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Piscirickettsia Infection in the Muskellunge Population of Lake St. Clair

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Abstract.−For decades, Lake St. Clair has supported an economically important sport fishery for muskellunge. The muskellunge population has been completely self-sustaining, thus any threat to the continued successful recruitment of muskellunge in the lake is a serious concern for fisheries managers. In 2002, muskellunge exhibiting external lesions were collected by the Michigan Department of Natural Resources (MDNR) from the Anchor Bay area of Lake St. Clair. The lesions were in the form of raised, reddish granulomatous-like sores that varied in diameter from 3 mm to 2 cm. An intracellular bacterium was found in affected tissues. Based on its morphological characteristics, cultural criteria, and gene sequencing, the bacterium found in abundance in muskellunge skin lesions and internal organs was determined to be a *Piscirickettsia* species. This two-year study was funded with the goal of addressing some concerns and questions regarding this emerging infection. The objectives of this study were: (1) to identify the *Piscirickettsia*-like bacterium that was the causative agent of the lesions; (2) to determine the *Piscirickettsia* infection rate in Lake St. Clair muskellunge and whether the rate varies spatially or temporally; (3) to determine if other fish species in the St. Clair System are infected by the bacteria; (4) to identify the impacts of the bacterium on the health of individual muskellunge; and (5) to determine if the disease can be vertically transmitted. This study found a high proportion of muskellunge caught in Anchor Bay were infected, but found no evidence of the infection in other fish species, except for yellow perch. Molecular analyses confirmed that muskellunge and yellow perch isolates were identical to each other but not identical to *Piscirickettsia salmonis* that devastates cultured salmonids, suggesting the causative agent was likely a new species of *Piscirickettsia*. Histopathology demonstrated the presence of severe skin edema and severe degeneration of the kidney glomeruli in infected muskellunge. In laboratory trials, mortality was high for fingerling muskellunge experimentally infected with *Piscirickettsia* bacteria, but mortality was low for rainbow trout and largemouth bass. No *Piscirickettsia* bacteria were retrieved from muskellunge ovarian fluids, eggs, or milt samples, suggesting likelihood of vertical transmission was minimal. Trap net survey and angler diary catch rates suggested that no major change in adult muskellunge abundance occurred from 2002 to 2007. However, the effects of a decline in fingerling survival, if it occurred in the lake, would not be apparent for several years. Subsequent to the identification of the *Piscirickettsia* infection, viral hemorrhagic septicemia virus was identified from muskellunge in Lake St. Clair. The impact of the combination of these two infectious agents on the muskellunge population of the lake remains unknown.

Introduction

Lake St. Clair is centrally located in the connecting channel between Lake Huron and Lake Erie in the Laurentian Great Lakes (Figure 1). Approximately 4 million people live within a one-hour drive of Lake St. Clair. Jamsen (1985) reported that nearly 22% of all Michigan Great Lakes sport fishing effort in 1981 was spent on Lake St. Clair. In 1983 and 1984, the annual estimated fishing effort on Lake St. Clair averaged 1.9 million angler hours (Haas et al. 1985). During creel surveys in 2003 and 2004, fishing effort on the Michigan waters of Lake St. Clair averaged 1.75 million angler hours (M. V. Thomas, unpublished), generating approximately $23.1 million in economic activity (based on $59.00 per trip: United States Department of the Interior, Fish and Wildlife Service and United States Department of Commerce, Bureau of Census 2008). Clearly, the intensive sport fishing effort on Lake St. Clair generates important socioeconomic benefits.

For decades, Lake St. Clair supported a recreational fishery renowned for smallmouth bass, muskellunge, walleye, and yellow perch (see Appendix 1 for scientific names of fishes). Muskellunge abundance tripled during the 1980s and 1990s as water clarity increased, and macrophyte densities and spatial coverage increased (MacLennan 1996). By 2000, muskellunge anglers across North America ranked the Lake St. Clair muskellunge fishery as one of the most productive on the continent (Warye 2002). The muskellunge population in Lake St. Clair has been completely self-sustaining, thus any threat to the continued successful recruitment of muskellunge in the lake is a serious concern for fisheries managers.

Since at least the late 1990s, adult muskellunge with raised, reddish granulomatous-like sores that varied in diameter from 3 mm to 2 cm, known among anglers as “Muskie Pox”, were observed in Lake St. Clair. Several unsuccessful attempts to identify the etiological agent of this syndrome were made in the late 1990s and early 2000s. *Aeromonas hydrophila*, *Serratia* spp., *Pseudomonas* spp., and other facultative pathogens were isolated from affected skin areas and were wrongly implicated as the causative agent. In 2002, light and electron microscopical analyses at Michigan State University revealed the presence of Gram-negative, intracellular bacterium that occurred as rings or curved rods. The organism grew only in cultured cell lines, in particular the Chinook salmon embryo cell line, CHSE-214. Based on its morphological and cultural criteria, the organism found in abundance in muskellunge skin lesions and internal organs was determined to be a *Rickettsia*-like species. The *Rickettsia*-like bacteria are an emerging group of pathogens among teleosts, wild and farmed (Fryer and Lannan 1996; Fryer and Mauel 1997). This group of bacteria is described as “emerging” because they have been rapidly increasing in incidence, in new species, and in new geographic locations. One such bacterium is *Piscirickettsia salmonis*, which causes serious mortalities among mari-cultured salmons in Chile (Bravo and Campos 1989). *Piscirickettsia* are Gram-negative, nonmotile, aerobic, and develop within vacuoles of the host cell cytoplasm (Fryer et al. 1992). In the United States, *Piscirickettsia*-like organisms have been detected in a few species of marine and freshwater species such as the blue-eyed plecostomus (*Panaque suttoni*, Khoo et al. 1996), the white seabass (*Atractoscion nobilis*, Chen et al. 2000),and *Tilapia* hybrids (Mauel et al. 2003).

Identification of clinical cases in Lake St. Clair muskellunge associated with the presence of *Piscirickettsia*-like organisms raised numerous questions and concerns regarding implications for the lake’s muskellunge population, the overall fish community, and the ecosystem. Concerns were further elevated in April and May 2003, when anglers reported many dead muskellunge floating on Lake St. Clair and drifting down the Detroit River, downstream from Lake St. Clair. The mortality event was estimated to have included at least 500 muskellunge, based on observations by MDNR staff. To this end, this two-year study was funded with the goal of addressing some of those concerns and questions. The objectives of this study were: (1) to identify the *Piscirickettsia*-like bacterium; (2) to determine the infection rate in Lake St. Clair muskellunge and whether the rate varies spatially or temporally; (3) to determine if other fish species in the St. Clair System are infected by the bacterium,

particularly migratory salmonids; (4) to identify the impacts of the organism on the health of individual muskellunge; and (5) to determine if the disease can be vertically transmitted.

Study Area

Lake St. Clair has a surface area of 1,114 km^2, an average depth of 3 m, and a maximum natural depth (i.e., excluding the dredged shipping channel which is maintained at a depth of 8.8 m below low water datum) of only 6.4 m (Bolsenga and Herdendorf 1993). The lake is located about midway between Lake Huron and Lake Erie, sandwiched between the St. Clair River and the Detroit River (Figure 1). Due to shallow depths and high flow volumes, the hydraulic retention time for Lake St. Clair is short and averages 9 days (Edsall et al. 1988). In general, the lake is characterized by two distinct water masses. The western portion of the lake, including the northern portion known as Anchor Bay, is dominated by Lake Huron water flowing through the St. Clair River channels and sweeping south to the Detroit River. The southeastern area of the lake is influenced by contributions of nutrients and silt from the Thames River. Sampling for this study occurred mainly in the northwest area of the lake, locally known as Anchor Bay. The Anchor Bay area of Lake St. Clair is heavily influenced by Lake Huron water flowing through the St. Clair River delta channels (Leach 1980).

Methods

Fish Collections

Most of the fish examined during this study were collected with trap nets during May 2004–07 in Anchor Bay, Lake St. Clair. Five trap nets were fished at the same locations in Anchor Bay each year during May. GPS was used to locate the same sampling locations each year. The trap nets had 1.8-m deep pots of 5.1-cm stretch mesh, 7.6-cm stretch mesh hearts and wings, and 91.4-m long leads of 10.2-cm stretch mesh. Five nets were fished throughout each sample period and were normally tended three times each week. The nets were typically set in early May and fished until the Thursday before Memorial Day weekend. However, due to scheduling conflicts, the sampling period in 2003 was delayed about a month.

Total length (mm) and weight (kg) were recorded for each muskellunge captured. Sex and maturity were recorded for muskellunge that exuded gametes. The anterior three dorsal fin rays were removed, later sectioned, and examined under a dissecting scope to estimate the age of each fish. All muskellunge captured in the trap nets were examined for external signs of *Piscirickettsia* infection. External signs included sores (Figure 2); puffy scales (Figure 3); sunken eyes; and hemorrhagic fins or patches of skin (Figure 4). Presence or absence of external signs of the infection was recorded for each muskellunge examined.

On prescheduled dates, two to eight muskellunge were randomly selected from the trap net catch and sacrificed for laboratory examination and tissue sampling. Sampling dates were determined by laboratory capacity for samples, personnel availability, and sometimes by availability of personnel for transporting the samples from Lake St. Clair to the laboratory located at East Lansing, Michigan. Initially, fish samples had to be delivered alive to the Aquatic Animal Health Laboratory at Michigan State University (MSU-AAHL). In 2006, MSU-AAHL personnel rode along on board the Research Vessel Channel Cat on scheduled dates and examined live specimens of various fish species caught in the trap nets. Blood samples were collected from live fish selected for lab examination and testing. Those fish were then sacrificed and transported to the MSU-AAHL for further examination and lab analyses.

We did not attempt to collect muskellunge with trap nets at locations outside of Anchor Bay because of conflicts with other survey commitments. Since live fish were required, we did not attempt

to capture muskellunge with gill nets at any location during this study because we anticipated mortality would be high with that gear type. Trawls were used for other surveys at locations outside Anchor Bay, but muskellunge catches with trawls were rare and unpredictable, leading to difficulties with laboratory preparation for samples. So, to investigate possible spatial and temporal differences in the infection rate of muskellunge in Lake St. Clair, we attempted to collect live muskellunge samples from volunteer anglers and muskellunge fishing tournaments. Collections from volunteer anglers were planned during July, August, and September 2003, to obtain fish during those months and from areas outside Anchor Bay. The Michigan-Ontario Muskie Club (MOMC) hosted monthly tournaments in June, July, August, and September and were another potential source of live fish samples from outside Anchor Bay.

Lesion Diaries

In collaboration with the Ontario Ministry of Natural Resources, a muskellunge lesion diary was designed and distributed to muskellunge anglers who volunteered to examine their catch for external signs of the *Piscirickettsia* infection. The diary booklet included glossy, colored, close-up photographs of both *Piscirickettsia* lesions and lamprey marks on Lake St. Clair muskellunge to help anglers discriminate between the two types of marks. At the end of the fishing season, the cooperating anglers were contacted, the diaries collected, and the data recorded.

Laboratory Investigations

Bacterial isolation on conventional media and cell line.–Attempts were made to isolate bacteria from muskellunge tissue samples on a number of culture media commonly used to isolate fish-pathogenic bacteria such as brain heart infusion agar, blood agar, Rimmler-Shott medium, and trypticase soy agar. In order to isolate intracellular bacteria, Chinook salmon embryo (CHSE) and fathead minnow (FHM) cell lines were inoculated with homogenates of muskellunge kidneys, spleens, and skin lesions according to the methods detailed in the American Fisheries Society 'Blue Book' (American Fisheries Society 2004) and the 2003 OIE code for aquatic animal disease diagnosis (Office International des Epizooties 2003). The cell lines were incubated for 21 days at 15°C in the case of CHSE and at 20°C in the case of FHM with daily microscopical observation. Tissues were not frozen or centrifuged and no antibiotics were added to the culture medium so as not to inactivate growth of *Rickettsia* and *Piscirickettsia* species.

Electron microscopy.–Samples of tissues and infected cell lines were fixed for electron microscopy. Inoculated cultured cells and swabs from skin lesions were fixed in methanol and stained with Giemsa. Supernatants of inoculated cells were collected individually, filtered (0.45 µm), centrifuged into a glass slide, and then stained with Gram stain. Infected cell lines and skin lesions were placed in Trump's fixative and stored at 4°C. Samples were then postfixed in 1% osmium tetroxide after rinsing in 0.1 M Sorenson sodium phosphate buffer (pH 7.2). Following resin embedding, ultrathin sections were stained with methanolic uranyl acetate and lead citrate.

Experimental infection.–Pathogenicity testing was performed at the MSU Research Containment Facility. Noninfected muskellunge, rainbow trout, and largemouth bass fingerlings were intraperitoneally injected with 100 µl containing 10^4 bacteria. Mortality of infected fish was monitored for a period of 30 days.

Molecular assays.–The discrimination between muskellunge isolate from *P. salmonis* was performed by molecular assays using the bacterial 16S ribosomal rDNA gene sequences. The following primers were used:

EubB (27F): AGAGTTTGATCMTGGCTCAG
EubA (1518R): AAGGAGGTGATCCANCCRCA

Amplicons (470 bp) were sequenced bidirectionally at the MSU Genomic Facility. A phylogenetic tree was then constructed by the neighbor-joining method, using the obtained sequence of Michigan isolate (bacterium isolated from Lake St. Clair muskellunge) and published sequences of a number of related and distant bacteria.

Results

Field Collections

From 2004 through 2007, 182 muskellunge were captured with trap nets in Anchor Bay. The fish ranged in total length from 564–1,330 mm (mean = 1,049 mm). Nearly all muskellunge over 1,100 mm exhibited external signs of infection by *Piscirickettsia* spp. (Figure 5). Weight of muskellunge ranged from 0.9–16.8 kg (mean = 8.5 kg). Ages were estimated for 172 of the fish and ranged 2–19 years (mean = 9.1 years). Nearly all muskellunge older than 8 exhibited external symptoms of the *Piscirickettsia* infection (Figure 6). The percentage of muskellunge captured that exhibited visible external symptoms of the infection varied each year, but overall was more than 80% (Table 1). Other species were also collected from the Anchor Bay trap nets and delivered to the MSU-AAHL for analyses (Table 2).

In 2004 and 2007, eggs were stripped from ripe female muskellunge and fertilized with gametes from spermiating male muskellunge. Milt, unfertilized eggs, fertilized eggs, and ovarian fluid samples were delivered to MSU-AAHL to examine the potential for vertical transmission.

Two muskellunge were also collected at the June 2003 Michigan-Ontario Muskie Club (MOMC) fishing tournament. We determined after this initial effort, that collecting live fish samples at the MOMC tournaments would not be cost effective and did not attempt to do so again. Similarly, our efforts to coordinate the collection of live muskellunge samples from volunteer anglers on prescheduled fishing days were minimally effective. Unquestionably, the necessity of keeping the muskellunge alive until they reached the MSU-AAHL greatly limited the options for sample collections. One fish, nearly dead, was found floating on the surface of Lake St. Clair during the mortality event in May 2006, and was collected and transported to the MSU-AAHL for analysis.

Bacterial Isolation and Identification

There was no bacterial growth of significance on any of the conventional cell-free bacterial media streaked with homogenates of affected muskellunge tissues. When the same homogenates were inoculated into confluent monolayers of CHSE-214 cells, cytopathic effects (CPE) in the form of cell rounding followed by detachment were observed 10 days post-incubation. Results of the Tissue Culture Infectious $Dose_{50}$ ($TCID_{50}$) assay were titers ranging from 10^4 to10^{10} $TCID_{50}$/gram tissues.

Giemsa-stained slides demonstrated the presence of intracellular coccoidal bacteria that ranged in size from 0.43 to 1.61 µm (Figure 7). These bacteria were Gram negative in Gram stained preparations. At the ultrastructural level, bacteria exhibited the typical Gram negative membrane and cell wall and were found exclusively intracellular within membrane-bound vacuoles in inoculated cell lines, in skin lesions, and in internal organs of affected fish (Figure 8). These observations were in accordance with those described for *Piscirickettsia salmonis* by Fryer et al. (1990; 1992) and Fryer and Mauel (1997).

Characterization of muskellunge *Piscirickettsia* spp. was performed on CHSE-214 and FHM cell lines. Results demonstrated that optimum growth temperature is 20°C. The bacterium retained infectivity to both cell lines up to 14 days at 32°C, 7 days at 34°C, 6 hr at 37°C, and 5 minutes at

56°C. The organism tolerated salt concentrations up to 30 ppt for 7 days. These results suggest a high tolerance of the organism to a wide range of environmental conditions.

Molecular analyses demonstrated that Michigan isolates sequences are members of the family Rickettsiaceae, and are closer to the genus *Piscirickettsia* than to other bacterial genera. Additional phylogenetic analyses were then performed with expanded sequencing that involved intergenic transcribed spacers (ITS) between the 16S and 23S rDNA genetic loci. These analyses confirmed that muskellunge and yellow perch isolates were identical to each other but not identical to *P. salmonis* that devastates cultured salmonids (Figure 9), likely representing a new species.

Upon experimental infection by injection, the isolated muskellunge *Piscirickettsia* spp. caused high mortalities (87%) in muskellunge fingerlings within 28 days postinfection. There were also mortalities in rainbow trout and largemouth bass fingerlings at much lower rates (Figure 10).

In naturally or experimentally infected muskellunge, *Piscirickettsia* spp. caused raised lesions, skin hemorrhages, wide spread cutaneous edema, and enlargement of internal organs. Histopathological examination on the affected muskellunge revealed severe degeneration of the kidney glomeruli (Figure 11) and widespread subcutaneous edema.

As displayed in Table 1, infection with *Piscirickettsia* spp. was widespread in muskellunge analyzed in 2004–06. The bacterium was found not only in fish exhibiting skin lesions, but also in apparently healthy fish. No *Piscirickettsia* spp. or other bacterial species were retrieved from muskellunge ovarian fluids, eggs, or milt samples. The fact that no bacteria were isolated from the gametes of infected fish exhibiting external and internal lesions, indicates that the likelihood of vertical transmission may be minimal.

Of all other fish species examined, *Piscirickettsia* spp. was only present in the yellow perch (skin and kidney) with an infection rate of 57% (Table 2). Molecular analysis indicated that both muskellunge and yellow perch isolates were identical (Figure 9).

From two muskellunge sampled in 2004, one in 2005, and several in 2006, a rhabdovirus was isolated from kidneys and spleen homogenates (Figure 12). The virus replicated in FHM cell lines at 25°C. Isolation, electron microscopy, and molecular evidence identified the rhabdovirus as the Viral Hemorrhagic Septicemia (VHS) Virus genotype IV, sublineage b (Elsayed et al. 2006). The virus was found in high titers in the internal organs of the lone dying muskellunge collected during the mortality episode in spring 2006.

Lesion Diaries

Nine muskellunge anglers returned lesion diaries that contained records of the presence or absence of lamprey wounds, red sores, or other abnormal wounds on the muskellunge they caught in 2003. A total of 501 muskellunge observations were recorded. The fish ranged in length from 508–1,372 mm. Red sores or *Piscirickettsia* lesions were observed on 20 fish (4%), while lamprey marks were observed on 38 fish (7.6%). Both red sores and lamprey marks were absent from fish less than 810 mm in length. Only two fish were recorded with both red sores and lamprey marks (0.3%). The number of muskellunge observations recorded by anglers varied by month, with nearly 65% of all observations recorded in July and August. Numbers of fish observations recorded in September, October, and November were much lower. Red sore observations were recorded from June to October, with the highest numbers of fish exhibiting sores in July and August. However, the highest proportion of fish observed with red sores was noted in June and October.

In May 2004, diaries were returned to eight of the cooperating anglers for their use during the 2004 muskellunge fishing season. One angler had moved and could not be contacted by mail or phone. Three lesion diaries were returned by cooperating anglers in the fall of 2004. A total of 76 muskellunge observations were recorded. No red sores or *Piscirickettsia* lesions were observed on

any of the fish, while lamprey marks were observed on 13 fish (17%). Due to reduced number of lesion diaries returned and low number of fish observations recorded, no summary of lesions and lamprey marks by season or fish size was attempted. As a result of reduced cooperation, lesion diaries were not distributed in 2005 or 2006.

Discussion

Investigation of the *Piscirickettsia* infection in muskellunge in Lake St. Clair was a challenge in a number of ways. First, *Piscirickettsia* bacteria are inactivated by freezing and preservation in methanol or formalin. As a result, live fish specimens or blood samples were required for bacteria isolation and identification. Second, muskellunge are large fish and easily succumb to stress from handling, warm water, and or low oxygen; therefore, their live transportation was extremely difficult. Third, muskellunge in Lake St. Clair are abundant, but widely distributed and dispersed. They are not schooling fish, so sampling with nets generally did not produce large catches during this study. Finally, capture of muskellunge in survey nets was unpredictable, while staff and laboratory capacity at MSU-AAHL was limited and sometimes working at capacity due to other fish health sampling commitments. Sampling fish was further complicated by the logistics of arranging for delivery of live fish samples to the MSU-AAHL. As a result, sometimes Lake St. Clair muskellunge samples were available, but laboratory space and personnel were not available. At other times, laboratory space and personnel were ready for muskellunge samples to be delivered, but no fish were caught in the survey gear. In combination, these factors resulted in suboptimal sampling. Despite these limitations, the causative agent was identified, its characteristics studied, and Koch’s postulates confirmed by experimental infection. Koch's postulates are four criteria designed to establish a causal relationship between a causative microbe and a disease. Those four criteria include: 1) the suspected causal organism must be constantly associated with the disease; 2) the suspected causal organism must be isolated from an infected animal and grown in pure culture; 3) when a healthy susceptible host is inoculated with the pathogen from pure culture, symptoms of the original disease must develop; and 4) the same pathogen must be reisolated from animals infected under experimental conditions.

Hemorrhagic lesions on Lake St. Clair muskellunge have been occasionally noted by MDNR personnel since at least the mid 1990s. Several attempts were made to identify the etiological agent of these lesions, but live fish were not sampled. As a result, *Aeromonas hydrophila*, *Serratia* spp., *Pseudomonas* spp., and other facultative pathogens were isolated from affected skin areas and were suspected as the causative agent, but *Piscirickettsia* bacteria were not identified until 2002 because of the difficulty in isolating these intracellular bacteria.

By 2004, this study found a high percentage of the muskellunge caught in trap nets exhibited external symptoms of the *Piscirickettsia* infection. Laboratory analyses further confirmed that the infection was widespread in muskellunge caught in Anchor Bay, with 100% of the muskellunge samples examined at the MSU-AAHL testing positive for presence of the bacteria. Unfortunately, we were unable to obtain samples from enough locations or throughout the open water season to assess the seasonal or geographical variability of the infection in Lake St. Clair muskellunge because of the difficulty in capturing live muskellunge in the open waters of the lake.

Our results indicated that the bacterial infection has the potential to severely compromise the health of individual muskellunge. In naturally or experimentally infected muskellunge, we found *Piscirickettsia* spp. infections caused enlargement of internal organs along with accumulation of yellowish transparent fluid in swim bladders of fish. Histopathological examination revealed severe degeneration of the kidney glomeruli and widespread subcutaneous edema. These conditions varied in intensity among infected fish. Fish with skin lesions often had more severe histopathological lesions than those without skin lesions. Fingerling muskellunge experimentally infected with bacteria by injection in the laboratory suffered high mortalities. *Piscirickettsia* was reisolated at titers of up to 10^9

plaque forming units/gram tissue from fish kidneys of the dead and moribund laboratory muskellunge. It remains unknown if such mortality rates are also experienced by wild muskellunge fingerlings in Lake St. Clair, but we would expect a large difference between the effects of injected bacteria and wild contracted bacteria. Unfortunately, management agencies have not been able to establish a muskellunge recruitment index survey on Lake St. Clair, mainly due to difficulties in consistently sampling age 0 muskellunge in the lake. As a result, any recent change in fingerling survival will be undetected until the abundance of older muskellunge is affected.

Lake St. Clair muskellunge are a potential source of gametes for muskellunge propagation and stocking in Michigan and other states. Based on internal examination of 20 muskellunge sampled from 2004 to 2006, we found that vertical transmission of *Piscirickettsia* spp. was unlikely. As part of a VHS egg disinfection study in 2007, an additional 14 samples of eggs from ripe Lake St. Clair muskellunge tested negative for *Piscirickettsia* spp. infection. However, we were unable to thoroughly assess the possibility of vertical transmission in resultant fry and fingerlings. Even though vertical transmission of the disease appears remote, gametes collected from Lake St. Clair muskellunge would need to be disinfected for VHS virus and other potential disease agents prior to being used for propagation. From the studies performed on *P. salmonis*, antibiotics and all disinfectants commonly used in aquaculture are effective against *Piscirickettsia* spp. (Fryer et al. 1990, 1992).

Muskellunge mortality events were evident on Lake St. Clair and the Detroit River in the springs of 2003 and 2006. Both years, numerous dead muskellunge were observed by anglers and agency personnel during April and May. Many anglers called and e-mailed Lake St. Clair Fisheries Research Station to report sightings of dead muskellunge. Dead muskellunge observed were typically bleached out and apparently had been dead for many days or even weeks. Some of the dead fish had skin lesions suggestive of *Piscirickettsia* spp. infection, but it was unclear whether the bleaching was due to postmortem changes or from the edema that accompany skin infection. Indeed, in some live infected fish, edematous areas appeared bleached out. In 2003, most of the dead muskellunge appeared to be larger and presumably older fish. Based on field observations, the 2006 die-off included a wider size/age range of muskellunge. Despite repeated efforts by MDNR personnel to collect samples fresh enough for laboratory analyses, only one suitable fish was collected. That muskellunge, collected in 2006, tested positive for both VHS and *Piscirickettsia* infection. The possible roles or interactions of *Piscirickettsia* spp. and VHS virus in the observed mortality episodes are unknown.

Despite apparently substantial muskellunge mortality events in 2003 and 2006, there was no obvious trend in muskellunge catch rates in the Anchor Bay trap net survey between 2002 and 2007 (Table 3). In fact, the muskellunge catch per net lift and catch per 24 hours increased from 2003 to 2004 and was essentially unchanged from 2006 to 2007. Similarly, we found no obvious change in the age distribution or sex ratio of muskellunge in the trap net catch from 2005 to 2007. This could be an indication that the muskellunge population changed little between 2006 and 2007. However, we also recognize the possibility that the MDNR trap net survey in a localized area of Anchor Bay, during spawning season, does not provide a representative picture of the overall abundance of muskellunge across Lake St. Clair.

The MDNR and Ontario Ministry of Natural Resources have collaborated since 1994 in an Angler Diary Program. Volunteer anglers provide fishing effort and catch data each year for their fishing activity on Lake St. Clair. Although participation has been on a downward trend, no trend in catch rate for muskellunge has been apparent (Table 4). While this lack of a trend could indicate the muskellunge population has been stable, we recognize that angler catch rates can be influenced by a wide variety of factors that could confound analysis. Additionally, volunteer angler diary data must be interpreted carefully. Other studies have found that participants in such programs tend to fish more frequently, belong to fishing clubs, and experience higher catch rates than the general fishing public (Prentice et al. 1995, Bray and Schramm 2001). Also, trends in fish abundance may not be well reflected in catch rates for avid anglers because those anglers are more persistent and effective at catching fish (Baccante 1995). Thus, actual trends in fish abundance could be masked by angler diary catch rates.

Muskellunge anglers cooperating in the lesion diary program in 2003 observed the highest frequencies of red sores on the muskellunge in June and October. Unfortunately, we were not able to verify a seasonal pattern to the appearance of external symptoms with survey-gear-caught muskellunge, because our samples were collected only during May. Similarly, we were unable to directly compare frequencies of red sores and lamprey marks on muskellunge recorded by lesion diary anglers in 2003, with those recorded for muskellunge caught in the trap nets because the presence of external symptoms and lamprey marks were not recorded by MDNR surveys until 2004.

Numerous fish species were sampled for analysis by MSU-AAHL for *Piscirickettsia* infection, but yellow perch was the only species, other than muskellunge, that tested positive. Yellow perch are ubiquitous in Lake St. Clair and are a common item in the diet for Lake St. Clair muskellunge. While no die-off of Lake St. Clair yellow perch was observed during this study, we did record incidence of red external lesions on some yellow perch caught in survey nets. Anglers reported similar lesions as well. What, if any, relation those lesions have to the *Piscirickettsia* spp. was not established. It is possible, however, that muskellunge can acquire the infection through predation on yellow perch.

We were not able to collect any salmonids from Lake St. Clair for lab testing for *Piscirickettsia*. Salmonids are present seasonally in Lake St. Clair and the St. Clair River and could potentially intermingle with muskellunge in both water bodies during late fall, winter, and early spring. Gene sequencing demonstrated that the muskellunge isolate was distinct from *P. salmonis* yet closely related. Additional characterization of the isolate and determination of its host range needs to be performed.

The source of the *Piscirickettsia* bacteria in Lake St. Clair remains unknown. In fact, we are unsure if *Piscirickettsia* is a new disease in Lake St. Clair, or an endemic organism that remained undetected until 2002. Based on angler and MDNR staff recollection of external lesions on muskellunge, it appears that *Piscirickettsia* has infected Lake St. Clair muskellunge since at least the mid-1990s. This study documented that the infection was prevalent among muskellunge in Anchor Bay, Lake St. Clair, by 2004.

Management Recommendations

1. Fisheries management agencies should attempt to establish a muskellunge fingerling index survey on Lake St. Clair to monitor trends in spawning success or fingerling survival.
2. While we are unsure if *Piscirickettsia* is a new disease in Lake St. Clair, or an endemic organism that remained undetected until 2002, Lake St. Clair has been the focal point of several recent exotic species introductions to the Great Lakes. Such introductions pose an enormous threat to the biodiversity of Lake St. Clair and the overall integrity of the Great Lakes ecosystem and should be prevented.
3. All transfers of fish or fish gametes originating from Lake St. Clair should require pathogen screening and certification and appropriate disinfection procedures.

Acknowledgments

This study was partially supported by funds from the Sport Fish Restoration Act F-81-R Study 230488, Michigan. Nearly all the fish sampled were captured in trap nets fished as part of that federal aid study. Numerous MDNR employees were instrumental in field collections. Boat captains Jack Hodge and Roy Beasley operated the *RV Channel Cat* and maintained the sampling gear. Ken Koster, Fisheries Research Technician, and Jeremy Maranowski, Fisheries Assistant, were also involved in field collections. Lab work was conducted at the Aquatic Animal Health Laboratory at Michigan State University. Gary Whelan and Phil Schneeberger provided critical reviews of the manuscript.

Figure 1.–Water flows south from Lake Huron, through the St. Clair River, Lake St. Clair, and the Detroit River, which drains to western Lake Erie.

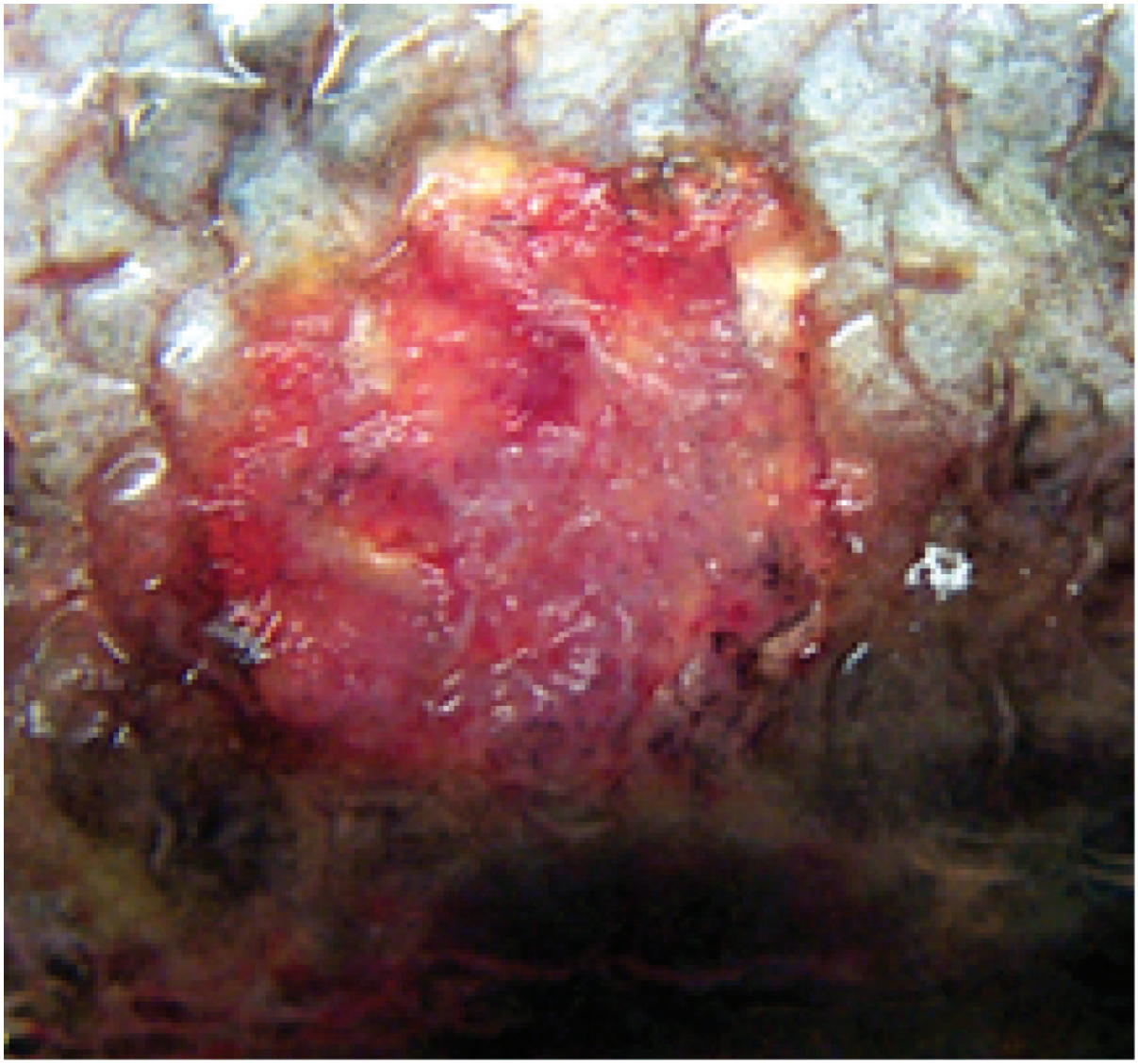

Figure 2.–Photograph of typical external raised lesion of Piscirickettsiosis in muskellunge, locally referred to as “muskie pox” by anglers.

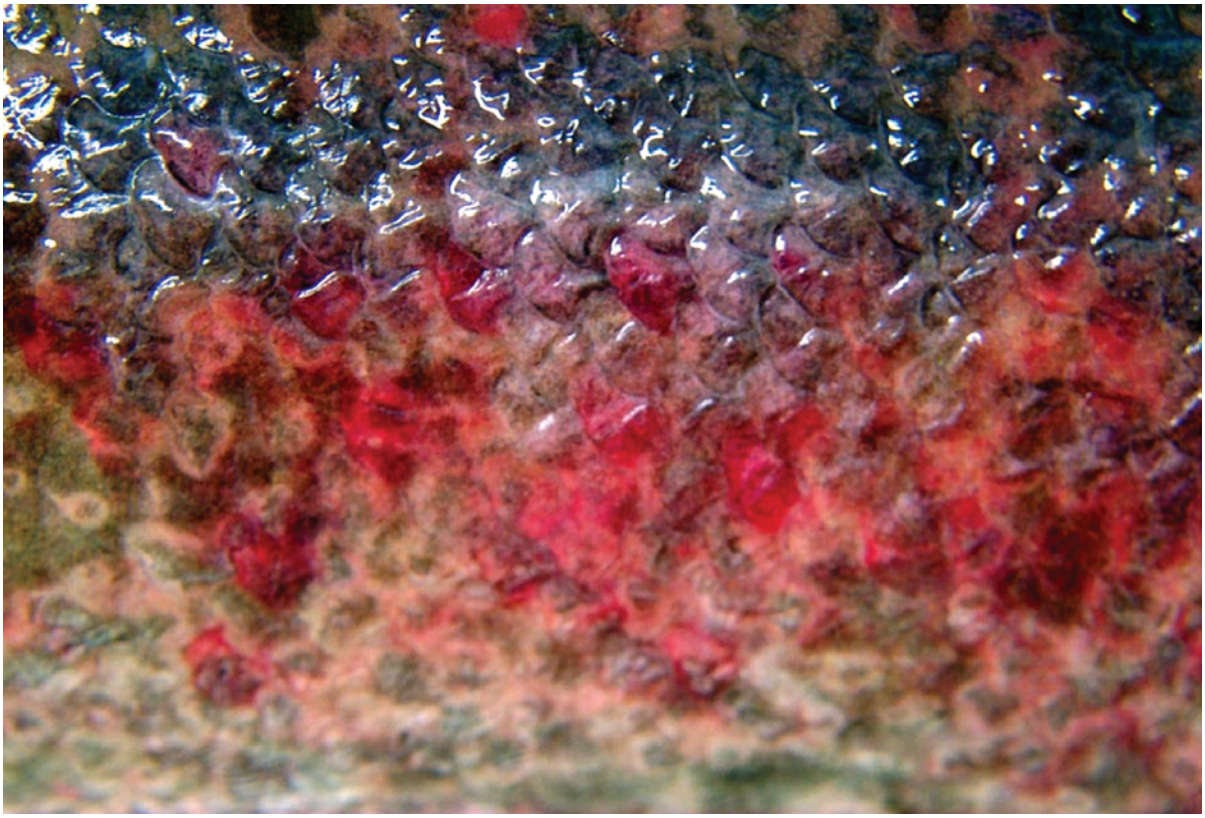

Figure 3.–Photograph showing condition described as “puffy scale”, produced by subcutaneous edema and hemorrhaging resulting from *Piscirickettsia* spp. infection.

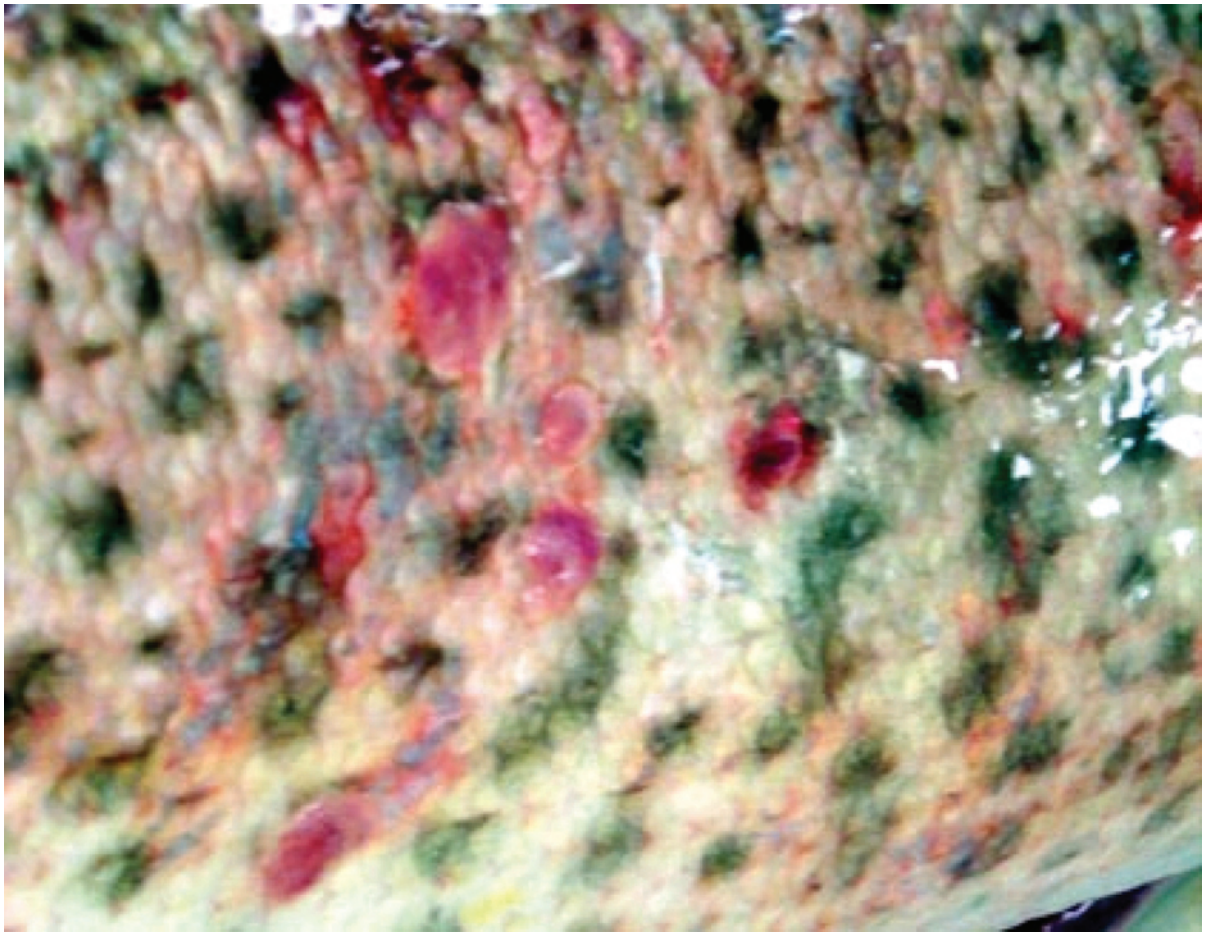

Figure 4.–Photograph of *Piscirickettsia* spp. affected muskellunge showing widespread subcutaneous hemorrhaging.

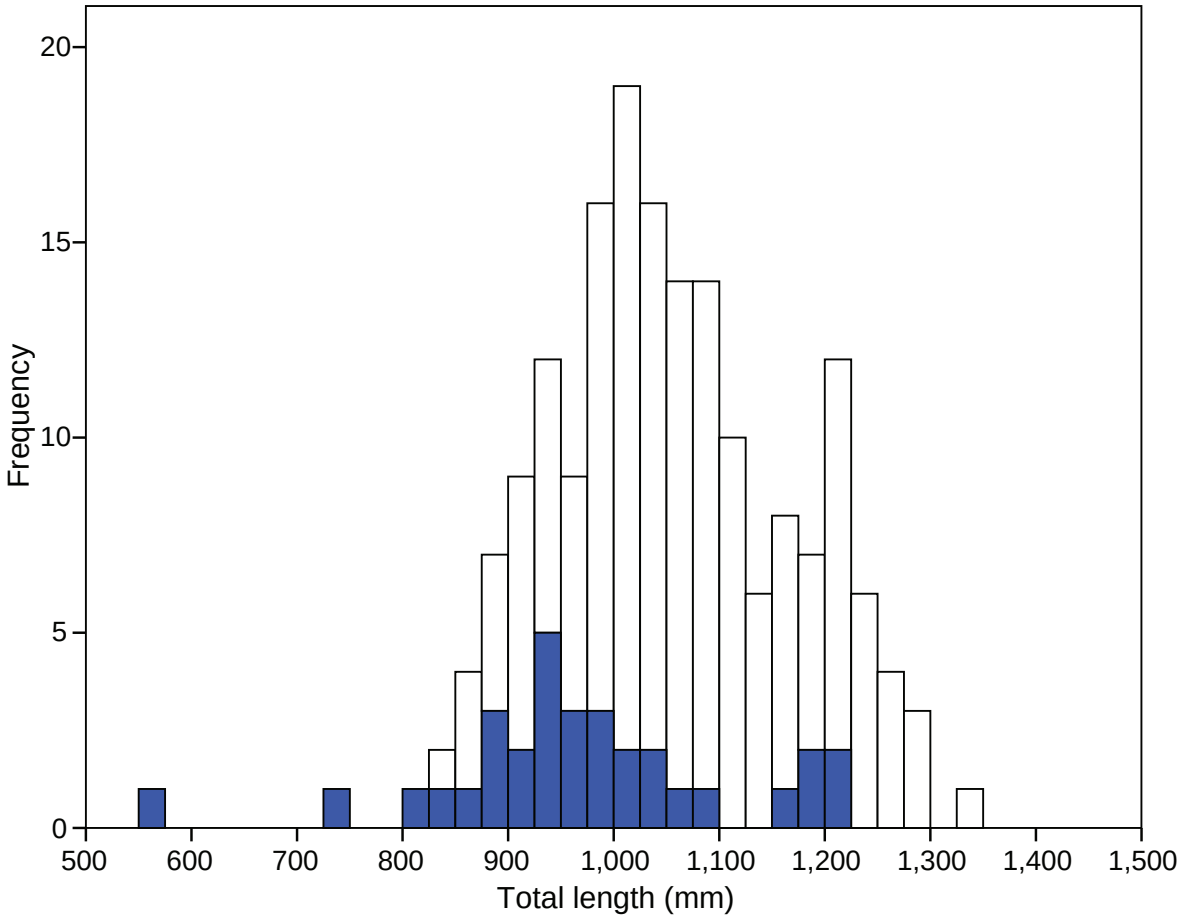

Figure 5.–Length frequency distribution for muskellunge captured in Anchor Bay trap nets with dark portion of the bars representing muskellunge with no external signs of *Piscirickettsia* infection and light portion of the bars representing those fish with visible external symptoms. External symptoms include: sunken eyes, red sores, puffy scales, and ragged or hemorrhagic fins.

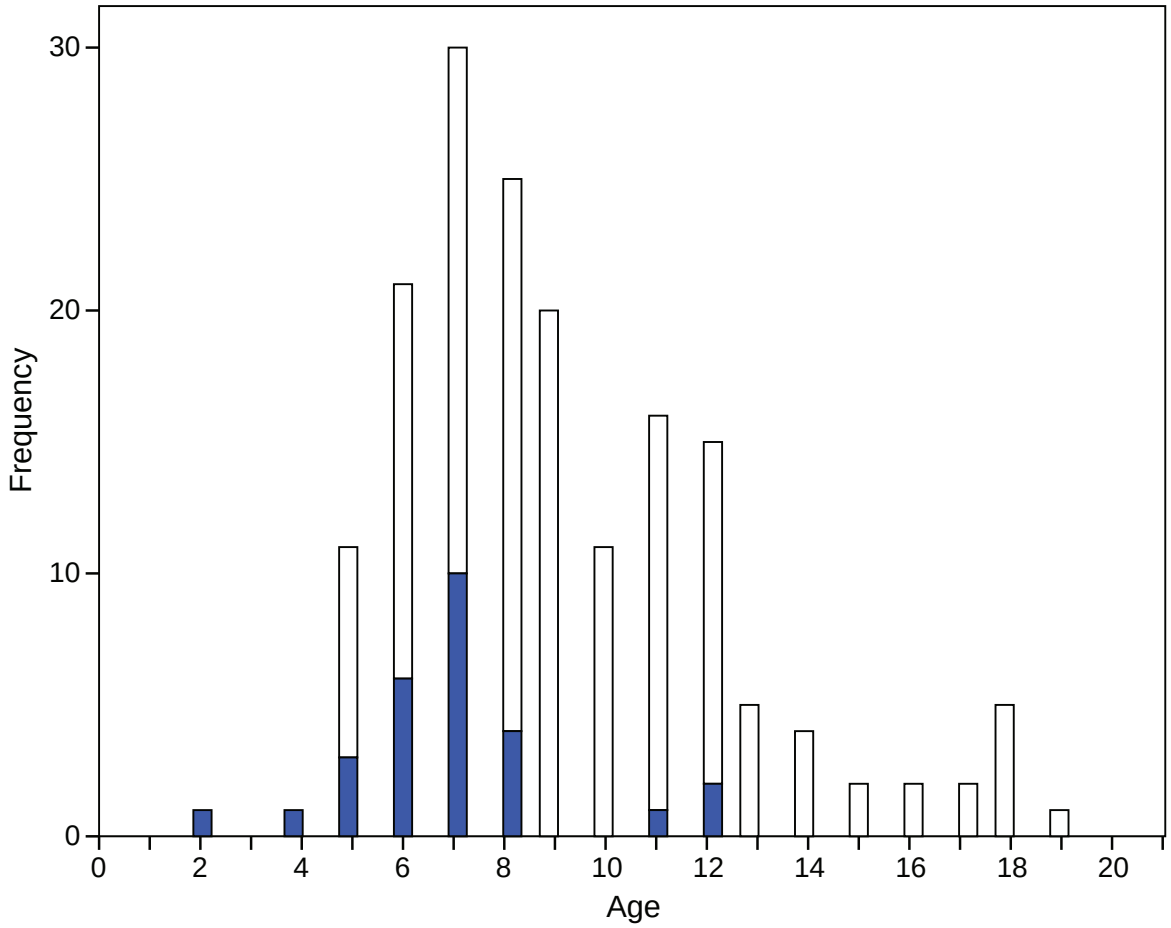

Figure 6.–Age composition for muskellunge collected with trap nets in Anchor Bay from 2004 through 2006 with dark portion of the bars representing muskellunge with no external signs of *Piscirickettsia* infection and light portion of the bars representing those fish with visible external symptoms. External symptoms include: sunken eyes, red sores, puffy scales, and ragged or hemorrhagic fins.

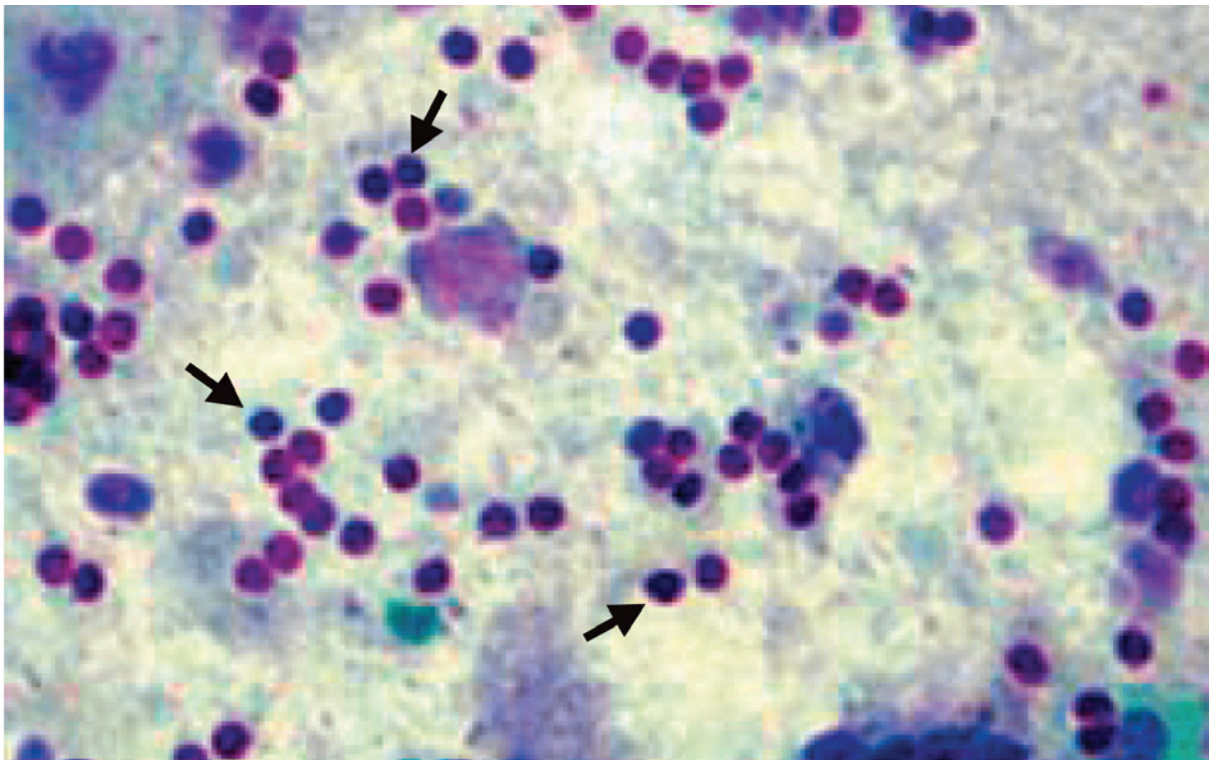

Figure 7.–Muskellunge *Piscirickettsia* spp. (indicated by arrows) in affected skin lesions stained with Giemsa (780X).

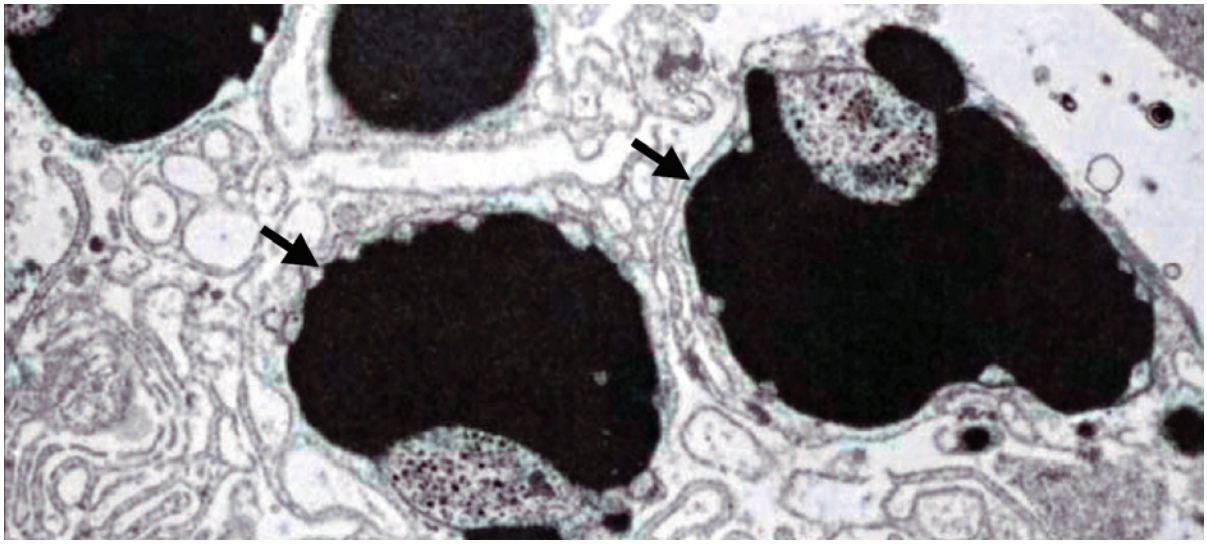

Figure 8.–Electron microscopy photograph of muskellunge *Piscirickettsia* spp. Notice the intracellular bacteria (indicated by arrows) are enclosed within membrane-bound vesicles (79,000 X).

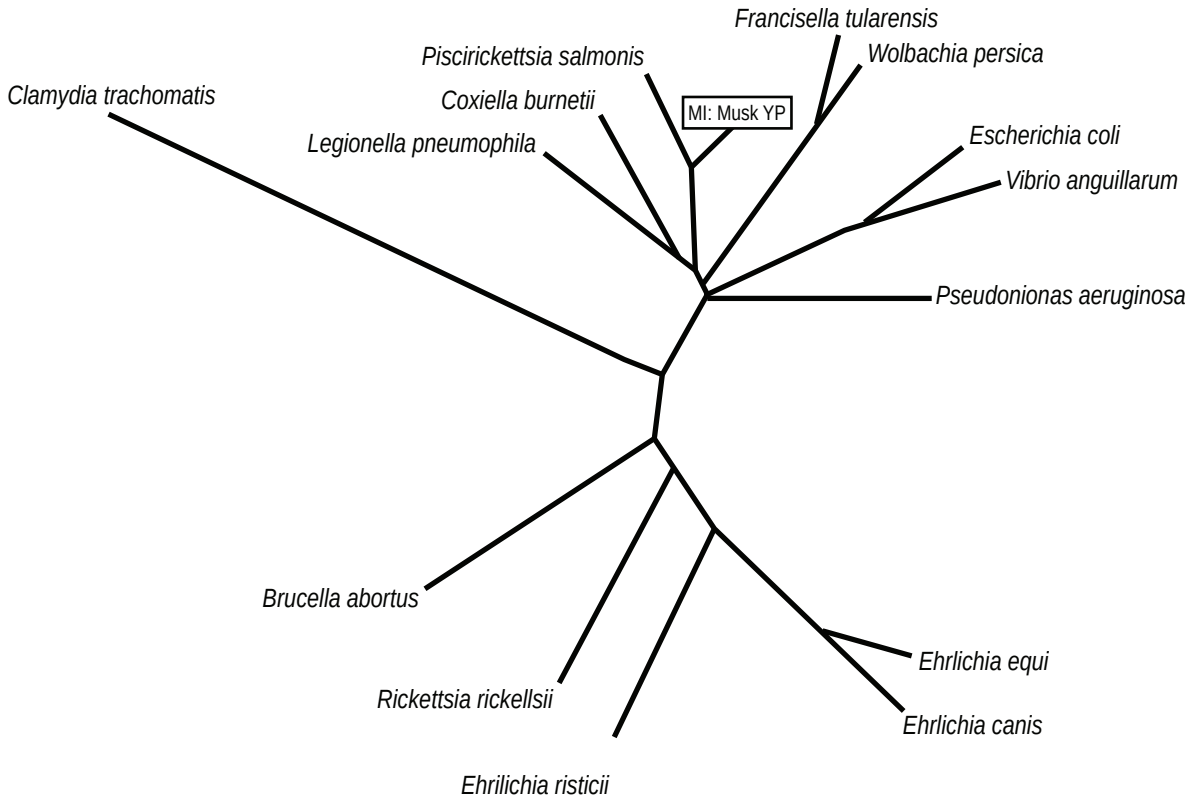

Figure 9.–Illustration of phylogenetic analyses involving sequences of the 16S, ITS, and 23S rDNA genes. These analyses confirmed that muskellunge and yellow perch isolates were identical to each other, but not identical to *P. salmonis*. Musk = Muskellunge isolate, YP = Yellow perch isolate.

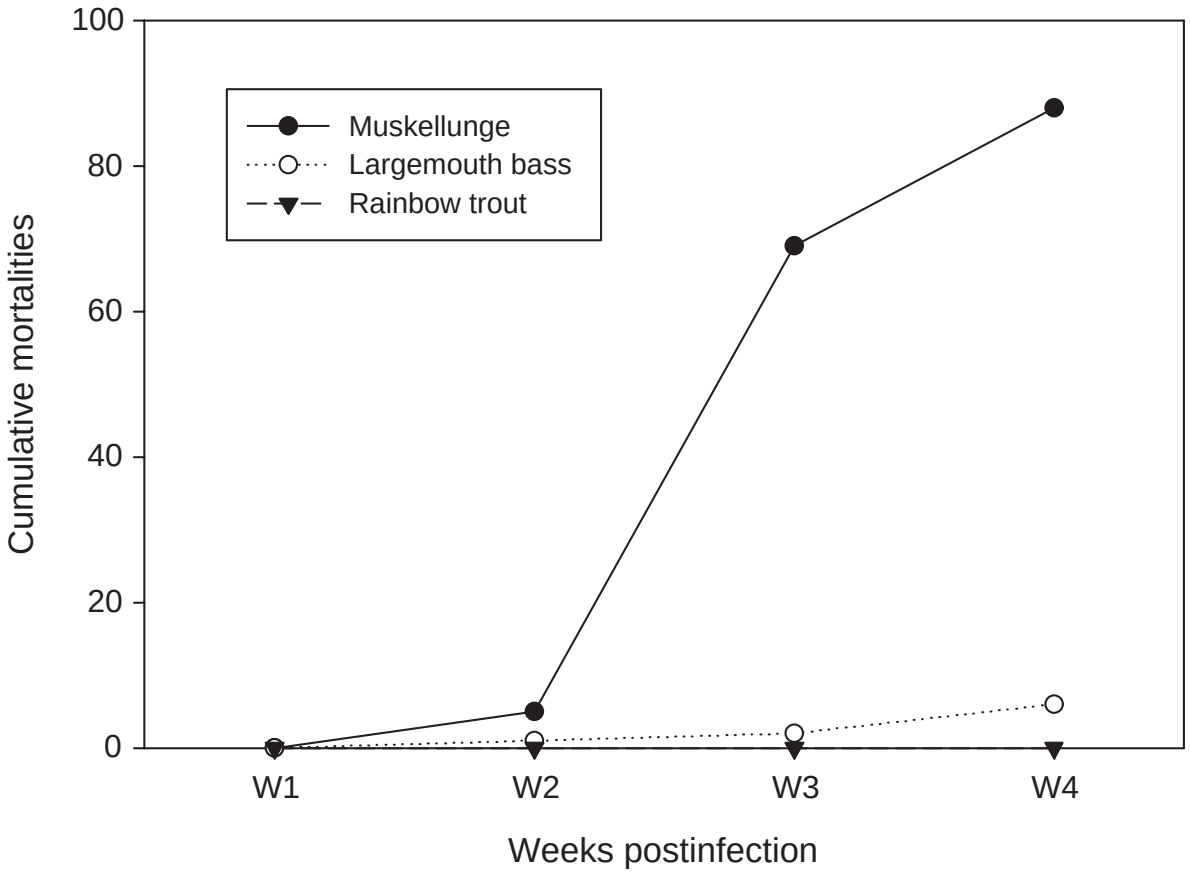

Figure 10.–Pathogenicity testing performed by intraperitoneal injection of healthy fish fingerlings with 100 µl of tissue culture supernatant containing 10^4 bacteria.

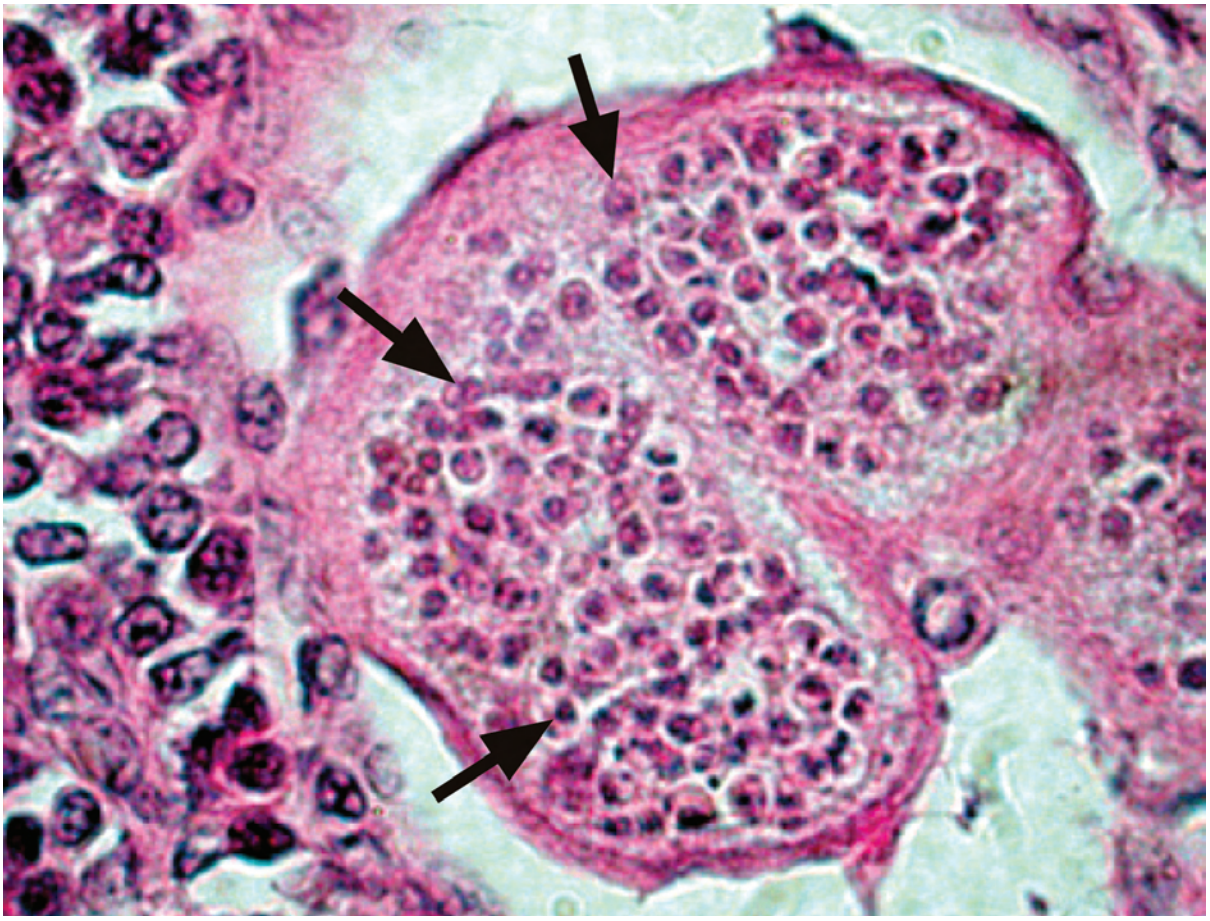

Figure 11.–Hematoxylene and eosin stained tissue section showing destroyed kidney glomeruli filled with multiplying *Piscirickettsia* spp. (indicated by arrows, magnification 1000X).

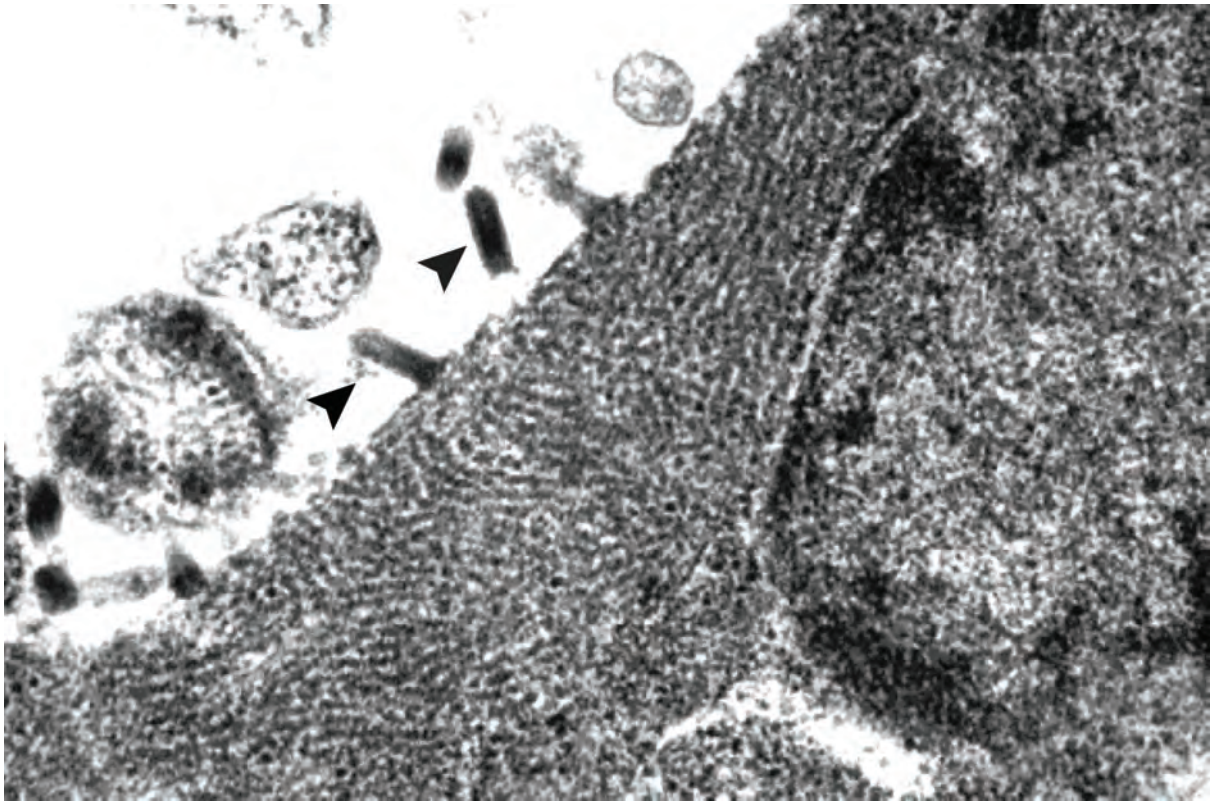

Figure 12.–Ultrastructural details of a rhabdovirus (indicated by arrows) isolated from Lake St. Clair muskellunge on FHM cell line (90,000 X).

Table 1.–Occurrence of various external symptoms of Piscirickettsia spp. infection in muskellunge caught in Anchor Bay trap nets and number of samples transferred to the Animal Health Laboratory at Michigan State University. External symptoms include: red sores, puffy scales, sunken eyes, and ragged or hemorrhagic fins.

	Year			
	2004	2005	2006	Total
Number of muskellunge captured in trap nets	78	56	48	182
Percent with red sores	19%	11%	4%	13%
Percent with puffy scales	81%	68%	94%	80%
Percent with sunken eyes	0%	0%	2%	<1%
Percent with ragged or hemorrhagic fins	6%	2%	8%	6%
Percent of muskellunge with any visible external signs of infection	84%	70%	96%	82%
Percent of muskellunge with at least 1 lamprey attachment mark	53%	62%	96%	67%
Number of muskellunge samples transferred to MSU AHL	7	9	6	20
Percent of muskellunge samples testing positive for *Piscirickettsia sp.*	100%	100%	100%	100%

Table 2.−Fish species other than muskellunge from Lake St. Clair that were analyzed for *Piscirickettsia* spp. infection in this study.

Year	Species (number)	Percent positive
2004	Yellow Perch (69)	57%
	Northern pike (19)	0%
	Smallmouth bass (2)	0%
	Largemouth bass (3)	0%
	Freshwater drum (3)	0%
	Shorthead redhorse (8)	0%
	Silver lamprey (1)	0%
	Crayfish (20)	0%
	Snails (2)	0%
2005	Walleye (5)	0%
	Northern pike (1)	0%
	Channel catfish (1)	0%
	Smallmouth bass (1)	0%
2006	Gizzard shad (38)	0%
	Yellow perch (1)	0%
	Northern pike (4)	0%
	Shorthead redhorse (2)	0%
	Freshwater drum (12)	0%
	Rock bass (1)	0%
	Silver redhorse (2)	0%
	Spottail shiner (60)	0%
	Emerald shiner (60)	0%

Table 3.–Anchor Bay trap net survey effort, physical conditions, and muskellunge catch rates from 2002 through 2007 (unpublished Michigan Department of Natural Resources data).

	Survey year					
	2002	2003	2004	2005	2006	2007
Number of net lifts	64	50	55	34	43	50
Hours fished	2,748	2,839	3,080	1,773	2,371	2,445
Starting date	03 May	28 May	03 May	11 May	04 May	01 May
Ending date	30 May	20 Jun	26 May	26 May	24 May	22 May
Starting water temp. (°C)	9	12	8	9	13	12
Ending water temp. (°C)	15	16	15	13	13	13
Average secchi depth (m)	1.7	2.2	1.2	2.2	1.7	2.6
Number of muskellunge caught	41	28	78	56	46	51
Muskellunge catch per lift	0.6	0.6	1.4	1.6	1.1	1.02
Muskellunge catch per 24 hours fished	0.4	0.2	0.6	0.8	0.5	0.5

Table 4.–Reported trips, effort, catch, and catch rates for muskellunge reported by anglers voluntarily participating in the Lake St. Clair Sport Fishery Diary Program (updated from Thomas and Haas 2004).

Year	Trips (targeted)	Effort (rod-hours)	Number caught	Number kept	Catch per rod-hour
1996	494	15,629	1,458	12	0.093
1997	425	15,199	1,573	11	0.103
1998	383	11,336	1,075	8	0.094
1999	318	9,370	645	5	0.069
2000	269	8,874	749	16	0.084
2001	241	7,248	851	2	0.117
2002	156	3,953	277	4	0.070
2003	141	3,731	341	10	0.091
2004	114	2,510	236	1	0.094
2005	109	2,468	209	0	0.085
2006	89	1,838	130	0	0.071
2007	65	1,264	149	0	0.118

Literature Cited

American Fisheries Society. 2004. Suggested procedures for the detection and identification of certain finfish and shellfish pathogens. American Fisheries Society, Bethesda, Maryland.

Baccante, D. 1995. Assessing catch inequality among walleye angling fisheries. North American Journal of Fisheries Management 15:661-665.

Bolsenga, S. J., and C. E. Herdendorf. 1993. Lake Erie and Lake St. Clair handbook. Wayne State University Press, Detroit, Michigan.

Bravo, S., and M. Campos, 1989. Coho salmon syndrome in Chile. American Fisheries Society Fish Health Section Newsletter 17:3.

Bray, G. S. and H. L. Schramm, Jr. 2001. Evaluation of a statewide volunteer angler diary program for use as a fishery assessment tool. North American Journal of Fisheries Management 21:606-615.

Chen M. F., S. Yun, G. D. Marty. 2000. A *Piscirickettsia*-like bacterium associated with mortality of white bass. Diseases of Aquatic Organisms 43:117-126.

Edsall, T. A., B. A. Manny, and C. N. Raphael. 1988. The St. Clair River and Lake St. Clair, Michigan: an ecological profile. U.S. Fish and Wildlife Service Biological Report 85(7.3).

Elsayed, E., M. Faisal, M. Thomas, G. Whelan, W. Batts, and J. Winton. 2006. Isolation of viral haemorrhagic septicaemia virus from muskellunge, *Esox masquinongy* (Mitchill), in Lake St. Clair, Michigan, USA reveals a new sublineage of the North American genotype. Journal of Fish Diseases 29:611-619.

Fryer, J. L., C. N. Lannan, L. H. Garces, J. J. Larenas, and P. A. Smith. 1990. Isolation of a rickettsia-like organism from diseased coho salmon *Oncorhynchus kisutch* in Chile. Fish Pathology 25:107–114.

Fryer, J. L., C. N. Lannan, S. J. Giovannoni, and N. D. Wood. 1992. *Piscirickettsia* salmonis gen. nov., sp. nov., the causative agent of an epizootic disease in salmonid fishes. International Journal of Systematic Bacteriology 42:120–126.

Fryer, J. L., and C. N. Lannan. 1996. Rickettsial infections of fish. Annual Review of Fish Diseases 6:3-13.

Fryer, J. L., and M. J. Mauel. 1997. The rickettsia: an emerging group of pathogens in fish. Emerging Infectious Diseases 3:137–144.

Haas, R. C., W. C. Bryant, K. D. Smith, and A. J. Nuhfer. 1985. Movement and harvest of fish in Lake St. Clair, St. Clair River, and Detroit River. Michigan Department of Natural Resources, Final Report, Winter Navigation Study, U. S. Army Corps of Engineers, Ann Arbor.

Jamsen, G. C. 1985. Michigan's 1981 and 1982 sport fishery. Michigan Department of Natural Resources, Fisheries Technical Report 85-4, Ann Arbor.

Khoo L, P. M. Dennis, G. Lewbartet. 1996. Rickettsial-like organism in the blue-eyed plecostomus, *Panaque suttoni* (Eigenmann & Eigenmann). Journal of Fish Diseases 18:157-164.

Leach, J.H. 1980. Limnological sampling intensity in Lake St. Clair in relation to distribution of water masses. Journal of Great Lakes Research 6:141-145.

MacLennan, D. S. 1996. Changes in the muskellunge fishery and population of Lake St. Clair after an increase in the minimum size limit. Pages 19-27 *in* S.J. Kerr and C. H. Olver, editors. Proceedings of the Managing Muskies in the '90s' Workshop. Ontario Ministry of Natural Resources and Muskies Canada, Inc., Kemptville, Ontario.

Mauel M. J., D. L. Miller, K. Frazieret, A. D. Liggett, L. Styr, D. Montgomery-Brock, and J. Brock. 2003. Characterization of a piscirickettsiosis-like disease in Hawaiian tilapia. Diseases of Aquatic Organisms 53:249-255.

Office International des Epizooties. 2003. Manual of diagnostic tests for aquatic animals, 4th edition. Office International des Epizooties, Paris

Prentice, J. A., B. W. Farquhar, and W. E. Whitworth. 1995. Comparison of volunteer angler-supplied fisheries catch and population structure data with traditional data. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 47(1993):666-678.

Thomas, M. V., and R. C. Haas. 2004. Status of the Lake St. Clair fish community and sport fishery, 1996–2001. Michigan Department of Natural Resources, Fisheries Research Report 2067, Ann Arbor.

Warye, R. 2002. Muskie: The premier waters of North America. Fishing Hotspots, Inc., Rhinelander, Wisconsin.

United States Department of the Interior, Fish and Wildlife Service and United States Department of Commerce, Bureau of Census. 2008. 2006 National survey of fishing, hunting and wildlife-associated recreation. United States Governmental Printing Office, Washington.

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Appendix 1.–Common and scientific names of fishes mentioned in this report.

Common name	Scientific name
Channel catfish	*Ictalurus punctatus*
Emerald shiner	*Notropis atherinoides*
Chinook salmon	*Oncorhynchus tshawytscha*
Fathead minnow	*Pimephales promelas*
Freshwater drum	*Aplodinotus grunniens*
Gizzard shad	*Dorosoma cepedianum*
Largemouth bass	*Micropterus salmoides*
Muskellunge	*Esox masquinongy*
Northern pike	*Esox lucius*
Rainbow trout	*Oncorhynchus mykiss*
Rock bass	*Ambloplites rupestris*
Shorthead redhorse	*Moxostoma macrolepidotum*
Silver lamprey	*Icthyomyzon unicuspis*
Silver redhorse	*Moxostoma anisurum*
Smallmouth bass	*Micropterus dolomieu*
Spottail shiner	*Notropis hudsonius*
Walleye	*Sander vitreus*
Yellow perch	*Perca flavescens*