AN ABSTRACT OF THE THESIS OF

Sophia Karandashova for the degree of Honors Baccalaureate of Science in Microbiology presented on August 13, 2008. Title: The introduction of myxozoan parasites through the release of feeder goldfish (*Carassius auratus*).

Abstract approved: _____

Jerri Bartholomew

Myxozoa have been found worldwide; these parasites infect a wide variety of hosts, but have been found most often in fish. *Carassius auratus* (goldfish), a popular aquarium fish and a widespread invasive species, have been identified as an intermediate host for several myxozoan species. Some myxozoan parasites are pathogenic and can lead to the death of the fish host; others merely decrease the market value of the fish due to unsightly cysts. Goldfish from three different types of sites were sampled— rearing facilities, pet shops, and natural waterways, in order to assess the potential for the introduction of myxozoan parasites to the environment through the pet shop trade. Four different Myxozoa were identified via their morphology and morphometrics: *Myxobolus cultus, Myxobolus diversus, Sphaerospora* sp., and *Hoferellus carassii*. The four myxozoan species detected were previously identified; the data indicates a seasonal variation in myxospore presence, more data collection is needed for conclusive results. This survey showed that feeder goldfish can introduce novel Myxozoa to natural waterways, although whether the Myxozoa can establish an infectious cycle in those waterways is unknown. If the Myxozoa can establish an infectious cycle, they could have a serious impact on fish residing in those waterways.

Key Words: *Carassius auratus*, goldfish, Myxozoa, parasitology

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The introduction of myxozoan parasites through the release of feeder goldfish (*Carassius auratus*).

by

Sophia Karandashova

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Dean, University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

_____

Sophia Karandashova, Author

I want to thank my mentor, Professor Jerri Bartholomew for giving the opportunity to carry out this research project in her lab.

Many thanks also to Sascha Hallett and Stephen Atkinson; without their help and advice this project would not have been possible.

I want to thank my family for their steadfast support and advice, especially my grandfather for his unrelenting belief in me.

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In loving memory of my grandmother, who taught me to never surrender, and that family is far more important than any accomplishment.

INTRODUCTION

Myxozoan parasites have been located worldwide; they infect a variety of hosts, including fish, trematodes (Overstreet 1976, Siau et al. 1981), reptiles (Lom 1990) amphibians (McAllister et al. 1995), and birds (Bartholomew et al. 2008), and mammals— specifically, shrews (Prunescu et al. 2007). *Carassius auratus*, or goldfish, have been identified as an intermediate host for several myxozoan species. Goldfish are a widespread invasive species; they have been found living in the rivers and lakes of every state in the U.S., excluding Alaska (Albert 2005). Initially, these fish either escaped the mud-bottom ponds they were kept in as pets, or were released into the wild by careless owners (GSMFC 2005). *Carassius auratus* is the one of the world's most popular aquarium fish (GSMFC 2005). Myxozoan parasites that infect goldfish can decrease the market value of the fish, and also lead to the death of the fish, since some Myxozoa are pathogens.

Myxozoa

There are two classes of the phylum Myxozoa, Myxosporea and Malacosporea. More than 2180 myxosporean species have been discovered, as well as 4 malcosporean species (Lom and Dyková 2006). Some myxozoan parasites are pathogenic. For example, *Myxobolus cerebralis* –the cause of whirling disease (O'Grodnick 1979), has been devastating salmon and trout populations in North America. *Ceratomyxa shasta* is also a pathogen of salmonid fish (Bartholomew et al. 1997). Several other serious diseases are also caused by Myxozoa; *Tetracapsula bryosalmonae* causes proliferative kidney disease in salmonids (Henderson and Okamura 2004), *Henneguya ictaluri* causes proliferative gill disease in catfish (Belem, Pote, 2001), and *Sphaerospora renicola* causes

swimbladder inflammation of carp (Kallert 2006). Nevertheless, not all myxozoan parasites are pathogenic.

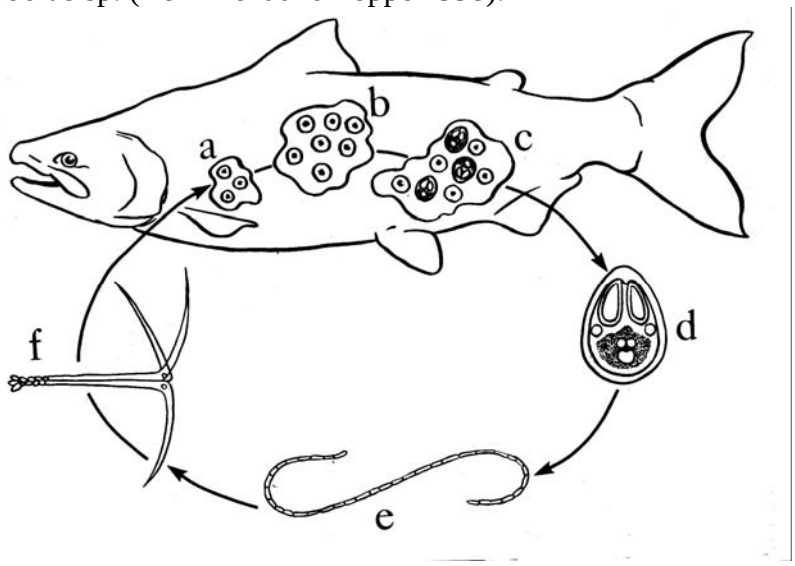
Identifying and studying myxosporean and Malacosporean species is difficult. Myxozoan parasites have a complex life cycle, consisting of a pair of alternating hosts, an aquatic invertebrate, such as a polychaete or oligochaete worm, and a vertebrate host, a fish. Myxozoa can be difficult to identify via morphology, due to the high variability in appearance even within species (Kallert 2006). The two mature forms of the parasite—the myxospore and actinospore, are drastically different morphologically, although they share some features; both have polar capsules, a sporoplasm, and valve cells. Unfortunately, there is no known correlation between a given actinospore shape and any Myxozoan genus; this makes the life cycles of myxozoan parasites difficult to describe. Actinospores are so remarkably different from myxospores morphologically, that they were initially identified as a separate class of myxozoan parasite (Lester et al. 1998).

Life Cycles

Although there are many myxozoan parasites that have been identified, only about 35 life cycles are currently known. The basic life cycle of a myxosporean involves a pair of alternating hosts (Figure 1). The intermediate host is a vertebrate, usually a fish, and an annelid worm, either polychaete or oligochaete, is the definitive host. Tubifex worms, *Tubifex tubifex*, are common annelid hosts (Lowers et al., 2006). The life cycles of myxozoans remain largely unknown. After the maturation of the myxospores in the intermediate host, which can occur in a variety of tissues, the spores are released into the water via urine or feces while the host is alive or after death of the intermediate host (Lowers et al., 2006). These shed spores infect the polychaete or oligochaete host

(Lowers et al., 2006). After typically two to four months, the infected worm begins to release actinospores, which in turn infect the vertebrate host, by attaching to gills or skin (Kallert 2006); the exact method of infection is difficult to identify. Because the release of actinospores and myxospores is often seasonal, a complete life cycle (transmission from definitive host to intermediate, and then back to definitive) can take one to two years to complete, despite the fact that spore development occurs in about two months in each host (Kallert 2006). There are many difficulties to describing life cycles of Myxozoa, one of the most prominent being the wide variety of possible definitive and intermediate hosts.

Figure 1. Simplified life cycle and development of the Myxozoa, exemplified by *Myxobolus* sp. (from Kent and Poppe 1998).



a and b vegetative development results in formation of a multinucleated plasmodium (trophozoite) with many daughter cells.

c daughter cells develop into multicellular myxospores.

d myxospores are released from the fish to complete development and transmission of the parasite - spores are released after death of the host, or through the urine and feces.

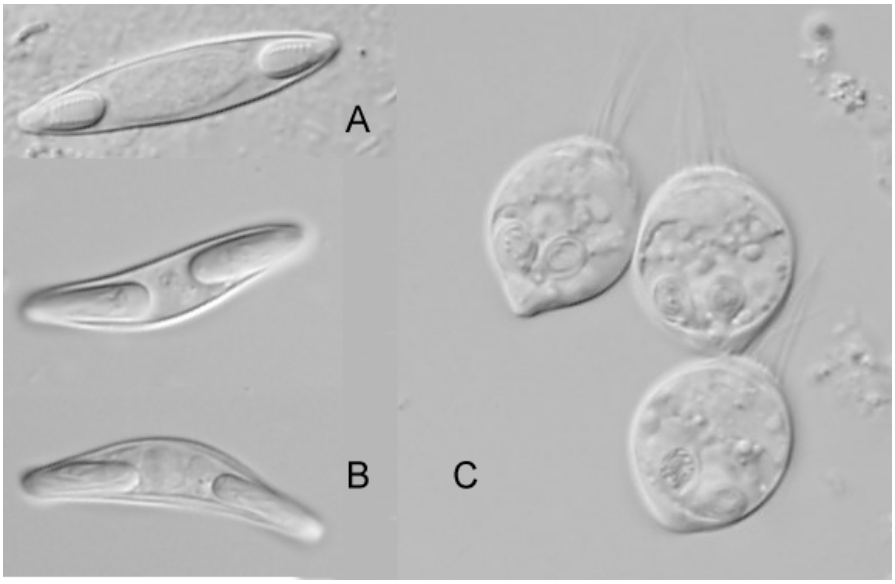
e annelid worms become infected after ingestion of myxospores from fish.

f following complex development, the "actinospore" is released from the worm and infects the fish host to complete the life cycle.

Characteristics of Myxospores

The taxonomy of myxozoan parasites is traditionally based solely on the shape and structure of the myxospores, which vary drastically (Figure 2). The vegetative (or trophic) stages offer few features important for classification (Svobodová, Vykusová 1991), although sequence analysis of the 18S rDNA or other genes is now recommended in species and type descriptions (Eszterbauer et al. 2006). Myxospores are "composed of several cells transfigured into 2 to 7 spore shell valves, 1 to 2 amoeboid infective germs (sporoplasms) and 2 to 7 polar capsules. The latter contain an extrudible filament of anchoring function" (Svobodová, Vykusová 1991). The most denominating feature of myxospores is the polar capsules (Kallert 2006), which contain the polar filaments. Myxospore dimensions are usually within 10 to 25 micrometers; vegetative stages can vary drastically in size and shape (Kallert 2006). The development of myxospores depends on factors including temperature and fish age (Kallert 2006). Myxospores are resilient and have adapted to cope with environmental changes (Kallert 2006); many resist freezing and remain viable after passage through alimentary tracts of cold-blooded animals (El- Matbouli and Hoffmann 1991). Myxospores are able to retain infectivity for 20 years or more under ideal conditions (El-Matbouli et al. 1992).

Figure 2. Variation of myxospore morphology.
(A = *Myxidium coryphaenoideum*, Image © Ivan Fiala)
(B = *Sphaeromyxa hellandi*, Image © Ivan Fiala)
(C = *Chloromyxum leydigi*, Image © Ivan Fiala)

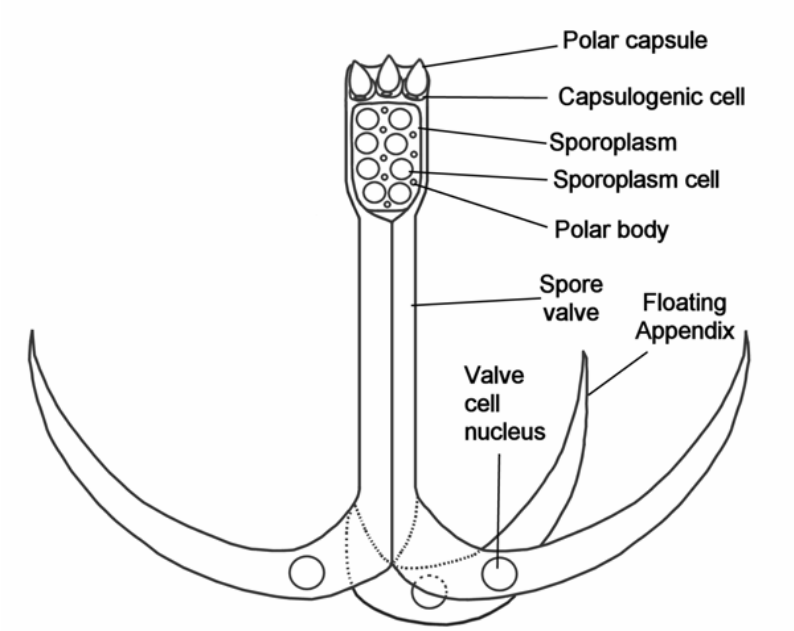


Characteristics of Actinospores

Actinospores also vary morphologically, although they typically have triradial symmetry, and softer valves than myxospores (Kallert 2006). Furthermore, they are usually drastically larger, having a size of up to 300 micrometers (Kallert 2006). Despite the drastic difference in size and shape, actinospores have a few similarities to myxospores. Both myxospores and actinospores contain teardrop-shaped polar capsules and circular valve cell nuclei; however, the placement of both components is different due to the dissimilar morphology of the two. Actinospores are released into the water from the invertebrate host, usually a polychaete or oligochaete worm, and infect fish by coming into contact with their gills or skin (Kallert 2006). Actinospores remain infective for several days (Yokoyama et al. 1993b, Xiao & Desser 2000). *Myxobolus cerebralis* triactinomyxons have longevity of up to 15 days at a temperature of up to 15°C; it is

unknown how long the spores would survive in natural waterways (El-Matboulia et al. 1998).

Figure 3. Schematical structure of a myxozoan triactinomyxon-type actinospore (from Kallert 2006).



An intermediate host of Myxozoa— common goldfish, *Carassius auratus*

The common goldfish, *Carassius auratus* (Linnaeus 1758) is a freshwater species native to Asia that flourishes in a variety of aquatic environments (Albert 2005). It is used as an ornamental species in aquaculture, as well as live food for fish in aquariums as well as live bait for fishing (Albert 2005). Goldfish are omnivorous, although wild goldfish have been described as benthic herbivores (GSMFC 2005). Goldfish are a widespread invasive species; they have been found living in the rivers and lakes of every state in the U.S., excluding Alaska (Albert 2005). Initially, these fish either escaped the mud-bottom ponds they were kept in as pets, or were released into the wild by careless owners (Albert

2005). *Carassius auratus* is one of the world's most popular aquarium fish, and can act as hosts for a plethora of parasites, including Myxozoa.

Approximately 39 species of Myxozoa have been isolated in goldfish (Table 1) (Lom and Dyková 1992). Some myxozoan parasites infect only closely related species (Kallert 2006), but others have a very diverse fish host range. Unfortunately, it appears that several of the Myxozoa that infect goldfish are members of the latter group (Table 1). A total of 15 of the 39 parasites mentioned above have also been isolated from *Cyprinus carpio* (common carp). *Cyprinus carpio* is another widespread invasive species that has been introduced into temperate freshwaters throughout the world as food and also as an ornamental fish (ISSG 2006). The common carp can be found in temperate freshwaters ranging from central Mexico to central Canada; it is one of the most widely distributed fish in North America (ISSG 2006). In the U.S., *Cyprinus carpio* is often regarded as a sportfish— a fish that is hunted for sport. *Sphearospora renicola*, a serious myxozoan pathogen of the common carp that causes swim bladder inflammation (SBI), has been previously isolated in goldfish (Lom and Dyková 1992). A second source stated that a different *Sphaerospora* sp. infected goldfish, one morphologically identical to *Sphaerospora renicola* but genetically distinct (Eszterbauer, Székely, 2004). *Sphaerospora* sp. is a pathogenic myxozoan parasite that has been shown to infect the respiratory epithelium of gills in goldfish (Hedrick et al. 1990), and also the renal tubules of the kidney (Eszterbauer, Székely, 2004). *Sphaerospora molnari*, a serious pathogen which causes sphaerosporosis of the gills, skin and blood in the common carp, can also be found in *Carassius carassius* (crucian carp) and goldfish (Lom and Dyková 1992).

Table 1. Synopsis of Myxozoa previously described from *Carassius auratus.*

Myxozoa	**Life Cycle**	**Definitive Host**	**Site of Infection**	**Pathogen**	**Other Fish Infected**
Chloromyxum auratum (Hallett et al. 2006)	Partially Described	Unknown oligochaete	Gall bladder	No	N/A
Chloromyxum ellipticum (Li and Nie in Chen 1973)	N/A	N/A	Gall bladder	N/A	*Ctenopharyngodon idella*
Chloromyxum fluviatile (Thélohan 1812)	N/A	N/A	Gall bladder	N/A	*Cyprinus carpio* *Blicca bjoerkna* *Carassius carassius* *Rutilus rutilus*
Henneguya miyairii (Kudo, 1919)	N/A	N/A	Body cavity	N/A	*Carassius carassius*
Henneguya zikaweiensis (Sikama 1938)	N/A	N/A	Gills, skin cornea	N/A	N/A
Hoferellus carassii (Akhmerov 1960)	Described	*Branchiura sowerbyi*	Kidney	Yes	N/A
Myxidium cuneiforme (Fujita 1924)	N/A	N/A	Gall bladder	N/A	*Cyprinus carpio* *Carassius carassius*
Myxidium ochengensis (Chen and Hsieh 1984)	N/A	N/A	N/A	N/A	N/A
Myxidium wupehensis (Chen and Hsieh 1984)	N/A	N/A	Kidney	N/A	N/A
Myxobolus acutus (Fujita 1912)	N/A	N/A	Kidney	N/A	*Cyprinus carpio*
Myxobolus bibullatus (Kudo 1934)	N/A	N/A	N/A	N/A	Members of Family *Catostomidae*

Myxobolus bilis (Akhmerov 1960)	N/A	N/A	N/A	N/A	N/A
Myxobolus carassii (Klokacheva 1914)	Described	*Tubifex tubifex*	Kidney, body cavity organs, muscle	No	*Cyprinus carpio*
Myxobolus cultus (Yokoyama et al. 1995)	Described	*Branchiura sowerbyi*	Cartilage, kidney	N/A	N/A
Myxobolus diversus (Nie and Li in Chen 1973)	Not Described	N/A	Fins, cartiledge	No	N/A
Myxobolus egregius (Nie and Li in Chen 1973)	N/A	N/A	N/A	N/A	N/A
Myxobolus ellipsoides (Thelohan 1892)	N/A	N/A	Connective tissue of swim bladder and gills	N/A	*Cyprinus carpio* and other cyprinids *Tinca tinca* *Rutilus rutilus* *Scardinius erythrophthalmus*
Myxobolus gigi (Fujita 1927)	N/A	N/A	N/A	N/A	N/A
Myxobolus koi (Kudo 1920)	N/A	N/A	Connective tissue of swim bladder and gills, subcutaneous tissue of the head	Yes	*Cyprinus carpio*
Myxobolus kubanicum (Bykhovskava and Bykhovski 1940)	N/A	N/A	N/A	N/A	N/A
Myxobolus musculi (Keysselitz 1908)	N/A	N/A	Muscle tissue	N/A	N/A

Myxobolus nemachili (Weiser 1949)	N/A	N/A	Brain, gills, intestine, muscle, heart, gall bladder	N/A	*Cyprinus carpio* *Luxilis cornutus* *Notropis heterolepis*
Myxobolus notropis (Fantham, Porer and Richardson 1939)	N/A	N/A	Brain, liver, kidney, intestine, muscle, spleen, swim bladder	N/A	*Carassius gibelio* *Luxilis cornutus* *Notemigonus crysoleucas*
Myxobolus orientalis (Schulan 1962)	N/A	N/A	N/A	N/A	N/A
Myxobolus rotundus (Lu et al. 2003)	N/A	N/A	skin	N/A	*Cyprinus carpio* *Carassius carassius*
Myxobolus rutilus (Nie and Li in Chen 1973)	N/A	N/A	N/A	N/A	N/A
Myxobolus solidus (Shulman 1962)	N/A	N/A	N/A	N/A	N/A
Myxobolus toyamai (Kudo 1915)	N/A	N/A	gills	No	*Cyprinus carpio*
Myxobolus velatus (Li and Nie in Chen 1973)	N/A	N/A	N/A	N/A	N/A
Myxobolus wulii (Landsberg and Lom 1991)	N/A	N/A	N/A	N/A	N/A
Sphaerospora angulata (Fujita 1912)	N/A	N/A	Kidney	No	*Cyprinus carpio*
Sphaerospora molnari (Lom, Dyková, Pavlásková and Grupcheva 1983)	N/A	N/A	Gills, skin,	Yes	*Cyprinus carpio*

Sphaerospora renicola (Dyková, Lom 1982)	Described	*Branchiura sowerbyi*	Kidney, swim bladder	Yes	*Cyprinus carpio*
Sphaerospora sp. (Hedrick et al. 1990)	Not Described	N/A	Kidney	N/A	N/A
Spirosuturia carassii (Chen and Hsieh 1984)	N/A	N/A	Urinary bladder	N/A	N/A
Thelohanellus carassii (Kashkovski 1974)	N/A	N/A	N/A	N/A	N/A
Thelohanellus dogieli (Akhmerov 1955)	N/A	N/A	Gills, fins, skin, liver	N/A	*Cyprinus carpio*
Thelohanellus fuhrmanni (Auerbach 1909)	N/A	N/A	gills	N/A	*Cyprinus carpio*
Thelohanellus gangeticus (Tripathi 1953)	N/A	N/A	N/A	N/A	N/A
Zschokkella carassii (Nie and Li in Chen 1973)	N/A	N/A	N/A	N/A	*Cyprinus carpio*

A Known Definitive Host of Goldfish Myxozoa— *Branchiura sowerbyi*

Branchiura sowerbyi (Bedard 1892) is an oligochaete of the family Tubificidae, and acts as a definitive host for *Hoferellus carassii* (Yokoyama et al. 1993a) and *Myxobolus cultus* (Yokoyama et al. 1995); both have been shown to infect goldfish. In fact, *Branchiura sowerbyi* is the only fully identified definitive host for myxozoan parasites that use goldfish as an intermediate host; all that is known about the definitive host of *Chloromyxum auratum* is that it is a freshwater oligochaete (Atkinson et. al.

2007). *Branchiura sowerbyi* has also been shown to act as a definitive host for several Myxozoa that infect *Cyprinus carpio,* the common carp (Kent et al. 2001). This oligochaete has several distinguishing characteristics, the most obvious being that it has gills, unlike any other tubificid worm (Brinkhurst et al., 1971). It is a relatively large worm, as it can reach the size of 185mm (Spencer 1932). *Branchiura sowerbyi* is known to be widespread in North and South America, Europe, and has also been reported in South Africa and Australia (Brinkhurst et al., 1971); it has been reported "on all continents except Antarctica" (Carroll et al., 1972). In the USA, it has been found in various lakes, streams, and reservoirs throughout the country, including places in Oklahoma (Carroll et al., 1972), and Lake Erie in 1961 (Hiltunen 1969).

Although some myxozoan parasites usually infect only closely related species (Kallert 2006), and indeed some of the Myxozoa in goldfish have also been found in the closely related species *Carassius carassius*, this is not necessarily true for all Myxozoa known to infect goldfish. A total of 15 species that infect goldfish have also been isolated from the common carp; no doubt there are other fish in the *Cyprinidae* family that can be infected with Myxozoa previously isolated in goldfish. Given the fact that both *Branchiura sowerbyi*, a known definitive host for myxozoan parasites, and goldfish are widespread throughout many parts of the world— goldfish may pose a serious problem to fish inhabiting natural waterways if they can introduce novel myxozoan parasites.

OBJECTIVE

The objective of this study is to survey the myxozoan infections in goldfish from three different locations (rearing facility, pet shop, and natural waterways) and determine the possibility of the introduction of myxozoans to the environment through the pet shop trade. To achieve this objective the following aims were pursued:

1. Describe myxozoans in goldfish from a rearing facility (farm)
2. Describe myxozoans in goldfish from a pet shop
3. Describe myxozoans in goldfish released into natural waterways
4. Assess the risk of goldfish introducing novel myxozoan parasites to the environment

MATERIALS AND METHODS

Source of Samples

Five sets of samples (fish and sediment) from three different farms were collected by Professor Andrew E. Goodwin and provided for this project (Table 1). The samples— AN1, FP1, FP2, FM1, FM2, originated from central Arkansas and were collected on August 22, 2007, and shipped overnight to Oregon. The fish were from privately owned ponds where they were reared to be feeder goldfish— to be used as bait and food for larger fish. Each sample set consisted of a Ziploc bag with approximately ten euthanized feeder goldfish and a second Ziploc bag containing sediment and water. The samples with identical letter codes (e.g. FP1 and FP2) originated from the same farm, but from ponds separated by more than a mile: this was done to maximize the diversity of the samples. The sediment collected was chosen from the pond randomly (i.e. grab samples).

These ponds were used exclusively for rearing feeder goldfish for commercial use; however, it is unknown if there were any other fish kept in ponds nearby.

Another three sample sets were collected by Professor Goodwin on May 1, 2008, (Table 4) and shipped overnight to Oregon. These were from a single farm that raised feeder goldfish exclusively, with ponds approximately 2-3 acres in size. These were labeled with the numbers 1 through 3. The first two ponds were in the process of being drained for harvest at the time of sample collection (Figure 4).

All of the sediment and water samples that were taken from the private ponds in Arkansas were approximately 1 liter in volume, and composed primarily of sediment. They were packaged in Ziploc plastic bags and shipped in a cooler which arrived in the lab on the day following collection.

Samples from Upper Duck Pond in Lithia Park, located in Ashland, Oregon (Figure 5), were provided by Mr. Paul Kay. These samples were collected in 2008 on April 25th through 29th (Table 9). Several liters of sediment were collected from two randomly selected sites in the Upper Duck Pond. The samples were collected during the first cleaning the pond had in 28 years. Before draining, the pond contained approximately 287000 gallons of liquid and sediment. The only sources of water for the pond were groundwater and precipitation; there was no outlet for the pond. Much of the sediment buildup resulted from park visitors feeding the ducks that frequented the pond year-round, as well as from the fecal matter of the birds themselves. The majority of the sediment collected was comprised of decomposed granite and organic matter, including manure. The extreme organic load likely reduced the habitable areas for fish to the shallow layer on top of the pond. Thus goldfish, because of their bright coloring, would

be easy prey to birds such as Great Blue Herons, which were observed feeding at the pond. Other species collected from the pond included several turtles— Western Pond Turtles and Red Eared Sliders, and two other species of fish— a single Koi, and one Brown Bullhead.

All of the aforementioned sediment samples were screened for oligochaete worms using a mesh screen with a limiting size of 0.175 mm. If worms were found, the water that sediment sample was kept in was screened for actinospores.

Figure 4. Picture of goldfish being harvested at a farm in Arkansas

Figure 5. Upper Duck Pond in Lithia Park in Ashland, Oregon.
(Photo taken by Stephen Atkinson)

Collection of Myxospores : Fish Description

From Arkansas, euthanized goldfish, stored in plastic bags, were shipped in a cooler along with the corresponding sediment samples. All of the fish arrived in various states of decomposition; the ones in the worst state had almost completely liquefied organs, with only a differentiable, intact kidney. At a minimum, kidney and muscle tissues were collected from each fish for examination.

Live goldfish were collected from Upper Duck Pond in Lithia Park in Ashland, Oregon. The fish were killed by placing them in a liter of water containing a teaspoon of MS 222—an anesthetic, or with the application of a scalpel to the brain, and then dissected. The tissues sampled were as follows: kidney, muscle, liver, gall bladder.

Eleven live feeder goldfish were purchased from PetCo on July 2, 2008. They were euthanized and frozen for later dissection. The origin of these feeder goldfish prior to PetCo, as well as the conditions they were reared in is unknown. The fish were dissected; due to the small size of the fish, the only tissues sampled were the kidney and muscle tissue.

Collection of Myxospores : Dissection Procedure

1. External Examination

   After either thawing of frozen fish, or euthanization of the goldfish via MS222 (a sedative which results in the fish dying through lack of oxygen) or a scalpel to the brain, the goldfish were prepared for dissection. They were rinsed with distilled water, and photographed beside a scalpel that was intended to be used as a reference for size. The total length of the fish was measured before dissection. The general condition of the fish was also recorded (e.g. presence of damage to the fins, tail, scales).

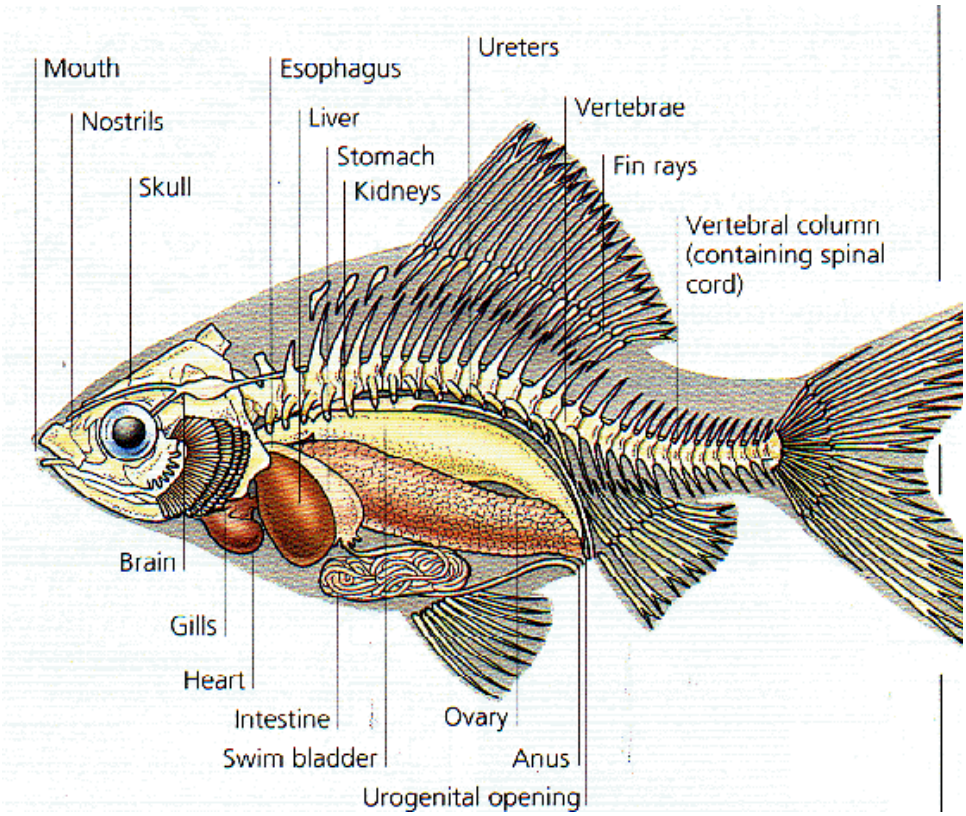
   a. Opercula and Gills

      The opercles were cut away and placed aside. For smaller fish, the gill arches were removed and placed on a slide and covered with a cover slip. Larger fish required the gill filaments to be cut away from the gill arches before being placed on a slide for examination.

2. Internal Examination

In order to minimize damage to the tissues, the fish were not refrozen; optimally, the fish were dissected immediately after euthanization. The fish was laid on one side and opened from the anal region to the opercula using a pair of scissors. This was followed by a pair of incisions from the ventral side of the fish to its spine at the operculum and anus. All of these were done carefully in order to minimize damage to the organs. The resulting "flap" of flesh was lifted to expose the abdominal cavity of the fish. Any connective tissue linking muscle tissue and ribs to the organs in the abdominal cavity was cut away carefully with a scalpel.

Figure 6. Basic internal anatomy of a fish. (From http://oceanblue2u.com)



a. Abdominal Cavity

The viscera were removed *in toto*. First the large intestine was cut at the anus and the majority of the entrails removed with tweezers. The pharyngeal/esophageal junction was cut before completing the removal of the entrails. All of this was done without rupturing the intestines within the abdominal cavity.

A smear of the gall bladder was made after removing the organ. For the larger fish, the gall bladder was placed in a Petri dish before being teased apart. The gall bladder of a smaller fish (if it was intact) was teased apart on a slide and used as a smear. Tissue squashes were prepared using pieces of the kidney and liver.

b. Musculature

On the larger fish, the musculature was examined by carefully cutting out a thin slice of muscle creating an organ squash. For all of the smaller fish, a small chunk of muscle tissue was used for a tissue squash.

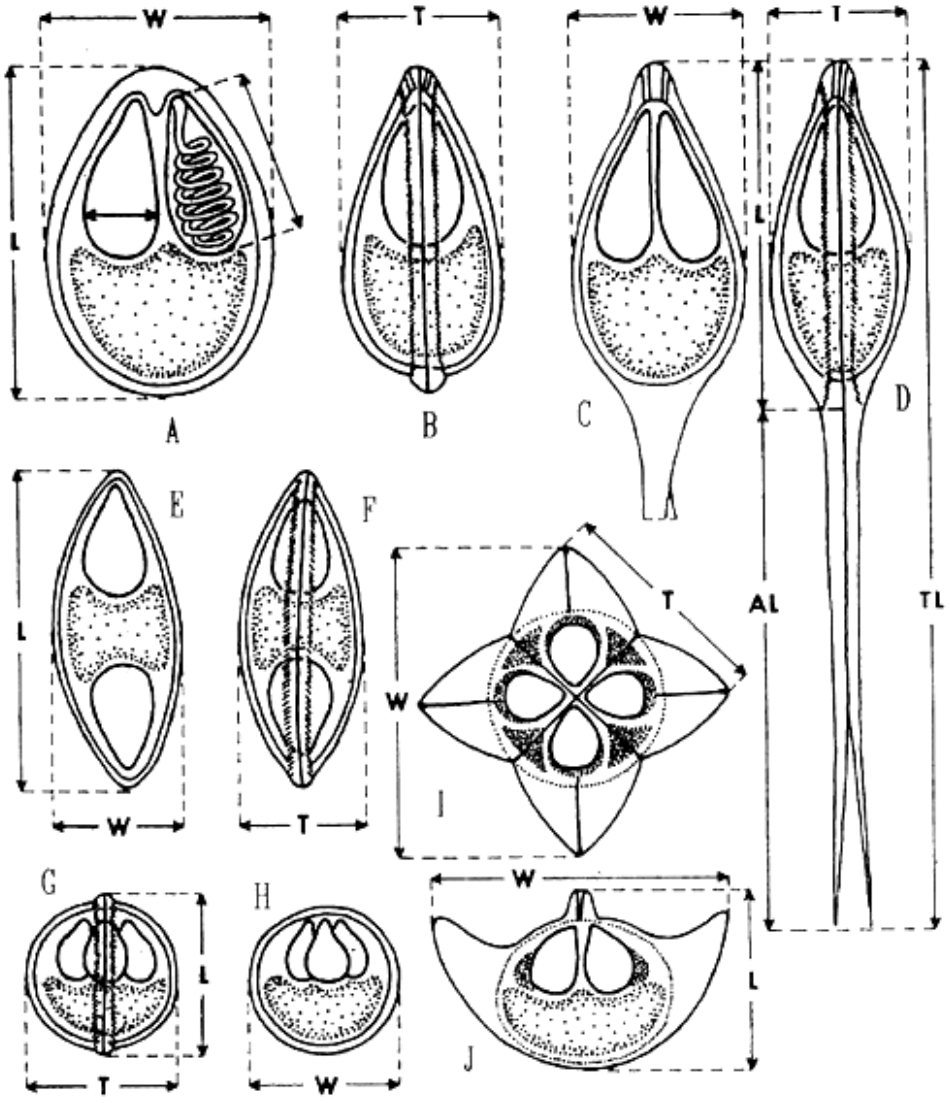
For the larger fish, tissue samples of organs containing myxospores were stored in 2.0ml test tubes and frozen for later use; smaller fish were refrozen for the same purpose.

Identification of Myxospores: Microscopy

The tissues sampled were examined via bright field microscopy at 400 times and 1000 times magnification (40x or 100x for objective lens plus 10x for oculars) in an

attempt to identify myxospores. After initial identification, a second microscope, connected to digital camera was utilized. Digital image capture (IM 50 software) was used to photograph the identified myxospores, either under bright field or Nomarski, otherwise known as differential interference contrast (DIC), microscopy. The contrast and brightness of the photographs was adjusted using Photoshop CS2 in order to maximize the clarity of the myxospore and its characteristic features. These photographs were analyzed using the SPOT imaging program to determine the dimensions of the myxospores, such as the spore body length, width, and thickness, as well as the dimensions of any outstanding features of the spores, including polar capsules, polar filaments, and any caudal appendages. Spores were characterized following the guidelines of Lom and Arthur (1989). The following procedure was used to determine the myxospore species. The first factor considered was morphology: the shape of the spore body, placement of polar capsules, and any outstanding features such as caudal appendages or spore body ridges. Next, the spores were measured (Figure 7) and the morphometrics compared to possible matches identified from literature via morphology. Last, the tissue specificity (kidney, liver, muscle) and host specificity (goldfish) were considered.

Figure 7. Methods of measurement of myxosporean spores of various genera. (from Z. Svobodová, B. Vykusová 1991).



A and B *Myxobolus* in frontal and side sutural view

C and D *Henneguya* in frontal and side view

E and F *Myxidium* in frontal and side view

G and H *Chloromyxum* in side or sutural and frontal view

I and J *Kudoa* in apical view and *Kudoa* in a possible side view (the diagonal one) (J).

(Measurement of the polar capsule is indicated in A. L - length of the spore, W - width of the spore, T - thickness of the spore; in spores with caudal appendages such as *Henneguya*, Al - length of the caudal appendage, Tl - total length of the spore.)

Identification of Actinospores

The water of samples that contained oligochaetes was screened for actinospores using a mesh with a limiting size of 20 µm after moistening the mesh with distilled water. Water kept in the same container as the sediment samples was poured through this mesh slowly, in order to minimize the amount of large particulates. Distilled water was used to wash retentate into the center of the mesh. A transfer pipette was used to transfer water and retentate to a small, shallow Petri dish. The retentate was screened for actinospores using polarized light, with each sample being observed for a minimum of 5 minutes. IM 50 software was used to photograph any actinospores evident.

RESULTS

Goldfish Samples

Arkansas Samples from 2007

The goldfish received from Arkansas on August 22 of 2007 were partially decomposed, despite the fact that after death they were kept cold (but not frozen) prior to and during transport. The mean total length of the goldfish was 39 mm (range 28 - 80 mm). The largest goldfish were in the best condition; the internal organs were still relatively easy to differentiate. Most of the fish were in such a degenerate condition that most of the internal organs as well as some of the muscle tissue was liquefied.

Two different Myxozoan species were detected in the goldfish received from Arkansas in 2007: *Myxobolus diversus* (Figure 8) and *Myxobolus cultus* (Figure 9). *Myxobolus diversus* was identified as a result of the distinct size difference of the two polar capsules. *Myxobolus cultus* was identified due to the ellipsoidal shape of the spore, thick sutural ridge, and a pair of polar capsules similar in size placed side by side in the

spore. Both myxospore types were detected in tissue squashes of the kidney, although due to the condition of the fish it was difficult to determine in which part of the organ they resided, or if they were from some other liquefied tissue. In several instances, for fish in groups AN and FP#1, goldfish were infected by both myxozoan species (Table 2). Most of the infections detected were light, consisting of half a dozen spores or less per fish. One infection was heavy: there were over a dozen spores in the kidney of the fish. For FM#1, only *Myxobolus cultus* myxospores were found. Goldfish in sample sets from FM#2 and FP#2 were not infected with myxozoan parasites. A summary of the measurements of the spores is in Tables 3 and 4.

Table 2. Myxozoan species detected in samples received from Arkansas in 2007.

	AN	**FP#1**	**FP#2**	**FM#1**	**FM#2**
Myxobolus diversus	2	2	0	4	0
Myxobolus cultus	1	0	0	0	0
Myxobolus diversus* and *Myxobolus cultus	1[1]	3	0	0	0
Ratio: (Infected Fish/Total Fish Sampled)	4/5	5/8	0/5	4/7	0/3

[1] heavy infection

Table 3. Summary of *Myxobolus diversus* measurements for samples from Arkansas (2007). (All measurements are in µm.) (Goldfish n = 1)(Spores n = 13)

	Average	**Maximum**	**Minimum**	**Standard Deviation**
Spore, Total Length	14.8	15.9	13.3	0.9
Spore Body, Length	14.8	15.9	13.3	0.9
Spore Body, Width	9.1	9.8	8.3	0.6
Spore Body, Thickness	7.8	8.7	7.3	0.6
Polar Capsules, Length	3.8	5.6	1.7	1.2
Polar Capsules, Width	2.6	3.9	1.4	0.8
Polar Filament (Turns)	5	5	5	0

Table 4. Summary of *Myxobolus cultus* measurements for samples from Arkansas

(2007). (All measurements are in µm.) (Goldfish n = 1)(Spores n = 1)

	Spore Measurements
Spore Body, Length	15.5
Spore Body, Width	N/A
Spore Body, Thickness	7.6
Polar Capsules, Length	4.9
Polar Capsules, Width	2.8
Polar Filament (Turns)	5

Figure 8. *Myxobolus cultus* myxospore, front view, (line = 10 µm). Wet tissue mount of the kidney from PetCo Fish 1, unfixed and unstained. Bright field microscopy.

(A) wet mount of kidney tissue containing myxospore
(B) Polar capsule with coiled polar filament

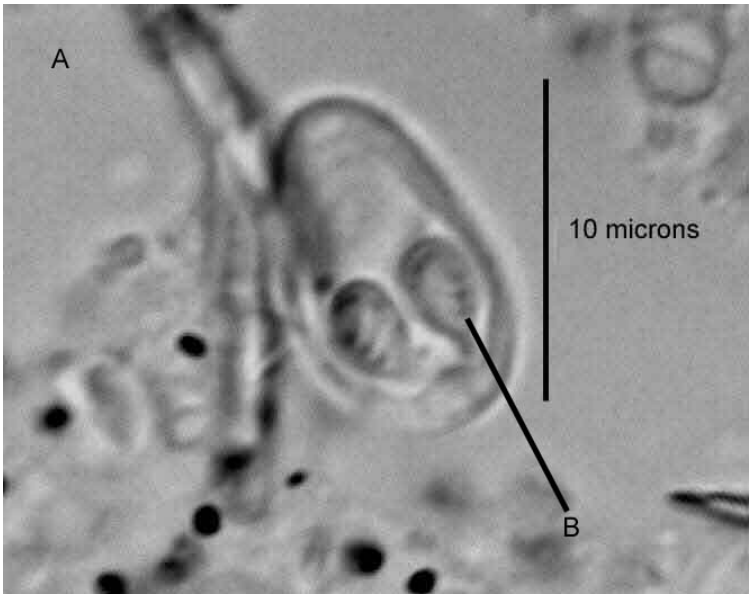
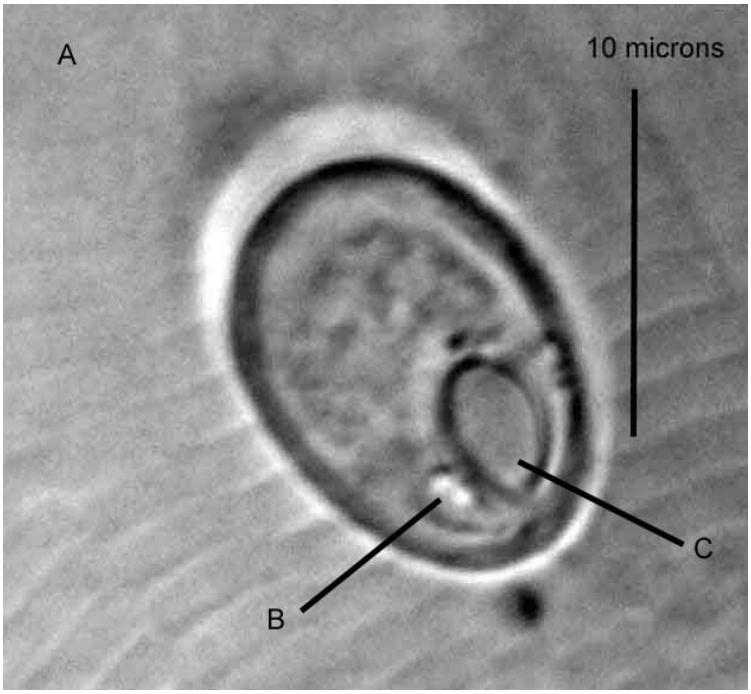


Figure 9. *Myxobolus diversus* myxospore front view, (line = 10 µm). Wet tissue mount of the muscle tissue from PetCo Fish 5, unfixed and unstained. Bright field microscopy.

(A) wet mount of muscle tissue containing encysted myxospore
(B) smaller polar capsule
(C) larger polar capsule



Arkansas Samples from 2008

A second set of euthanized goldfish was received from Arkansas on May 1 of 2008. They were in better condition than those received the year prior. The mean total length of the goldfish was 54 mm, (range 39 mm - 75 mm). Like in the samples received from Arkansas in 2007, the largest goldfish were in the best condition; they had differentiable organs in the body cavity. Although most of the fish were in various stages of putrefaction, few were in such an advanced stage as to have liquefied muscle tissue.

Two myxozoan species were detected in the goldfish received from Arkansas in 2008: *Myxobolus diversus* (Figure 8) and *Sphaerospora* sp. (Figure 10). *Myxobolus*

diversus was identified in the same manner as listed above. *Sphaerospora* sp. was identified due to its small size, distinct circular shape, and mucus coating. Both of the myxozoan species were found in the kidney of the goldfish. There were no instances of multiple infections in the same fish. Goldfish from sample set #1 contained *Myxobolus diversus*, and those from sample set #3 contained *Sphaerospora* sp. (Table 5). Fish from sample set #2 were not infected with a myxozoan parasite. A summary of the measurements taken is presented in Table 6. There is no summary for measurements of the *Myxobolus diversus* myxospores, due to the fact that the spores were small in number.

Table 5. Myxozoan species detected in samples received from Arkansas in 2008.

	1	**2**	**3**
Myxobolus diversus	0	0	1[1]
***Sphaerospora* sp.**	2	0	0
Ratio: (Infected Fish / Total Fish Sampled)	2/7	0/10	1/9

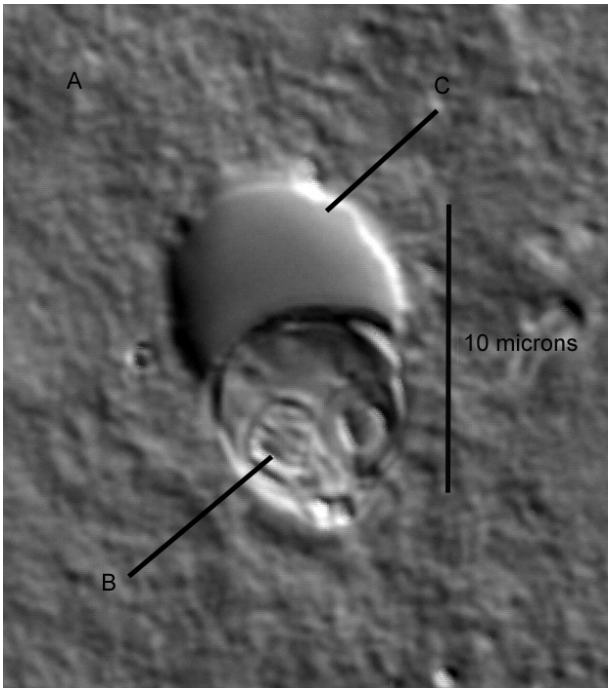
[1] heavy infection

Table 6. Summary of *Sphaerospora* sp. measurements for samples from Arkansas (2008). (All measurements are in µm.) (Goldfish n = 1)(Spore n = 4)

	Average	**Maximum**	**Minimum**	**Standard Deviation**
Spore Body, Length	6.3	6.5	6.1	0.2
Spore Body, Width	5.4	5.9	5.1	0.4
Spore Body, Thickness	5.0	5.0	5.0	N/A
Polar Capsules, Length	2.5	2.8	2.1	0.3
Polar Capsules, Width	1.8	2.4	1.3	0.4
Polar Filament (Turns)	N/A	N/A	N/A	N/A

Figure 10. *Sphaerospora* sp. myxospore, front view, (line = 10 µm). Wet tissue mount of the kidney tissue from Ashland Fish 9, unfixed and unstained. Nomarski (DIC) microscopy.

(A) wet mount of kidney tissue containing myxospore
(B) polar capsule with coiled polar filament
(C) distinct mucus coat extruding behind the myxospore



PetCo Samples

Live goldfish received from a PetCo in Corvallis, Oregon on July 2 of 2008 were in good condition. The fish did not have any evident injuries, externally or internally. The mean total length of the goldfish was 32 mm, with a maximum of 40 mm and minimum of 26 mm. Since the goldfish were frozen after euthanization and not dissected immediately, some decomposition occurred. However, most of the organs were still differentiable.

Three myxozoan species were detected in the goldfish: *Myxobolus diversus* (Figure 1), *Myxobolus cultus* (Figure 2), and *Sphaerospora* sp. (Figure 3). All three of the

species were identified as stated in previous sections. The three different myxozoan parasites were found in the kidney of the dissected fish. *Myxobolus cultus* was also found encysted in the muscle tissue (Figure 2). There were no fish with two or more myxozoan infections (Table 7). Measurements for *Sphaerospora* sp. were not recorded, due to the small amount of spores present in the tissue sample and difficulty capturing them on camera.

Table 7. Myxozoan species detected in samples received from PetCo.

	Sample Set #1
Myxobolus cultus	2
Myxobolus diversus	1
***Sphaerospora* sp.**	1
Ratio: (Infected Fish / Total Fish Sampled)	4/11

Table 8. Summary of *Myxobolus diversus* measurements for samples from PetCo Corvallis, Oregon (2008). (All measurements are in µm.)
(Goldfish n = 1)(Spore n = 2)

	Average	**Maximum**	**Minimum**	**Standard Deviation**
Spore Body, Length	13.7	13.9	13.5	0.3
Spore Body, Width	10.2	11.0	9.4	1.1
Spore Body, Thickness	N/A	N/A	N/A	N/A
Polar Capsules, Length	3.6	4.9	2.2	1.3
Polar Capsules, Width	2.0	1.7	1.1	0.8

Table 9. Summary of *Myxobolus cultus* measurements for samples from PetCo Corvallis, Oregon (2008). (All measurements are in µm.)
(Goldfish n = 1)(Spore n = 5)

	Average	**Maximum**	**Minimum**	**Standard Deviation**
Spore Body, Length	10.7	11.1	10.2	0.4
Spore Body, Width	5.8	6.3	5.4	0.4
Spore Body, Thickness	N/A	N/A	N/A	N/A
Polar Capsules, Length	3.7	3.9	3.5	0.2
Polar Capsules, Width	2.1	2.4	1.7	0.2
Polar Filament (Turns)	5.0	5.0	5.0	N/A
Polar Filament (Turns)	4.0	4.0	4.0	N/A

Ashland, Oregon Samples

The live fish received from Upper Duck Pond in Lithia Park (Ashland, Oregon) on May 1 of 2008 were in good condition. Several fish had lost scales and had ripped tails, but no significant injuries were evident; it is possible these minor injuries occurred during collection and transport. The mean total length of the goldfish was 135 mm, with a maximum of 190 mm and minimum of 55 mm. The fish were also healthy internally, with no evidence of illness or injury. Since the goldfish were euthanized immediately before dissection, the organs were easily differentiable and fully intact.

Two myxozoan species were detected in the goldfish: *Hoferellus* sp. (Figure 11) and *Sphaerospora* sp. (Figure 10). *Sphaerospora* sp. was identified in the same manner as listed above. *Hoferellus* sp. was identified due to the unique longitudinal ridges or striations that extend to short caudal filaments. Both myxozoan species had spores evident in the kidney. The goldfish infected with *Hoferellus* sp., had an especially heavy infection (Table 10), with many intermediate stages of the parasite evident in the heavily inflamed kidney (Figure 12).

Table 10. Myxozoan species detected in samples received from Upper Duck Pond in Lithia Part in Ashland, Oregon (2008).

	Sample Set #1
***Hoferellus* sp.**	1[1]
***Sphaerospora* sp.**	1
Ratio: (Infected Fish / Total Fish Sampled)	2/15

[1] heavy infection

Table 11. Summary of *Hoferellus* sp. measurements for samples from Ashland, Oregon (2008). (All measurements are in µm.)

(Goldfish n = 1)(Spore n = 29)

	Average	**Maximum**	**Minimum**	**Standard Deviation**
Spore, Total Length	14.0	15.4	12.0	1.0
Spore Body, Length	10.2	11.7	8.9	0.8
Spore Body, Width	6.5	7.1	5.2	0.4
Spore Body, Thickness	6.3	7.0	4.8	0.7
Bristle Length	3.8	5.8	1.9	0.9
Polar Capsules, Length	3.5	4.7	2.6	0.5
Polar Capsules, Width	2.1	2.9	1.6	0.4
Polar Filament (Turns)	4.0	5.0	4.0	0.4
Polar Filament (Turns)	4.5	5.0	4.0	0.7
Polar Filament, Length	11.7	11.7	11.7	N/A

Table 12. Summary of *Sphaerospora* sp. measurements for samples from Ashland, Oregon (2008). (All measurements are in µm.)
(Goldfish n = 1)(Spore n = 11)

	Average	**Maximum**	**Minimum**	**Standard Deviation**
Spore Body, Length	7.1	7.8	6.2	0.4
Spore Body, Width	6.1	6.4	5.6	0.3
Spore Body, Thickness	5.4	5.9	5.0	0.5
Polar Capsules, Length	3.0	3.5	2.6	0.3
Polar Capsules, Width	2.1	2.6	1.7	0.3
Polar Filament (Turns)	5.0	5.0	5.0	0.0

Figure 11. *Hoferellus* sp. myxospore, front view, (line = 10µm). Wet tissue mount of the kidney tissue from Ashland Fish 14, unfixed and unstained. Nomarski (DIC) microscopy.

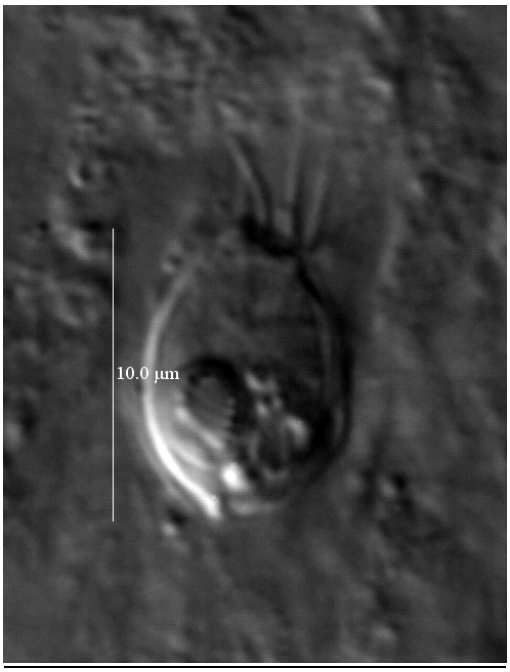


Figure 12. *Hoferellus* sp. myxospores and proliferating states in kidney renal tubules, (line = 50µm). Wet tissue mount of the kidney tissue from Ashland Fish 14, unfixed and unstained. Bright field microscopy.

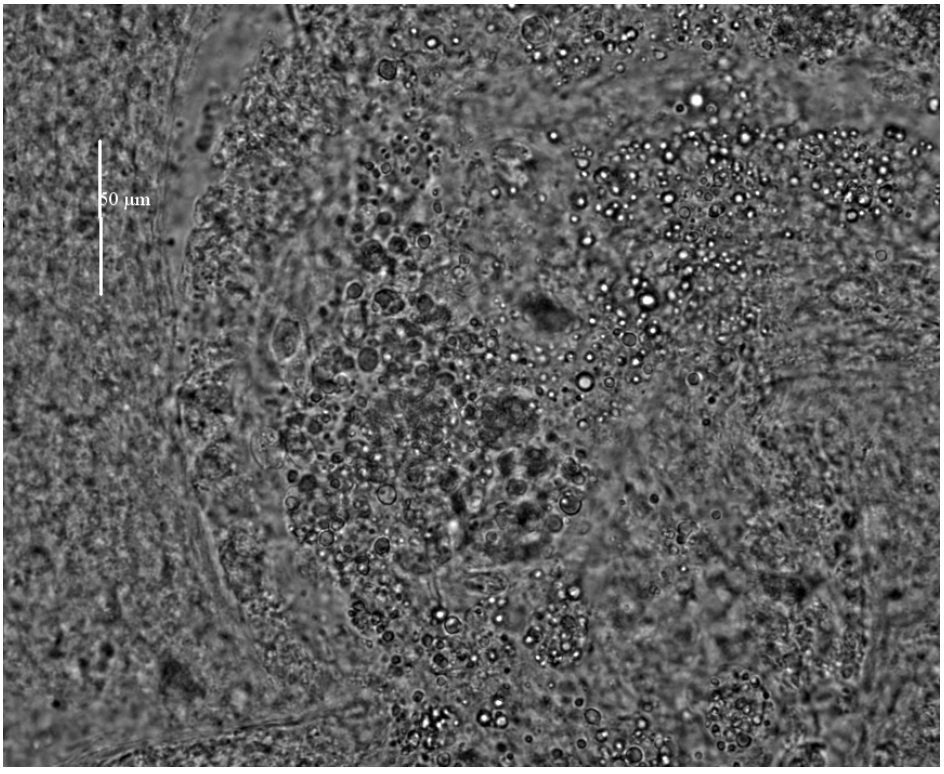


Table 13. Average Spore Measurements for *Myxobolus diversus*
(All measurements are in µm.)

	Average Spore Measurements		**Range of Measurements**
	Arkansas 2007	**PetCo Fish**	***Myxobolus diversus*** (Molnár and Székely 2003)
Spore Body, Length	14.8	13.7	12-14
Spore Body, Width	9.1	10.2	8-9.5
Spore Body, Thickness	7.8	N/A	6-7
Polar Capsules, Length	3.8	3.6	4-5.5
Polar Capsules, Width	2.6	N/A	2.5-3.5
Polar Filament (Turns)	5	N/A	6
Polar Filament (Turns) [1]	N/A	N/A	2-3

[1](Smaller Polar Capsule)

Table 14. Average Spore Measurements for *Myxobolus cultus* and a *Myxobolus cultus* like species (from Arkansas 2007). (All measurements are in µm.)

	Average Spore Measurements		**Range of Measurements**
	Arkansas 2007	**PetCo Fish**	***Myxobolus cultus*** (Yokoyama et al. 1995)
Spore Body, Length	15.5	10.7	9.3-11.3
Spore Body, Width	N/A	5.8	5.2-7.2
Spore Body, Thickness	7.6	N/A	3.6-4.6
Polar Capsules, Length	4.9	3.7	3.1-4.9
Polar Capsules, Width	2.8	2.1	1.5-2.1
Polar Filament (Turns)	5	5.0	3-5
Polar Filament (Turns)	N/A	4.0	3-5

Table 15. Average Spore Measurements for *Sphaerospora* sp.
(All measurements are in µm.)

	Average Spore Measurements		
	Arkansas 2008	**Ashland, Oregon Fish**	***Sphaerospora renicola*** (Dyková and Lom, 1982)
Spore Body, Length	6.3	7.1	7.3
Spore Body, Width	5.4	6.1	6
Spore Body, Thickness	5.0	5.4	7.2
Polar Capsules, Length	2.5	3.0	2.8
Polar Capsules, Width	1.8	2.1	2.4
Polar Filament (Turns)	N/A	5.0	4-6
Polar Filament (Turns)	N/A	N/A	4-6

Table 16. Average Spore Measurements for *Hoferellus* sp.
(All measurements are in µm.)

	Average Spore Measurements		
	Ashland Fish	***Hoferellus carassii* (Lom and Dyková 1992)**	***Hoferellus carassii*** [1] **(Lom and Dyková 1992)**
Spore, Total Length	14.0	17.5-19	14.5-16
Spore Body, Length	10.2	13	10
Spore Body, Width	6.5	7.5	6
Spore Body, Thickness	6.3	N/A	N/A
Bristle Length	3.8	4.5 - 6	4.5 - 6
Polar Capsules, Length	3.5	4.2	4.2
Polar Capsules, Width	2.1	2.4	2.4
Polar Filament (Turns)	4.0	5-6	5-6
Polar Filament (Turns)	4.5	5-6	5-6
Polar Filament, Length	11.7	N/A	N/A

[1]populations from feral *Carassius auratus* in Europe yielded smaller *Hoferellus carassii* myxospores, which are not known to cause disease (Lom and Dyková 1992).

Table 17. Summary of Myxozoa detected at different sites.

Species Present	**Arkansas (2007)**	**Arkansas (2008)**	**Lithia Park (Ashland, OR)**	**PetCo (Corvallis, OR)**
Myxobolus diversus	Yes	No	No	Yes
Myxobolus cultus	Yes	Yes	No	Yes
***Sphaerospora* sp.**	No	Yes	Yes	Yes
Hoferellus carassii	No	No	Yes	No

Sediment Samples

Arkansas Samples from 2007

Accompanying the goldfish samples sent in August of 2007 by Professor Goodwin, were five liters of sediment, one from each pond. Only one sample yielded an oligochaete worm, specifically *Branchiura sowerbyi* (Table 18). However, the oligochaete was damaged, likely during collection or transport, and no actinospores were

ever detected from the water sample corresponding with the sediment. None of the water samples yielded actinospores.

Table 18. Summary of oligochaete and actinosopre data for Arkansas samples (2007)

	AN	**FP#1**	**FP#2**	**FM#1**	**FM#2**
Oligochaete Worms	Present [1]	0	0	0	0
Actinospores Detected	0	0	0	0	0
Number of Sediment Samples Provided	1	1	1	1	1

[1] only one worm was found

Arkansas Samples from 2008

Although oligochaetes were present in all three sample sets received from Arkansas in May of 2008, after three months of monthly screening the water samples, no actinospores were yielded from the water the sediment samples were kept in (Table 19). The species of the oligochaetes were not investigated.

Table 19. Summary of oligochaete and actinosopre data for Arkansas samples (2008)

	1	**2**	**3**
Oligochaete Worms	Present	Present	Present
Actinospores Detected	0	0	0
Number of Sediment Samples Provided	1	1	1

Ashland, Oregon Samples

Oligochaetes were present in one of the two sediment samples collected from Upper Duck Pond in Lithia Park from Ashland, Oregon in May of 2008. The species of the oligochaetes were not investigated. Although the water was screened regularly for three months, no actinospores were found (Table 20).

Table 20. Summary of oligochaete and actinosopre data for Ashland, Oregon samples (2008)

	Sample Set #1
Oligochaete Worms	Present[1]
Actinospores Detected	0
Number of Sediment Samples Provided	2

[1] only one of the two samples contained oligochaete worms

DISCUSSION

Carassius auratus, or goldfish, are important as a cheap and popular decorative fish for ponds and aquariums, and are also reared to be used as feeder fish, in other words as sustenance for larger fish, and fishing bait. Goldfish can act as an intermediate host for myxozoan parasites such as *Hoferellus carassii* (El-Matbouli et al. 1992b, Yokoyama et al. 1993a), *Myxobolus cultus* (Yokoyama et al. 1995), *Chloromyxum auratum* (Hallett al. 2006, Atkinson et al. 2007), *Myxobolus diversus* (Molnár and Székely 2003), *Myxobolus rotundus* (Lu et al. 2003), and *Sphaerospora* sp. (Hedrick et al. 1990).

A survey of myxozoan parasites infecting goldfish collected from a rearing facility, a pet shop, and natural waterways was conducted to determine the possibility of introducing novel Myxozoa to the environment. Goldfish from a total of eight different ponds from four different rearing farms were studied. PetCo was the source of goldfish from a pet shop. Goldfish collected from Upper Duck Pond in Lithia Park in Ashland, Oregon were assumed released by their previous owners into the pond. These goldfish were dissected and their tissues screened for myxospores using a compound microscope in order to assess the risk of goldfish introducing novel myxozoan parasites to the environment. The four species of Myxozoa detected in the goldfish are described below.

Myxobolus diversus was identified as a result of the disparity in size of the polar capsules. From the samples collected in Arkansas in 2007 and PetCo in 2008, the average

measurements (Table 13) for spore body and polar capsule dimensions fall within the ranges given in literature data (Molnár and Székely 2003), except for the spore body width of the PetCo goldfish, and the polar capsule length for both the PetCo and Arkansas 2007 goldfish.

Myxobolus cultus was identified due to the ellipsoidal shape of the myxospore, as well as the pair of polar capsules similar in size placed side by side in the spore. A *Myxobolus cultus* like species that was detected in goldfish from Arkansas in 2007 (Table 13); there was a disparity in size when compared to the literature values for spore body length and polar capsule width of the myxospores (Table 14). The rest of the measurements, including those taken from spores inhabiting PetCo goldfish in 2008, were within the range given in literature (Yokoyama et al. 1995). Due to the disparity in myxospore size, the like species found in Arkansas in 2007 was not identified as *Myxobolus cultus*, but as a *Myxobolus cultus* like species.

The *Sphaerosphora* sp. that was identified in both the samples received from Arkansas in 2008 and the samples from Ashland, Oregon was similar morphologically to *Sphaerospora renicola*, which has been found in *Cyprinus carpio* (common carp). *Sphaerospora renicola* has been shown to be morphologically identical to *Sphaerosphora* sp. isolated from *Carassius auratus*; however, the two are genetically different (Eszterbauer and Székely 2004). However, *Sphaerospora renicola* has also been shown to infect goldfish (Lom and Dyková 1992). From the samples collected in Arkansas in 2008, and the samples from PetCo goldfish, spores of similar morphology and size were found. The *Sphaerosphora* species that was isolated in those two sets of fish had measurement values (Table 15) close to those reported for *Sphaerospora renicola*

(Dyková and Lom, 1982). Direct transmission of *Sphaerospora renicola* to both carp and goldfish has been shown previously (Odening et al. 1989). There are no reported values for the *Sphaerosphora* sp. previously isolated in goldfish.

The *Hoferellus* sp. which was identified from samples taken from Upper Duck Pond in Lithia Park in Ashland, Oregon had spores that were smaller than those previously identified as *Hoferellus carassii* (Table16) (Lom and Dyková 1992), except in the case where nonpathogenic *Hoferellus carassii* myxospores were found in European feral goldfish (Table 16) (Lom and Dyková 1992). Since the myxospores observed were close in dimension to those previously found in feral European goldfish (Lom and Dyková 1992), the myxospores can be tentatively identified as belonging to the nonpathogenic strain of *Hoferellus carassi*i.

Similar infections were detected for different survey sites (Table 17). In rearing facilities *Myxobolus cultus, Myxobolus diversus,* and *Sphaerospora* sp. were detected. In goldfish collected from a pet shop, *Sphaerospora* sp. and *Myxobolus diversus* were detected. Finally, in goldfish that had been released into natural waterways, *Hoferellus* sp. and *Sphaerospora* sp. were detected. In order to conclusively identify any of the myxozoan parasites that were isolated, a genetic analysis must be done. Partial DNA sequences have been recorded for *Sphaerospora* sp., *Sphaerospora renicola*, *Myxobolus cultus*, specifically for the 18S rRNA. No sequencing has been done for *Myxobolus diversus* and *Hoferellus carassii*. Therefore, *Myxobolus diversus* and *Hoferellus carassii* could not be identified via genetic analysis, since there is no sequence for comparison.

Some myxozoan parasites have limited host specificity, and others can infect a variety of species, closely related or not. Therefore, members of the *Carassius* genus, as

well as other freshwater fishes including *Cyprinus carpio*, both of which have been shown to be infected by Myxozoa that also proliferate in goldfish, could acquire novel infections due to the release of goldfish into natural waterways. In this survey, it was established that goldfish can indeed introduce myxozoan parasites to natural waterways. Due to the lack of data on the annelid worms inhabiting the ponds we sampled, as well as a lack of actinospores, there is no conclusive data on whether these myxozoans establish a cycle of infection after introduction. On the other hand, *Branchiura sowerbyi*, a known host for several species of Myxozoa that infect common carp and goldfish, is widespread in temperate waters. Given that fish that inhabit natural waterways (e.g. common carp) can acquire Myxozoa that are known to infect goldfish, if these parasites can establish an infectious cycle in natural waterways there could be a significant impact on fish populations. Thus, feeder goldfish released into natural waterways may pose a significant risk to fish naturally residing there as a result of the myxozoan parasites they carry, and further surveys should be carried out to analyze the true severity of this risk.

Different types of infection were shown for samples collected during different seasons. For example, the goldfish retrieved from Arkansas in spring of 2007 were infected with *Myxobolus cultus* and *Myxobolus diversus*. The goldfish retrieved from Arkansas in summer of 2008 were infected with *Myxobolus diversus* and *Sphaerospora* sp.. Despite the fact that it takes approximately three months for an infection to fully develop in an oligochaete definitive host, the release of actinospores is seasonal (Kallert 2006). The presence or absence of a given parasite in a fish host depends on whether they are exposed to viable actinospores of a myxozoan parasite that infects them, which depends on the season. This difference in infection presence due to season has been

observed previously; for example, with *Hoferellus gilsoni* in *Anguilla rostrata* (American eels) (Melendy et al. 2001). However, since the goldfish were collected from different ponds, it is difficult to draw conclusions about the seasonality of these infections. There were low numbers of myxospores gathered, and only a few fish were infected; future experiments would require an increase in both spores gathered and the number of fish sampled for spores. This survey was rather limited in its scope, although it indicates a possibility for seasonal variability of myxozoan infection in goldfish parasites. Surveys with a wider scope, with more sites being analyzed on a seasonal or even monthly basis are needed to further the knowledge about myxozoan goldfish infections and their possible impact on the environment, as well as if there is a seasonal variability in the infection rates.

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