fitness component to show a statistically significant decrease compared to progeny biomass with no myxospores. The production of triactinomyxons and the infection prevalence of susceptible *T. tubifex* did not differ among strain A alone or 50 strain A + 50 strain B treatments. Therefore resistant individuals did not affect the transmission of the parasite by susceptible *T. tubifex*, and my work does not support deactivation of myxospores by the particular strain of resistant *T. tubifex* I used. However my results warrant further experiments to examine other

strains of *T. tubifex*, to determine possible mechanisms of resistance and to examine interactions under other conditions.

I demonstrated in Chapter 3 that the dose of myxospores required for 100% infection differed between strains of *T. tubifex*. One hundred percent infection of the susceptible strain of *T. tubifex* was reached at a dose of 50 myxospores per oligochaete. Progeny biomass production of *T. tubifex* and *L. hoffmeisteri* was always density dependent. The adult growth of *T. tubifex* was density dependent in monoculture, however in treatments where *L. hoffmeisteri* replaced *T. tubifex*, *T. tubifex* growth was not density dependent. Further, the adult growth of *L. hoffmeisteri* was not affected by increasing density with either *T. tubifex* or *L. hoffmeisteri*. *Tubifex tubifex* was compatible with *M. cerebralis*, whereas *L. hoffmeisteri* was clearly incompatible (Kerans et al. submitted). Kerans et al. (submitted) showed that *L. hoffmeisteri* did not produce TAMs and its fitness was not negatively affected by high doses of myxospores. However, in the same experiment (Kerans et al. submitted) *T. tubifex* produced TAMs and its fitness was negatively affected by myxospores. My results revealed infection prevalence of *T. tubifex* was both density dependent and myxospore dose dependent. An increase in the density of oligochaetes decreased the prevalence of infected individuals, whereas an increase in myxospores increased the prevalence of infection. Further, the interaction among *T. tubifex* and *L. hoffmeisteri* did not reduce the prevalence of infection of *T. tubifex*. My work does not support deactivation of myxospores by *L. hoffmeisteri*.

Several important ideas emerge from the results of chapter two and three. First, the interactions among strains of *T. tubifex* are similar and the transmission of *M.*

cerebralis is largely unaffected by the interactions among resistant and susceptible *T. tubifex*. Also, the prevalence of infection and triactinomyxon production of susceptible *T. tubifex* did not differ between inter-strain treatments and intra-strain treatments. Second, the interactions between two oligochaete species, *T. tubifex* and *L. hoffmeisteri*, are similar to intraspecific interactions, however their association affected the transmission of *M. cerebralis*. The prevalence of infection of *T. tubifex* in interspecific treatments of *T. tubifex* and *L. hoffmeisteri* was different than the intraspecific treatments of *T. tubifex* at a density of 100. The interactions among *T. tubifex* and *L. hoffmeisteri* had a positive effect on the success of *M. cerebralis*. This suggests that the interactions among different species of oligochaetes will have a greater effect on the success of *M. cerebralis* than the interactions among strains of *T. tubifex*.

One of the most consistent results of these experiments was the density dependent response of total and progeny biomass, suggesting the density of individuals had a strong influence upon oligochaete reproduction. Another interesting similarity between the two experiments was infected susceptible *T. tubifex* had higher adult growth than uninfected susceptible *T. tubifex*. *Tubifex tubifex* may have benefited from a low dose of myxospores or been subjected to parasite manipulation.

Myxobolus cerebralis has been documented in many cold-water streams, but there have been few severe whirling disease incidents reported (Nickum 1999). Many factors could influence the occurrence of severe whirling disease incidents such as low abundance of myxospores, fish and oligochaete host dynamics. Possibly, natural *T. tubifex* communities contain a higher frequency of resistant or moderately susceptible

strains; otherwise severe whirling disease incidents might be more common. *Tubifex tubifex* examined in the Colorado River, CO, identified individuals producing TAMs as susceptible strains and were in greater numbers than resistant individuals, whereas individuals not producing TAMs were identified as resistant strains and were few in numbers compared to susceptible strains (Beauchamp 2002). Hypothetically, moderately susceptible *T. tubifex* rather than resistant *T. tubifex* may be capable of reducing the number of available triactinomyxons, and potentially myxospores in the environment. In previous challenges moderately susceptible *T. tubifex* did not produce many triactinomyxons (Stevens et al. 2001). Resistant *T. tubifex* may simply ingest and expel intact myxospores, allowing susceptible *T. tubifex* to eventually encounter these myxospores. Therefore, it seems possible that moderately susceptible *T. tubifex* strains would be more influential in reducing the production of triactinomyxons than resistant strains, due to their low triactinomyxon production.

To test the hypothesis that moderately susceptible *T. tubifex* would be more effective than resistant *T. tubifex* at reducing infection in susceptible *T. tubifex*, the hypothesis of deactivation must be re-examined. The myxospore deactivation hypothesis assumes that myxospore capsules open in the gut of non-susceptible oligochaetes, and sporoplasms fail to develop. I predicted in both interaction experiments that deactivation of myxospores may be a mechanism to reduce infection among susceptible *T. tubifex*. In Objective 1, the infection prevalence and TAM production did not differ among oligochaete combinations, whereas, in Objective 2 infection prevalence was different

among oligochaete combinations. Neither experiment produced results that would agree with the predictions of deactivation.

The previous work of El-Matbouli et al. (1998, 1999b) does not test the myxospore deactivation hypothesis adequately. The experimental design of El-Matbouli et al.'s (1999b) test for deactivation was not replicated, oligochaetes were exposed for the duration of the experiment and only two density-oligochaete combinations were examined (50 susceptible *T. tubifex* + 50 resistant *T. tubifex* and 50 susceptible *T. tubifex*). The experimental design of El-Matbouli et al. (1999b) neglected the effect of oligochaete density on the prevalence of infection, shown by my work. I suggest an experimental design that allows 3 infection prevalence comparisons between 1) 50 susceptible *T. tubifex* and 100 susceptible *T. tubifex*, 2) 50 susceptible *T. tubifex* and 50 susceptible *T. tubifex* with 50 resistant *T. tubifex*, and 3) 100 susceptible *T. tubifex* and 50 susceptible *T. tubifex* with 50 resistant *T. tubifex*.

I propose a proper test of deactivation by both resistant *T. tubifex* and *L. hoffmeisteri* that considers the effects of density dependence. Replicated treatments of 50 and 100 susceptible *T. tubifex*, and 50 resistant *T. tubifex* exposed to a dose of at least 100 myxospores per individual for 30 days. Once all myxospores had been consumed, 50 previously unexposed susceptible *T. tubifex* would be added to the 50 exposed resistant *T. tubifex*. The 30-day exposure time would ensure that all myxospores had been consumed and the addition of susceptible *T. tubifex* to resistant *T. tubifex* would test if myxospores had been deactivated or passed through the digestion system unharmed. The treatments of 50 and 100 susceptible *T. tubifex* would provide prevalence of infection and parasite

production for comparison, and characterize the effects of density on infection. This design could also be completed with *L. hoffmeisteri*. Further, the physical state of a myxospore as it passes through a susceptible and resistant *T. tubifex* or an incompatible *L. hoffmeisteri* using light and electron microscopy, should be examined. This will improve our understanding of how myxospores successfully complete their life cycle within *T. tubifex*.

Future investigations suggested from this work include: examining the parasite production and biomass production of *T. tubifex* in communities that may contain several species of oligochaetes, determining the success of *T. tubifex* "hybrids" and their rate of parasite infection, genetically characterizing *T. tubifex* in natural systems, testing susceptible strains for parasite manipulation, and determining the physiological effects to myxospores once within and expelled by non-compatible hosts. My recommendation for understanding whirling disease-affected streams begins with characterizing the oligochaete communities by the strain of *T. tubifex* and species of oligochaete present, which could be used to assess the importance of the experimental interaction effects found in my study. Once we increase our understanding of oligochaete community ecology, we may be able to predict the potential that individual streams have in maintaining protection from the parasite or in successful parasite invasion.

REFERENCES CITED

- Anderson, R. M. and May, R. M. 1979. Population biology of infectious diseases: Part I. - *Nature*. 280: 361-367.
- Andree, K. B., Gresoviak, S. J., and Hedrick, R. P. 1997. Small subunit ribosomal RNA sequences unite alternate actinosporean and myxosporean stages of *Myxobolus cerebralis* the causative agent of whirling disease in salmonids. - *J. Euk. Microbiol.* 44: 208-215.
- Andree, K. B., El-Matbouli, M., Hoffmann, R. W., and Hedrick, R. P. 1999. Comparison of 18S and ITS-1 rDNA sequences of selected geographic isolates of *Myxobolus cerebralis*. - *Intl. J. Parasitol.* 29: 771-775.
- Anlauf, A. 1994. Some characteristics of genetic variants of *Tubifex tubifex* (Müller, 1974) (Oligochaeta: Tubificidae) in laboratory cultures. - *Hydrobiologia*. 278: 1-6.
- Anlauf, A. 1997. Enzyme variability of *Tubifex tubifex* (Müller) (Oligochaeta, Tubificidae) and seven other tubificid species. - *Arch. Hydrobiol.* 139: 83-100.
- Bayne, C. J. 1991. Invertebrate host immune mechanisms and parasite escapes. In - Toft, C. A., Aeschlimann, A. and Bolis, L. (eds.) *Parasite-host associations. Coexistence or conflict?* Oxford, pp. 299-315.
- Beauchamp, K. A., Kathman, R. D., McDowell, T. S., and Hedrick, R. P. 2001. Molecular phylogeny of tubificids oligochaetes with special emphasis on *Tubifex tubifex* (Tubificidae). - *Mol. Phyl. Evol.* 19: 216-224.
- Beauchamp, K. A., Gay, M., Kelley, G. O., El-Matbouli, M., Kathman, R. D., Nehring, R. B., and Hedrick, R. P. 2002. Prevalence and susceptibility of infection to *Myxobolus cerebralis*, and genetic differences among populations of *Tubifex tubifex*. - *Dis. Aquat. Org.* 51: 113-121.
- Begon, M., Bowers, R. G., Kadianakis, N., and Hodgkinson, D. E. 1992. Disease and community structure: the importance of host self-regulation in a host-host-pathogen model. - *Am. Nat.* 139: 1131-1150.
- Block, E. M., Moreno, G., and Goodnight, C. J. 1982. Observations on the life history of *Limnodrilus hoffmeisteri* (Annelida, Tubificidae) for the Little Calumet River in temperate North America. - *Int. J. Invertebr. Repro.* 4: 239-247.
- Bonacina, C., Bonomi, G. and Monti, C. 1989. Density-dependent processes in cohorts of *Tubifex tubifex*, with special emphasis on the control of fecundity. - *Hydrobiologia* 180: 135-141.

- Bonomi, G. and Di Cola, G. 1980. Population dynamics of *Tubifex tubifex*, studied by means of a new model. - In: Brinkhurst, R. O. and Cook, D. G. (eds.). Aquatic Oligochaete Biology. Plenum Press, pp.183-203.
- Bonsall, M. B. and Hassell, M. P. 2000. The effects of metapopulation structure on indirect interactions in host-parasitoid assemblages. - Proc. R. Soc. Lond. B. 267: 2207-2212.
- Brinkhurst, R. O. 1974. Factors mediating interspecific aggregation of Tubificid oligochaetes. - J. Fish. Res. Bd. Canada 31: 460-462.
- Brinkhurst, R. O. and Jamieson, B. G. M. 1971. Aquatic Oligochaeta of the World. - Univ. Toronto Press.
- Brinkhurst, R. O., Chua, K. E. and Kaushik, N. K. 1972. Interspecific interactions and selective feeding by tubificid oligochaetes. - Limn. Oceanography 17: 122-132.
- Bruno, J. F., Stachowicz, J. J. and Bertness, M. D. 2003. Inclusion of facilitation into ecological theory. - Trends Ecol. Evol. 18: 119-125.
- Bull, J. J. 1994. Virulence. - Intl. J. Org. Evol. 48: 1423-1437.
- Calabrese, E. J. and Baldwin, L. A. 2003. Toxicology rethinks its central belief. Hormesis demands a reappraisal of the way risks are assessed. - Nature 421: 691-692.
- Callaway, R. M. and Walker, L. R. 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. - Ecology 78: 1958-1965.
- Chapman, P. M., Farrell, M. A., and Brinkhurst, R. O. 1982. Relative tolerances of selected aquatic oligochaetes to combinations of pollutants and environmental factors. - Aquatic Toxicology 2: 69-78.
- Dudgeon, S. and Petraitis, P. S. 2001. Scale-dependent recruitment and divergence of intertidal communities. - Ecology 82: 991-1006.
- Ebert, D. 1994. Virulence and local adaptation of a horizontally transmitted parasite. - Science 265: 1084-1086.
- Ebert, D., Zschokke-Rohringer, C. D., and Carius, H. J. 1998. Within- and between-population variation for resistance of *Daphnia magna* to the bacterial endoparasite *Pasteuria ramosa*. - Proc. R. Soc. Lond. B 265: 2127-2134.

- El-Matbouli, M. and Hoffmann, R. 1989. Experimental transmission of two *Myxobolus* spp. developing bisporogeny via tubificid worms. - Parasitol. Res. 75:461-464.
- El-Matbouli, M., Fisher-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. - Annu. Rev. Fish Dis. 3: 367-402.
- El-Matbouli, M., Hoffmann, R.W. and Mandok, C. 1995. Light and electron microscopic observations on the route of the triactinomyxon-sporoplasm of *Myxobolus cerebralis* from epidermis into rainbow trout cartilage.- J. Fish Biology 46: 919-935.
- El-Matbouli, M., McDowell, T. S., Mukkatira, K. Hedrick, R. P. 1998. Susceptibility of two different populations of tubificids to *Myxobolus cerebralis*. - In: Proceedings of Whirling Disease Symposium, Fort Collins, CO. Whirling Disease Foundation, pp. 117-119.
- El-Matbouli, M., McDowell, T. S., Antonio, D. B., Andree, K. B. and Hedrick R. P. 1999a. Effect of water temperature on the development, release and survival of the triactinomyxon stage of *Myxobolus cerebralis* in its oligochaete host. - Int. J. Parasitol. 29: 627-641.
- El-Matbouli, M., Gay, M., McDowell, T. S., Georgiadis, M. P., Hedrick, R. P. 1999b. The potential for using biological control technologies in the management of whirling disease. - In: Proceedings of Whirling Disease Symposium, Missoula, MT. Whirling Disease Foundation, pp. 191-195.
- Erséus, C., Prestegard, T. and Källersjö, M. 2000. Phylogenetic analysis of Tubificidae (Annelida, Clitellata) based on 18S rDNA sequences. - Mol. Phyl. Evol. 15: 381-389.
- Ewald, P. W. 1983. Host-parasite relations, vectors, and the evolution of disease severity. - Ann. Rev. Ecol. Syst. 14: 465-485.
- Forbes, M. R. L. 1993. Parasitism and host reproductive effort. - Oikos 67: 444-450.
- Freeman, S. and Herron, J. C. 2001. Evolutionary Analysis, 2nd Edition. Prentice-Hall, Inc., New Jersey.
- Gilbert, M. A. and Granath, Jr., W. O. 2001. Persistent infection of *Myxobolus cerebralis*, the causative agent of salmonids whirling disease, in *Tubifex tubifex*. - J. Parasit. 87: 101-107.

- Gilbert, M. A. and Granath, Jr., W. O. 2003. Whirling disease of salmonid fish: life cycle biology and disease. - J. Parasitol. 89: 658-667.
- Goldberg, D. E. and Scheiner, S. M. 2001. ANOVA and ANCOVA-Field competition experiments. - In: Scheiner, S. M. and Gurevitch, J. (eds.), Design and analysis of ecological experiments, 2nd edition. Oxford University Press, pp. 77-98.
- Grosholz, E. D. 1992. Interactions of intraspecific, interspecific and apparent competition with host-pathogen population dynamics. - Ecology 73:507-514.
- Halliday, M. M. 1973. Studies of *Myxosoma cerebralis*, a parasite of salmonids. I. The diagnosis of infection. - Nordisk Veterinaermedicin 25: 345-348.
- Hedrick, R. P., El-Matbouli, M., Adkison, M. A. and MacConnell, E. 1998. Whirling disease: re-emergence among wild trout. - Immunol. Rev. 166: 365-376.
- Hoffmann, G. L., Dunbar, C. E. and Bradford, A. 1962. Whirling disease of trouts caused by *Myxosoma cerebralis* in the United States. - In: United States Fish and Wildlife Service, Special Scientific Report, Fisheries 427: 1-14.
- Hurd, H. 2001. Host fecundity reduction: a strategy for damage limitation? - Trends in Parasitol. 17: 363-368.
- Kathman, R. D. and Brinkhurst, R. O. 1998. Guide to freshwater oligochaetes in North America. - Aquatic Resources Center, College Grove.
- Kennedy, C. R. 1966. The life history of *Limnodrilus hoffmeisteri* Clap. (Oligochaeta: Tubificidae) and its adaptive significance. - Oikos 17: 158-168.
- Kent, M. L., Andree, K. B., Bartholomew, J. L., El-Matbouli, M., Dessler, S. S., Delvin, R. H., Feist, S. W., Hedrick, R. P., Hoffman, R. W., Khattra, J., Hallett, S. L., Lester, R. J. G., Longshaw, M., Palenzeula, O., Siddall, M. E. and Xiao, C. 2001. Recent advances in our knowledge of the myxozoa. - J. Eukaryot. Microbiol. 48: 395-413.
- Kerans, B. L. and Zale, A. V. 2002. The ecology of *Myxobolus cerebralis*. - In: Bartholomew, J. L. and Wilson, J. C. (eds.), Whirling Disease: Reviews and Current Topics. American Fisheries Society, Symposium 29, Bethesda, pp. 145-166.
- Kerans, B. L., Rasmussen, C., Stevens, R., Colwell, A. E. L. and Winton, J. R. The oligochaete host as a modulator of whirling disease in salmonids. - Submitted to J. Parasitol.

- Kneib, R. T. 1988. Testing for indirect effects of predation in an intertidal soft-bottom community. - *Ecology* 69: 1795-1805.
- Kohler, S. L. 1992. Competition and the structure of a benthic stream community. - *Ecol. Monog.* 62: 165-188.
- Kosiorek, D. 1974. Development cycle of *Tubifex tubifex* Müll. in experimental culture. - *Pol. Arch. Hydrobiol.* 21: 411-422.
- Krueger, R. C. 2002. Correlations among environmental features, *Myxobolus cerebralis* infection prevalence in oligochaetes, and salmonid infection fish in the Madison River, Montana. - Masters Thesis. Montana State University, Bozeman.
- Lazim, M. N. and Learner, M. A. 1986. The life-cycle and productivity of *Tubifex tubifex* (Oligochaeta: Tubificidae) in the moat-feeder stream, Cardiff, South Wales. - *Holarctic Ecology* 9: 185-192.
- Lorz, H. V., Amandi, A., Banner, C. R. and Rohovec, J. S. 1989. Detection of *Myxobolus* (Myxosoma) *cerebralis* in salmonid fishes in Oregon. - *J. Aqua. Anim. Health* 1: 217-221.
- MacConnell, E. and Vincent, E. R. 2002. Review: the effects of *Myxobolus cerebralis* on the salmonid host. - *American Fisheries Society Symposium* 29: 95-107.
- McCall, P. L. and Fisher, J. B. 1980. Effects of tubificid oligochaetes on physical and chemical properties of Lake Erie sediments. - In: Brinkhurst, R. O. and Cook, D. G. (eds.), *Aquatic oligochaete biology*. Plenum Press, pp. 262.
- McMurtry, M. J., Rapport, D. J., and Chua, K. E. 1983. Substrate selection by tubificid oligochaetes. - *Can J. Fish. Aquat. Sci.* 40: 1639-1646.
- Markiw, M. E. 1986. Salmonid whirling disease-dynamics of experimental production of the infective stage-the triactinomyxon spore. - *Can. J. Fish. Aquat. Sci.* 43: 521-526.
- 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*. - *J. Aquat. Anim. Health* 4: 40-43.
- Markiw, M. E. and Wolf, K. 1983. *Myxosoma cerebralis* (Myxozoa: Myxosporaea), etiological agent of salmonids whirling disease requires tubificid worm (Annelida: Oligochaeta) in its life cycle. - *J. Protozool.* 30: 561-564.

- Marler, M. J., Zabinski, C. A. and Callaway, R. M. 1999. Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. - *Ecology* 80: 1180-1186.
- Martinez, G. and Medel, R. 2002. Indirect interactions in a microcosm-assembled cladoceran community: implications for apparent competition. - *Oikos*. 97: 111-115.
- May, R. M. and Anderson, R. M. 1979. Population biology of infectious diseases: Part II. - *Nature* 280: 455-461.
- May, R. M. and Anderson, R. M. 1990. Parasite-host coevolution. - *Parasitol.* 100: S89-S101.
- Mermillod-Blondin, F., Gerinl, M., Degrange, V., Lensi, R., Chasse, J., Rard, M. and Creuze des Chatelliers, M. 2001. Testing the functional redundancy of *Limnodrilus* and *Tubifex* (Oligochaeta, Tubificidae) in hyporheic sediments: and experimental study in microcosms. - *Can. J. Fish. Sci.* 58: 1747-1759.
- Milbrink, G. 1987. Mutualistic relationships between cohabiting tubificid species. - *Hydrobiologia* 155:193.
- Milbrink, G. 1993. Evidence for mutualistic interactions in freshwater oligochaete communities. - *Oikos* 68:317-322.
- Miller, T. E. and Kerfoot, W. C. 1987. Redefining indirect effects. - In: Kerfoot W. C. and Sih, A. (eds.), *Predation: Direct and indirect impacts on aquatic communities*. - University Press of New England, pp. 33-37.
- Minchella, D. J. 1985. Host life-history variation in response to parasitism. - *Parasitol.* 90: 205-216.
- Minchella, D. J and Scott, M. E. 1991. Parasitism: A cryptic determinant of animal community structure. - *Trends in Ecol. Evol.* 6: 250-254.
- Modin, J. 1998. Whirling disease in California: a review of its history, distribution, and impacts, 1965-1997. - *J. Aquat. An. Health* 10: 132-142.
- Moore, J. 1984. Altered behavioural responses in intermediate hosts: an acanthocephalan parasite strategy. - *Am. Nat.* 123: 572-577.

- Nayar, J. K., Knight, J. W., Kaiser, P. E., Seawright, J. A., and Narang, S. K. 1992. Comparative susceptibility of species A, B and C of *Anopheles quadrimaculatus* complex to infection with subperiodic *Brugia malayi* and *Brugia pahangi* (Nematoda: Filarioidea). - J. Am. Mosquito Control Assc. 8: 61-64.
- Nehring, B. and Walker, P. G. 1996. Whirling disease in the wild: the new reality in the intermountain west. - Fisheries 12: 28-30.
- Nickum, D. 1996. Whirling Disease in the United States: Overview and guidance for research and management. - Trout Unlimited Report. Arlington, VA.
- Nickum, D. 1999. Whirling Disease in the United States: A summary of poregress in research and management. - Trout Unlimited Report. Denver, CO.
- O'Grodnick, J. 1975. Whirling disease concentration using the continuous plankton centrifuge. - J. Wildlife Dis. 11: 54-57.
- Paine, R. T. 1966. Food web complexity and species diversity. - Am. Nat. 100: 65-75.
- Park, T. 1948. Experimental studies of interspecific competition. I. Competition between flour beetles, *Tribolium confusum* Duval and *Tribolium castaneum* Herbst. - Ecol. Monog. 18: 265-308.
- Parsons, P. A. 2001. The hormetic zone: an ecological and evolutionary perspective based upon habitat characteristics and fitness selection. - The Quarterly Review of Biology 76: 459-467.
- Pennak, R. W. 1978. Fresh-water invertebrates of the United States, 2nd edition. John Wiley and Sons.
- Poddubnaya, T. 1980. Life cycle of mass species of Tubificidae. - In Brinkhurst, R. O. and Cook, D. G. (eds.), Aquatic oligochaete biology. - Plenum Press, pp. 175-184.
- Poulin, R. 1994. The evolution of parasite manipulation of host behaviour: a theoretical analysis. - Parasitology 109 (Suppl.): S109-S118.
- Power, M. E., Tilman, D., Estes, J., Menge, B. A., Bond, W. J., Mills, L. S., Daily, G., Castilla, J. C., Lubchenco, J. and Paine, T. 1996. Challenges in the quest for keystones. - Bioscience 46: 609-620.
- Price, P. W., Westoby, M., Rice, B. Atsatt, P. R., Fritz, R. S., Thompson, J. N. and Mobley, K. 1986. Parasite mediation in ecological interactions. - Ann. Rev. Ecol. Syst. 17: 487-505.

- Price, P. W., Westoby, M., and Rice, B. 1988. Parasite-mediated competition: some predictions and tests. - *Am. Nat.* 131: 544-555.
- Rasmussen, C., Colwell, A. and Winton, J. R. 1998. Detecting genetic diversity among strains of North American *Tubifex tubifex* using Randomly Amplified Polymorphic DNA (RAPD) markers. - In: Whirling Disease Symposium, Fort Collins, CO. Whirling Disease Foundation, pp. 141-145.
- Reynoldson, T. B., Rodriguez, P., and Madrid, M. M. 1996. A comparison of reproduction, growth and acute toxicity in two populations of *Tubifex tubifex* (Muller, 1774) from the North American Great Lakes and Northern Spain. - *Hydrobiologia* 334: 199-206.
- Rognlie, M. C. and Knapp, S. E. 1998. *Myxobolus cerebralis* in *Tubifex tubifex* from a whirling disease epizootic in Montana. - *J. Parasitol.* 84: 711-713.
- Ryce, E. K. N., Zale, A. V. and Nehring, R. B. 2001. Lack of selection for resistance to whirling disease among progeny of Colorado River rainbow trout. - *J. Aquat. Anim. Health* 12: 63-68.
- Ryce, E. K. N. 2003. Factors affecting the resistance of juvenile rainbow trout to whirling disease. - Doctoral Thesis. Montana State University, Bozeman.
- SAS Institute. 2001. SAS/STAT for Windows. Version 8.1, SAS Institute, Cary, North Carolina, USA.
- Schall, J. J. 1992. Parasite-mediated competition in *Anolis* lizard. - *Oecologia* 92: 58-64.
- Schmitt, R. J. 1987. Indirect interactions between prey: apparent competition, predator aggregation, and habitat segregation. - *Ecology* 68: 1887-1897.
- Settle, W. H. and Wilson, L. T. 1990. Invasion by the variegated leafhopper and biotic interactions: parasitism, competition and apparent competition. - *Ecology* 71: 1461-1470.
- Sheldon, B. C. and Verhulst, S. 1996. Ecological immunity: costly parasite defenses and trade-offs in evolutionary ecology. - *Trends Ecol. Evol.* 11: 317-321.
- Siddall, M. E., Martin, D. S., Bridge, D., Dessler, S. S. and Cone, D. K. 1995. The demise of a phylum of protists: phylogeny of Myxozoa and other parasitic Cnidaria. - *J. Parasitol.* 81: 961-967.

- Stevens, R., Kerans, B. L., Lemmon, J. C. and Rasmussen, C. 2001. The effects of *Myxobolus cerebralis* myxospore dose on triactinomyxon production and biology of *Tubifex tubifex* from two geographic regions. - J. Parasitol. 87: 315-321.
- Sturmbauer, C., Opadiya, G. B., Niederstätter, H., and Riedman, A. 1999. Mitochondrial DNA reveals cryptic oligochaete species differing in cadmium resistance. - Mol. Biol. Evol. 16: 967-974.
- Théron, A., Gérard, C., and Moné, H. 1992. Early enhanced growth of the digestive gland of *Biomphalaria glabrata* infected with *Schistosoma mansoni*: side effect or parasite manipulation? - Parasitol. Res. 78: 445-450.
- Thompson J. N. 1988. Variation in interspecific interactions. - Ann. Rev. Ecol. Syst. 19: 65-87.
- Thompson, S. N., and Kavaliers, M. 1994. Physiological bases for parasite-induced alterations of host behaviour. - Parasitology 109: S119-S138.
- Vandame, R., Colin, M. E., Morand, S., and Otero-Colina, G. 2000. Levels of compatibility in a new host-parasite association: *Apis mellifera*/*Varroa jacobsoni*. - Can. J. Zool. 78: 2037-2044.
- Vincent, E. R. 1996. Whirling disease and wild trout: the Montana experience. - Fisheries 21: 328-333.
- Whitham, T. G., Young, W. P., Martinsen, G. D., Gehring, C. A., Schweitzer, J. A., Schuster, S. M., Wimp, G. M., Fisher, D. G., Bailey, J. K., Lindroth, R. L., Woolbright, S., and Kuske, C. R. 2003. Community and ecosystem genetics: a consequence of the extended phenotype. - Ecology 84: 559-576.
- Wolf, K. and Markiw, M. E. 1984. Biology contravenes taxonomy in the Myxozoa: new discoveries show alternation of invertebrate and vertebrate hosts. - Science 225: 365-368.
- Wolf, K., Markiw, M. E. and Hiltunen, J. K. 1986. Salmonid whirling disease: *Tubifex tubifex* (Müller) identified as the essential oligochaete in the protozoan life cycle. - J. Fish Dis. 9: 83-85.
- Wootton, J. T. 1994. The nature and consequences of indirect effects in ecological communities. - Ann. Rev. Ecol. Syst. 25: 443-466.
- Yan, G. 1996. Parasite-mediated competition: a model of directly transmitted macroparasites. - Am. Nat. 148: 1089-1112.

- Yan, G., Stevens, L., Goodnight, C. J. and Schall, J. J. 1998. Effects of a tapeworm parasite on the competition of tribolium beetles. - Ecology 79: 1093-1103.
- Zendt, J. S. and Bergersen, E. P. 2000. Distribution and abundance of the aquatic oligochaete host *Tubifex tubifex* for the salmonids whirling disease parasite *Myxobolus cerebralis* in the Upper Colorado River Basin. - N. Am. J. Fish. Manag. 20: 502-512.

APPENDICES

APPENDIX A

ANOVA Tables from Chapter 2

Analyses of variance of the effects of days of growth and oligochaete combination on biomass variables of strain A *Tubifex tubifex*. All biomass response variables are expressed as a per capita rate.

Response					
variable(†)	Source of variation	ss§	F	df	P
Total biomass(sqrt)	days of growth	7.46	0.25	1	0.6325
	oligochaete combination	3132.32	53.24	2	0.0002
	interaction	51.41	0.87	2	0.4644
	error	176.49		6	
Adult growth(nt)	days of growth	7.89	1.96	1	0.2111
	oligochaete combination	27.07	3.36	2	0.1048
	interaction	14.87	1.85	2	0.2372
	error	24.15		6	
Progeny biomass(nt)	days of growth	0.04	0.01	1	0.9409
	oligochaete combination	2474.44	159.73	2	<0.0001
	interaction	18.97	1.23	2	0.358
	error	46.47		6	

(†) = the transformation: (nt) = no transformation, (sqrt) square root.

§ = sums of squares.

Analysis of variance of the effects of number of days triactinomyxon production observed and oligochaete combination for total triactinomyxon production per day by strain A *Tubifex tubifex*.

Response					
variable(†)	Source of variation	ss§	F	df	P
Total triactinomyxon production/day(nt)	end date	1.14E+07	0.2	1	0.6703
	oligochaete combination	9.92E+07	0.87	2	0.4651
	interaction	6.02E+07	0.53	2	0.6143
	error	511841332		11	

(†) = the transformation: (nt) = no transformation.

§ = sums of squares.

APPENDIX B

Total triactinomyxon production from Chapter 2

The total triactinomyxons produced and the mean daily production of triactinomyxons per replicate. Total triactinomyxons is the sum of each weekly count of triactinomyxons. The mean daily triactinomyxon production is the total triactinomyxons produced divided by the number of days observed producing triactinomyxons.

Replicate identification	Oligochaete combination	Number of days observed producing TAMs	Myxospore dose per container	Total TAMs produced	Mean daily production of TAMs
30	50 A	34	2500	982878.63	28908.19
37	50 A	34	2500	382515.04	11250.44
16	50 A	61	2500	1271757.36	20848.48
17	50 A	61	2500	569727.23	9339.79
10	100 A	34	2500	366454.40	10778.07
27	100 A	34	2500	430272.61	12655.08
9	100 A	61	2500	1006727.14	16503.72
23	100 A	61	2500	1531636.02	25108.79
3	50A/50B	34	2500	220454.50	6483.96
21	50A/50B	34	2500	374090.82	11002.67
29	50A/50B	61	2500	634401.29	10400.02
36	50A/50B	61	2500	967136.29	15854.69

APPENDIX C

Strain A and strain B progeny identified from Chapter 2

The number of progeny that were examined using randomly amplified polymorphic DNA primers. Each individual *T. tubifex* was identified by a minimum of two primers. Two progeny were identified as sharing bands of both strain A and strain B *T. tubifex* and referred to as hybrid progeny.

Replicate number	Myxospore dose per container	Oligochaete combination	Total # of progeny identified by PCR	Number identified strain A	Number identified strain B	Number identified hybrid
3	2500	50A/50B	42	13	27	2
21	2500	50A/50B	48	22	26	-
29	2500	50A/50B	30	14	16	-
36	2500	50A/50B	29	12	17	-
19	0	50A/50B	30	15	15	-
25	0	50A/50B	30	9	21	-
4	0	50A/50B	30	17	13	-

APPENDIX D

Infection prevalence from Experiment 1
Chapter 3

The number of infected individuals of each strain of *Tubifex tubifex* and the corresponding myxospore dose in the threshold infection experiment.

Myxospore dose per oligochaete	Number of infected strain A	Number of infected strain B
1	1.4	0
5	4.2	0
5	5.6	0
10	34.72	0
10	99	0
50	100	0
50	100	0
500	100	0
500	100	0
1000	100	0
1000	100	0
5000	100	0
5000	100	0

APPENDIX E

Triactinomyxon production from Chapter 3

The total and mean daily triactinomyxon production for 20 days per individual. Total triactinomyxon produced for 20 days is the sum each day of production. Mean daily triactinomyxons production is the total triactinomyxon production divided by 20 days of observation.

Replicate identification	Oligochaete combination	Myxospore dose per oligochaete	Total TAMs produced for 20 days	Mean daily TAM production
19	50 TT	10	1781.8164	445.4541
4	50 TT	10	872.7264	87.27264
40	50 TT	10	3309.0876	551.5146
32	100 TT	10	727.272	181.818
26	100 TT	10	3890.9052	648.4842
36	100 TT	10	2509.0884	418.1814
2	50 TT/ 50 LH	10	6436.3572	1072.7262
44	50 TT/ 50 LH	10	3563.6328	509.0904
42	50 TT/ 50 LH	10	31963.6044	3196.36044
37	50 TT	100	618.1812	61.81812
33	50 TT	100	23490.8844	2349.08844
12	50 TT	100	3090.9058	386.363225
34	100 TT	100	545.454	109.0908
7	100 TT	100	4327.2684	540.90855
31	100 TT	100	509.0904	169.6968
11	50 TT/ 50 LH	100	2072.7252	207.27252
18	50 TT/ 50 LH	100	1127.2716	125.2524
16	50 TT/ 50 LH	100	1781.8164	178.18164

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