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**Oligochaete Sampling to Determine the Presence of  
*Tubifex tubifex* in Four Study Lakes in Interior  
Alaska, 2007**

by

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and

**April Behr**

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April 2010

Alaska Department of Fish and Game

Divisions of Sport Fish and Commercial Fisheries



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<b>Weights and measures (metric)</b>		<b>General</b>		<b>Measures (fisheries)</b>	
centimeter	cm	Alaska Administrative Code	AAC	fork length	FL
deciliter	dL	all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.	mid-eye-to-fork	MEF
gram	g	all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.	mid-eye-to-tail-fork	METF
hectare	ha	at	@	standard length	SL
kilogram	kg	compass directions:		total length	TL
kilometer	km	east	E		
liter	L	north	N	<b>Mathematics, statistics</b>	
meter	m	south	S	<i>all standard mathematical signs, symbols and abbreviations</i>	
milliliter	mL	west	W	alternate hypothesis	H <sub>A</sub>
millimeter	mm	copyright	©	base of natural logarithm	<i>e</i>
		corporate suffixes:		catch per unit effort	CPUE
<b>Weights and measures (English)</b>		Company	Co.	coefficient of variation	CV
cubic feet per second	ft <sup>3</sup> /s	Corporation	Corp.	common test statistics	(F, t, $\chi^2$ , etc.)
foot	ft	Incorporated	Inc.	confidence interval	CI
gallon	gal	Limited	Ltd.	correlation coefficient (multiple)	R
inch	in	District of Columbia	D.C.	correlation coefficient (simple)	r
mile	mi	et alii (and others)	et al.	covariance	cov
nautical mile	nmi	et cetera (and so forth)	etc.	degree (angular)	°
ounce	oz	exempli gratia (for example)	e.g.	degrees of freedom	df
pound	lb	Federal Information Code	FIC	expected value	<i>E</i>
quart	qt	id est (that is)	i.e.	greater than	>
yard	yd	latitude or longitude	lat. or long.	greater than or equal to	≥
		monetary symbols (U.S.)	\$, ¢	harvest per unit effort	HPUE
<b>Time and temperature</b>		months (tables and figures): first three letters	Jan, ..., Dec	less than	<
day	d	registered trademark	®	less than or equal to	≤
degrees Celsius	°C	trademark	™	logarithm (natural)	ln
degrees Fahrenheit	°F	United States (adjective)	U.S.	logarithm (base 10)	log
degrees kelvin	K	United States of America (noun)	USA	logarithm (specify base)	log <sub>2</sub> , etc.
hour	h	U.S.C.	United States Code	minute (angular)	'
minute	min	U.S. state	use two-letter abbreviations (e.g., AK, WA)	not significant	NS
second	s			null hypothesis	H <sub>0</sub>
<b>Physics and chemistry</b>				percent	%
all atomic symbols				probability	P
alternating current	AC			probability of a type I error (rejection of the null hypothesis when true)	$\alpha$
ampere	A			probability of a type II error (acceptance of the null hypothesis when false)	$\beta$
calorie	cal			second (angular)	"
direct current	DC			standard deviation	SD
hertz	Hz			standard error	SE
horsepower	hp			variance	
hydrogen ion activity (negative log of)	pH			population	Var
parts per million	ppm			sample	var
parts per thousand	ppt, ‰				
volts	V				
watts	W				

***FISHERY DATA SERIES NO. 10-25***

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*TUBIFEX TUBIFEX* IN FOUR STUDY LAKES IN INTERIOR ALASKA,  
2007**

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## ABSTRACT

The first documentation of *Tubifex tubifex* and *Myxobolus cerebralis* (*Mc*) in Alaska occurred in 2006 when a representative sample from a single cohort of rainbow trout *Oncorhynchus mykiss* from the Alaska Department of Fish and Game Elmendorf Fish Hatchery identified genetic material unique to *Mc* spores at very low levels and *T. tubifex* lineages I, III, IV, and VI were found in a nearby river system. *Mc* is the causative agent of whirling disease. It requires two hosts to complete its life cycle, an aquatic oligochaete (*T. tubifex*) and a susceptible salmonid. Lineages I and occasionally lineage III of *T. tubifex* are susceptible to *Mc*, whereas lineages IV and VI are not susceptible to the *Mc* spore. Rainbow trout from the Elmendorf Fish Hatchery are stocked into various water bodies throughout Interior Alaska and fishery managers were concerned about potential outbreaks of whirling disease. Managers wanted to know whether *T. tubifex* was present in Interior Alaska lake systems, potentially enabling *Mc* spores to complete their life cycle and infect local fish populations. In 2007 sampling for oligochaetes was conducted at four study lakes (Birch Lake, Quartz Lake, West Twin Lake, and Wilderness Lake) in Interior (central) Alaska during July and September to further investigate the presence of *T. tubifex* throughout Alaska. Collected oligochaetes were analyzed using quantitative Polymerase Chain Reaction (qPCR) to determine the presence of *T. tubifex* lineages. Laboratory results indicated that low levels of lineage III *T. tubifex* DNA was present in one sample collected from one lake and that traces of lineage V DNA was present in samples from three lakes.

Key words: oligochaeta, *Tubifex tubifex*, Alaska, lakes, *Myxobolus cerebralis*, rainbow trout, *Oncorhynchus mykiss*

## INTRODUCTION

In 2006, a graduate student from Oregon State University-Corvallis conducted a qualitative risk assessment of the probability of *Myxobolus cerebralis* (*Mc*) introduction and establishment in two regions: the state of Alaska and the Willamette River basin, Oregon (Arsan 2006). Alaska was included in the project with the assumption that *Mc* was not present in Alaska waters. *Mc* is the parasite responsible for whirling disease in salmonid fishes. The parasite requires two hosts to complete its lifecycle, a specific aquatic oligochaete, (*Tubifex tubifex*), and a susceptible salmonid. Kathman and Brinkhurst (1999) describe *T. tubifex* as a cosmopolitan species that is found in a range of waters from very polluted to pristine. There are six known lineages of the *T. tubifex* worm, which vary in susceptibility to *Mc* (lineages I and occasionally III - susceptible; lineages IV and VI - not susceptible; Beauchamp et al. 2002; Beauchamp et al. 2005; Arsan 2006). Arsan (2006) sampled and positively identified four lineages of *T. tubifex* (I, III, IV, and VI) in Southcentral Alaska. In the same study, Arsan also found genetic material unique to *Mc* spores that indicated extreme low level presence of spores in rainbow trout *Oncorhynchus mykiss* collected from Elmendorf Fish Hatchery in Anchorage. To date, there has not been a clinical case of whirling disease in Alaska; however, these findings provide the first documentation of *T. tubifex* and *Mc* in the state.

Because genetic material of *Mc* spores were found in fish from the Elmendorf Fish Hatchery, and because fish from this and other Alaska hatcheries have been stocked in Interior (central) Alaska since 1957, it is important to know whether *T. tubifex* is present in the Interior. Although there is no evidence that fish stocked in Interior waters have been infected by *Mc*, obtaining knowledge of the distribution of *T. tubifex* and identifying the lineages is valuable when assessing the risk whirling disease poses to Interior fish populations. Prior to this study, it was unknown whether *T. tubifex* existed in Interior Alaska because identification of oligochaetes has not been pursued beyond the family or genus level (Holmquist 1975; Butler et al. 1980).

In 2007, oligochaete sampling was added to a project conducted by the Alaska Department of Fish and Game (ADF&G) Division of Sport Fish to document species distributions in Interior Alaska lakes. Understanding of the distribution of *T. tubifex* lineages that are susceptible to *Mc*

was important because: 1) this oligochaete is an essential host for *Mc* and its existence in Interior Alaska is unknown; 2) even if *Mc* was found in lake waters or stocked fish, the parasite will not persist without the appropriate lineages of *T. tubifex* present; and, 3) given the low concentrations of *Mc* spores observed thus far (Arsan 2006), it would not be as effective to sample fish or lake waters as an initial step (J. Bartholomew, Oregon State University Corvallis; personal communication). Consequently, sampling of fine benthic sediment where oligochaetes typically reside was added to the sampling protocol. This study will give managers the information needed to better assess the risk whirling disease poses to fish populations in Interior Alaska.

## **STUDY AREA**

The criteria for selecting lakes for this study included: 1) the importance of the information for management needs; 2) the number of public inquiries or requests for development of a fishery, or for information about the lake or species present in the lake; and, 3) proximity to other lakes under consideration to reduce transportation costs. Four lakes originally scheduled for sampling in 2007 were Birch Lake (315 ha), Quartz Lake (584 ha), and two unnamed lakes located near Rainbow Lake Unnamed Lake #1 (N64.01332 W146.21195) and Unnamed Lake #2 (N64.02152 W146.22535). Due to conflicting land-use schedules with the US Army that prohibited access to the two unnamed lakes, two alternative lakes were chosen: West Twin Lake (683 ha) and Wilderness Lake (79 ha) (Figure 1). All four lakes are in the Tanana River drainage.

### **Birch Lake**

Birch Lake is the second largest roadside lake fishery in Interior Alaska based on effort and harvest. It is in the Lower Tanana River Management Area along the Richardson Highway 97 km south of Fairbanks. The lake surface area is 315 ha, its maximum depth is 11.5 m, and its surface elevation is 251 m. Birch Lake is stocked with rainbow trout, Arctic char *Salvelinus alpinus*, Chinook salmon *O. tshawytscha*, coho salmon *O. kisutch*, and Arctic grayling *Thymallus arcticus*.

### **Quartz Lake**

Quartz Lake is in the Upper Tanana River Management Area, 21 km north of Delta Junction on the Richardson Highway. It is the largest roadside lake fishery in Interior Alaska. The lake surface area is 584 ha, its maximum depth is 11 m, and its surface elevation is 293 m. Quartz Lake is stocked with rainbow trout, Arctic char, Chinook salmon, and coho salmon.

### **West Twin Lake**

West Twin Lake is 87 km from Nenana, west of the Kantishna River in the Lower Tanana River Management Area. West Twin Lake is the deepest of the four lakes with a maximum depth of 33 m. Its surface elevation is 228 m and the lake surface area is 683 ha. West Twin Lake was last stocked in 1989 with lake trout (*S. namaycush*) and supports a wild population of northern pike *Esox lucius*.

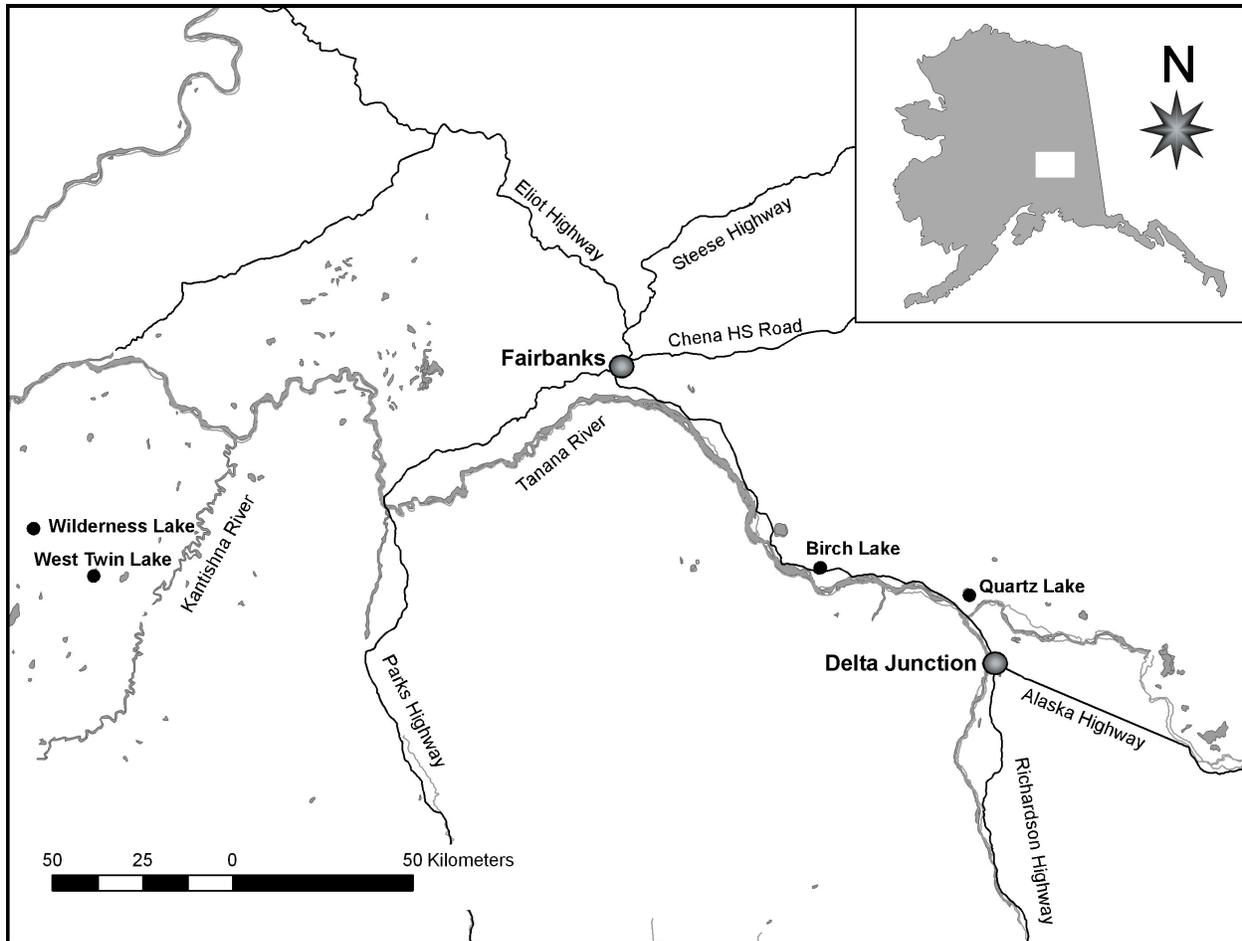


Figure 1.—Location of Birch, Quartz, West Twin and Wilderness lakes.

### Wilderness Lake

Wilderness Lake is also in the Lower Tanana River Management Area about 19 km northwest of West Twin Lake, about 95 km from Nenana. Wilderness Lake is 12.9 m at its deepest, and its elevation is 226 m. Wilderness Lake has not been previously stocked; however, northern pike are present.

## METHODS

Previous studies have found *T. tubifex* in soft mud/silt substrates (sometimes with organic matter; Zendt and Bergersen 2000; Lazim and Learner 1987; Arsan 2006) and near inlets and outlets of lakes or ponds (B. Nehring, Colorado Division of Wildlife; personal communication). Zendt and Bergersen (2000) found the densities of *T. tubifex* worms to be highly variable; however, the worms were present in all samples of fine sediments. Studies have consistently shown that the organisms are found in the first several centimeters of sediment (Bartholomew et al. 2007; Arsan

2006). Based on these findings, sampling was conducted by first surveying the perimeter of each lake in a small skiff, staying within 3 m of shore (or where water was  $\leq 2$  m deep). Potential inlets or outlets and reaches containing areas of fine benthic material were then marked on a field map or with a handheld global positioning system (GPS). One half (two to four) of the samples collected were taken near inlets or outlets (if present) and the rest were collected in an approximate systematic manner from the previously marked areas containing fine benthic material.

A preliminary sampling event was conducted at the end of June 2007 at Birch Lake to evaluate the efficacy of sampling methods. First a petite Ponar grab sampler (152 mm X 152 mm sample area) was tested. The sampler did not perform as hoped as the apparatus did not grab sufficient sediment from the lake bottom, perhaps due to interference of vegetation or compact sediment. As a result, we tried using a 500  $\mu$ m mesh D-frame dip net, to scoop sediment. The D-frame dip net collected more sediment and was chosen as the preferred gear type for this project. Three sediment sweeps with the dip net were combined to form one composite sample. The composite sample was then processed through a series of sieves, one sieve at a time (ending with a 500  $\mu$ m screen), to remove debris and coarse matter until approximately 1 L of material remained. Each sieve was inspected for oligochaetes before debris was discarded. Oligochaetes were transferred to a sample bottle with lake water. After the sample was sent through the last two sieves, the remaining sediment was placed in the same sample bottle and the bottle was placed in a cooler with ice. The samples were brought back to the lab at ADF&G Fairbanks office and stored in a refrigerator until they could be inspected. Because Quartz and Birch lakes are located on the road system, several trips were made during July to obtain the number of oligochaetes required for laboratory analysis. In September, inclement weather and rough water on Birch Lake prohibited individual sample collection; therefore, multiple net sweeps were put in a 5-gallon bucket with lake water, brought back to the lab, sent through sieves, and inspected. Daytime temperature readings were taken at approximately 0.5–1.0 m beneath the surface at each sample location.

Oligochaetes were sorted from benthic material in the laboratory by first emptying the contents of each sample bottle into a white tray. Water was added and samples were agitated slightly by shaking the pan to spot movement of live worms. Oligochaetes were removed and observed under a microscope to determine the presence of hair chaetae (a distinguishing characteristic of *T. tubifex*). The presence, location, and type of chaetae were used to separate out oligochaetes thought to be *T. tubifex* (Kathman and Brinkhurst 1999). In some cases, worms that weren't clearly identified as *Tubifex* were added to the samples of worms that were more assured to be *Tubifex*. Fifty oligochaetes thought to be *T. tubifex* from each sample were placed into a 50 ml vial with 2-3 ml of 70% ethanol. When 50 oligochaetes were not found in one sample, multiple samples from a lake were combined until 50 worms were collected.

Identification of *T. tubifex* is difficult, even for experienced researchers, and as a result quantitative Polymerase Chain Reaction (qPCR) methods have been developed to eliminate the uncertainty in identification, especially for researchers unfamiliar with oligochaetes (Hallett et al. 2005). Vials of collected oligochaetes were sent to Pisces Molecular LLC<sup>1</sup> for DNA analysis using qPCR methods to determine species and lineages present. Pisces Molecular LLC protocol for qPCR analysis is as follows:

*Sample Preparation:*

Worm samples were prepared by pouring off the ethanol, removing the worms with clean forceps, and placing them in a microcentrifuge tube with lysis buffer. Total DNA was extracted with a spin-column DNA purification procedure.

*qPCR Assay:*

Each DNA prep was assayed for the presence and quantity of each of the four known *Tubifex tubifex* subspecies or lineages, known to be present in North America (lineage I, lineage III, lineage V, and lineage VI) (Beauchamp et al. 2002) with a TaqMan qPCR assay directed at a region of the mitochondrial 16S rRNA gene, using two universal primers and four differently fluorescently labeled, lineage-specific TaqMan probe oligonucleotides (Wood et al. 2004), in a Stratagene MX4000 real-time PCR instrument. A ten fold dilution series of four plasmid DNAs, mixed equimolar, was used to generate a standard curve; each plasmid DNA contained the target sequences for the PCR primers and one of the lineage-specific probes.

## RESULTS

Field personnel had difficulty locating worms in July. In most cases, only enough oligochaetes were found to fill one vial per lake, the one exception being Wilderness Lake, from which three vials were filled. Pisces Molecular LLC laboratory qPCR results indicated that *T. tubifex* was not present in any of the July samples.

To maximize the number of worms collected during September, areas found to contain worms during July were targeted. In general, field personnel observed that more worms were present in less compact (soft) fine sediment with little vegetation, and in Wilderness Lake samples collected from the outlet contained the most worms. September sampling efforts were focused extensively in these areas.

Pisces Molecular LLC laboratory qPCR results indicated that *T. tubifex* lineage V was present in September samples collected from Birch Lake, West Twin Lake, and Wilderness Lake (Table 1). Wilderness Lake also tested positive for lineage III *T. tubifex* in four of the 10 vials analyzed; however, the percentage of this lineage was very low. There were also very weak signals for lineage V in the three lakes listed above, but these readings were at the limit of detection (45 cycles) in the PCR process and considered background noise in the assay.

Average daily water temperature for July (and August for Quartz Lake) ranged between 16.9 °C and 21.6 °C. September temperatures ranged between 9.8°C and 17.0°C (Table 2).

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<sup>1</sup> Pisces Molecular LLC, 2200 Central Avenue, Suite F, Boulder, CO 80301-2841.

Table 1.–*Tubifex tubifex* lineages found in sample lakes during 2007.

	Number of Vials Sent to DNA Lab		Number of Vials with Lineage III Detected		Number of Vials with Lineage V Detected <sup>a</sup>	
	July	September	July	September	July	September
Birch L	1	5	0	0	0	1
Quartz L	1	7	0	0	0	0
West Twin L	1	2	0	0	0	1
Wilderness L	3	10	0	4	0	2

<sup>a</sup> Lineage V signals were at the limit of detection and believed to be background noise; however, results are reported for completeness.

Table 2.–Average daily water temperatures taken at each sample location during July (August for Quartz Lake) and September 2007. Temperatures were taken at approximately 0.5-1.0 m below the water surface.

	Average Daily Water Temperature (°C)		
	July	August	September
Birch L	21.6	-	9.8
Quartz L	18.9	16.9	10.8
West Twin L	18.0	-	14.3
Wilderness L	19.9	-	17.0

## DISCUSSION

There was some concern from the Pisces Molecular LLC that DNA degradation may have occurred prior to lab analysis. Pisces Molecular LLC ran an aliquot of DNA from five of the Wilderness Lake samples (samples containing *T. tubifex* DNA) and compared those results with samples from Colorado. They found that our samples had very low molecular weights (<100 base pairs in size) compared to the Colorado samples (~2000 base pairs average size). From this finding, it was suggested that worms be preserved in 100% ethanol and the samples shipped to the testing lab as soon as possible. In its lab, Pisces Molecular LLC uses 100% ethanol, which is equivalent to Fisher catalog #A962-P4.

Ideal water temperature for *Mc* spore release from *T. tubifex* is between 10°C and 15°C (El-Matbouli et al. 1999). Interior Alaska lakes typically reach maximum temperatures of 20°C to 25°C in summer consistent with the measurements taken in the four study lakes. An experimental study by El-Matbouli et al. (1999) found at a constant 15°C, 3 months post-exposure to *Mc*, *T. tubifex* were releasing *Mc* spores. It is unknown whether the duration of ideal water temperatures is long enough to sustain *Mc* in Interior Alaska, but laboratory findings show that colder temperatures do not prohibit *Mc* production (El-Matbouli et al 1999); the development rate of *Mc* in oligochaetes is slowed in cold-water systems and may proliferate without any impact to trout populations (Kerans et al. 2005). *Mc* spores can even survive freezing at -20°C for at least 3 months (El-Matbouli and Hoffmann 1991).

This study developed the groundwork for oligochaete sampling and determined that *T. tubifex* is present in Interior Alaska. Additional work is necessary to determine the extent of their presence in Alaskan waters.

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