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Evaluation of Natural Steelhead Recruitment in the Muskegon River, Michigan

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EVALUATION OF NATURAL STEELHEAD RECRUITMENT IN THE MUSKEGON
RIVER, MICHIGAN

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ABSTRACT

The lower Muskegon River is one of the most heavily fished rivers in the state of Michigan and is a valuable component of the multi-billion dollar sport fishery in the Great Lakes. Although significant stocking effort has been invested to maintain and improve the steelhead (*Oncorhynchus mykiss*) fishery in the Muskegon River, natural recruitment has been severely limited due to high summer water temperatures. The goal of this research project was to evaluate the success of a diffuser system installed in 2008 at Croton Dam to moderate high summer water temperatures in the lower Muskegon River. I estimated natural juvenile steelhead abundance, survival, and production in the Muskegon River and compare that with previous work to see if there has been a population level response to the installation of the diffuser. In addition, I used heat shock protein analysis to confirm whether juvenile steelhead are experiencing thermal stress in the Muskegon River.

First, I used water temperature data from the USGS gauging station on the Muskegon River from 2006-2008 and 2010-2012 to compare stream temperatures before and after the installation of the diffuser. Based on the summary of the mean monthly average temperature, temperatures in August remained similar before and after the installation of the diffuser even though temperatures in July 2010-2012 were 0.8°C higher than July 2006-2008. Based on the results from this study, stream temperatures in the Muskegon River do not appear to have improved since the installation of the diffuser.

Pass depletion surveys were used to estimate parr survival, production and growth in the Muskegon River and Bigelow Creek during 2011-2013. Average fall density of parr in Bigelow Creek was 48-fold higher than in the Muskegon River. Average summer daily mortality rate of

parr in the Muskegon River was nearly six-fold higher than in Bigelow Creek. High mortality rates in the Muskegon River corresponded to average summer water temperatures exceeding 21^0C. My results were similar to previous work completed on the Muskegon River and suggest that natural steelhead production in the Muskegon River is still severely limited due to high summer water temperatures.

The final object of this study was to investigate whether juvenile steelhead in the Muskegon River and Bigelow Creek are experiencing thermal stress as a result of high summer water temperature using fin tissue to conduct heat shock protein analysis. I found that there were significant differences in fin heat shock protein 70 (hsp70) levels across sites and seasons. Relative hsp70 levels for each site in August varied significantly from June and October at all of my sites, except for one site in Bigelow Creek, which had lower summer water temperatures. Collectively, these results suggest that juvenile steelhead in the Muskegon River are still experiencing prolonged exposure to elevated temperatures and thermal stress, which result in physiological consequences that could ultimately affect the survival of naturally reproducing steelhead in the Muskegon River.

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Chapter 1

INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is a species of salmonid native to tributaries of the Pacific Ocean. Their native range extends from the Kamchatka Pensinsula (Russia) eastward to Alaska and as far south as Mexico. Within this range, they display highly variable and plastic life history strategies that include two primary forms; the stream resident rainbow trout and anadromous forms (Raleigh 1984). The anadromous form of rainbow trout is commonly known as steelhead and it has a life expectancy of 4 to 8 years (Raleigh 1984). The steelhead life cycle begins in freshwater streams where adults will return to spawn after maturing in the ocean. The two major returns of steelhead to freshwater streams including winter and summer-run steelhead. Winter-run steelhead return from the ocean between the fall and winter, while summer-run fish will return between spring and early-summer with both groups spawning in the spring and early summer months. Upon their return, female steelhead will select a location with relatively swift current and a streambed composed of course gravels. Females begin constructing a redd by turning on their side and vigorously fanning their caudal fin against the streambed. This causes the gravel to loosen and get swept downstream by the current. This process continues until a depression is formed in the streambed. When the female is ready to spawn, male steelhead will swim alongside the female and release milt when the female deposits eggs into the depression. Following this, the female steelhead will swim to the upstream side of the pit to loosen the gravel that will be swept downstream to cover the eggs. Spawning will then continue sporadically as the female enlarges her redd and deposits more eggs. Following spawning, many of the adult steelhead will die, but some return to the ocean to spawn again.

The incubation time for steelhead eggs in the gravel is dependent on temperature, but will generally take 3 to 4 weeks to hatch with temperatures from 10 - 15^{0}C (Moyle 2002). After hatching, juvenile steelhead (alevins) will remain in the gravel for 2 to 3 weeks before emerging. Following emergence, steelhead fry usually live in small schools in the shallow water along stream banks. As the steelhead grow, the schools break up and they establish individual feeding territories. Most juvenile steelhead will live in the riffles during their first year, but larger juvenile steelhead will reside in deep pools and runs (U.S. Fish and Wildlife Service 1995). In the river, they will predominantly feed on aquatic and terrestrial insects and other small invertebrates. The optimum temperature range for juvenile steelhead and fry is 12.8^{0}C to 15.5^{0}C, with temperature ranges of 15.6^{0}C to 20^{0}C, 20.1^{0}C to 22.5^{0}C, and greater than 22.5^{0}C corresponding to thermal stress levels of low, medium, and high, respectively (U.S. Fish and Wildlife Service 1995). Juvenile steelhead will reside in freshwater for 1 to 4 years before migrating out to the ocean as smolts. There they will primarily feed on fish, squid, and crustaceans (Moyle 2002). In the ocean, steelhead primarily inhabit the surface waters of the Pacific Ocean (Ruggerone et al. 1990) and have been found to be attracted to areas of sharp temperature variation (Höök et al. 2004), such as vertical fronts in the Great Lakes (Aultman and Haynes 1993). Steelhead may move to these areas because they provide the preferred thermal conditions and prey densities to maximize growth (Brandt 1980, 1993). In the ocean, steelhead will spend another 1 to 4 years in the ocean maturing. Once they have reached maturation, they will return to their natal freshwater streams to spawn.

While steelhead and resident rainbow trout are native to the tributaries of Pacific Ocean, they have been introduced to other bodies of water including the Great Lakes for sport fishing. As a result of their introduction and subsequent hatchery supplementation efforts in the Great

Lakes, steelhead have become an important component in the Great Lakes both ecologically and economically, and significantly contribute to the Great Lakes sport fishery, which is valued at approximately $7 billion dollars (American Sportfishing Association 2008).

The first documented introduction of steelhead into the Great Lakes came in the late 1800s (MacCrimmon 1972). Supplementation efforts continued until naturalized populations became well established around Lake Michigan by the 1920s with most of the natural reproduction occurring in Michigan tributaries due to the abundance of suitable spawning habitat (MacCrimmon 1972). Additional supplemental efforts in Lake Michigan did not occur again until the 1950s following a decline in the lake-wide steelhead population as a result of overfishing, the population explosion of parasitic sea lampreys (*Petromyzon marinus*), and the unintentionally introduction of alewives (*Alosa pseudoharengus*), which depleted food sources and altered the food web. (MacCrimmon 1972). During this time, hatchery supplementation primarily utilized the Michigan winter-run strain which is derived from returning adults to the Little Manistee River, the only egg-take facility in Michigan. While the Michigan strain is currently and historically been used around the Lake Michigan basin, additional hatchery strains like the summer-run Skamania strain from the Washougal River in Washington have been used since the mid-1980s to take advantage of strain-specific variation in life history characteristics (i.e. run timing) which are valued by recreational anglers.

Although significant effort has been put into supplementing the naturalized populations with hatchery steelhead, their relative contribution to Lake Michigan stocks did not significantly increase until stocking practices changed in 1983 when steelhead were stocked as large yearlings (>150mm). Prior to that time, steelhead were stocked as small fall fingerlings (<110mm; Seelbach 1987). This change resulted in an increase in the number of hatchery raised steelhead

surviving to the smolt stage (Rand et al. 1993). Subsequently, the contribution of hatchery steelhead on spawning runs increased from 0-30% (Seelbach and Whelan 1988) to 13-79% (Barton and Scribner 2004) in six Michigan rivers during the 1998-1999 spawning run.

Currently, the steelhead fishery is maintained primarily through hatchery supplementation, but significant natural reproduction still occurs in some Lake Michigan tributaries such as the Little Manistee River and Pine Creek (Seelbach 1993; Woldt and Rutherford 2002). Rand et al. (1993) estimated that in Lake Michigan natural origin steelhead comprised approximately 20-30% of adult steelhead caught in the sport fishery. While natural reproduction is occurring, and this production is important to management of the sport fishery, little is known about the factors regulating natural recruitment.

Questions about natural steelhead recruitment lead to a number of studies that were conducted on several Lake Michigan tributaries. For example, Seelbach (1988) found the contribution of natural origin steelhead to the adult population in seven Lake Michigan tributaries was substantially higher in rivers considered to be trout streams with substantial ground water inputs compared to rivers that are considered marginal trout streams. A study by Newcomb and Coon (1997) on the Betsie River and its connecting tributaries found small tributaries contributed disproportionally to overall production of steelhead parr in the Betsie River watershed. They found that the abundance and survival of steelhead parr in these connecting tributaries was higher than that of the Betsie River due the lower summer water temperatures and favorable thermal habitat quality for steelhead parr. In another study by Woldt and Rutherford (2002), they investigated the production and survival of steelhead parr in the large regulated Manistee River and compared those values with those for parr in the adjacent free-flowing Little Manistee River. Results from this study indicated that survival and production

of steelhead parr in the Manistee River was significantly lower than in the Little Manistee River as a result of high summer water temperatures. Similar to the study by Woldt and Rutherford (2002) on the Manistee River, a study was conducted from 1998 to 2001 by Godby et al. (2007) on the Muskegon River, a tributary to Lake Michigan. The purpose of this study was to investigate factors regulating age-0 steelhead production in a large regulated river and its connecting tributary creek that is characteristic of many Great Lakes watersheds. Results of this study indicated that high summer temperatures caused by Croton Dam were severely limiting natural steelhead production. As a result of this study by Woldt and Rutherford (2002), diffuser systems were designed and installed on the Manistee, Muskegon, and Au Sables Rivers as part of a $1.75 million commitment by Consumers Energy to address temperature and dissolved oxygen issues associated with hydropower dams. By installing the diffuser system, Consumers Energy hoped to address those concerns by upwelling colder bottom water behind the dam with oxygen to help alleviate elevated water temperatures and low dissolved oxygen levels downstream of hydropower dams. Increases in natural reproduction of anadromous salmonids and abundance of age-0 salmonids have been linked to changes in hydropower dam operations in other studies (Raymond 1988). Changes in hydropower dam operations have improved salmonid nursery habitat, increased availability to spawning areas, and subsequently increased natural recruitment and production of salmonids downstream of hydropower dams (Horne et al. 2004; Raymond 1988). Therefore, a primary objective of this study was to determine if natural steelhead abundance has changed following the installation of the diffuser system.

Over a billion dollars is spent annually in the United States on watershed restoration and in-stream habitat improvement projects (Bernhardt et al. 2005). Many of these restoration projects are designed to increase in-stream habitat quality, riparian zone integrity, bank

stabilization, fish passage, and improve water quality (Bernhardt et al. 2005). These projects are a widely used management strategy to lessen the anthropogenic impact on watersheds by mimicking natural conditions and thereby helping restore aquatic ecosystems. Restoration projects, such as building a diffuser system on Croton Dam, are geared toward lowering stream temperature because of the overall influence it has on all levels of biological organization (Tait et al. 1994; Beitinger el al. 2000). One way to assess the effectiveness of the Croton Dam diffuser system is to determine natural steelhead production and compare pre- and post-diffuser estimates in the treatment reaches (i.e., river reaches on the Muskegon River immediately downstream of Croton Dam) and reference (i.e., control) sites on Bigelow Creek. Another way to evaluate the diffuser system is to determine if there have been any decreases in water temperature and increases in dissolved oxygen downstream of Croton Dam since the installation of the diffuser system. Also, to determine if water temperatures downstream are suitable for juvenile steelhead, I measured cellular levels of heat shock proteins in the tissue of juvenile steelhead residing in the Muskegon River and Bigelow Creek.

At the population level, temperature influences the distribution of fish in an ecosystem (Torgersen et al. 1999) and ultimately their viability (Li et al. 1994). As such, temperature can be considered a master controller in fish distribution, life-history strategies, fecundity, and energy budget. In short, all aspects of fish ecology are influenced by temperature which is why temperature is such a vital factor in determining habitat quality. However, it is difficult to differentiate between what is biologically significant and what is statistically significant. At the cellular level, temperature virtually affects all components of the cellular process, including protein stability and enzymatic rates (Hochachka and Somero 2002). At the individual level, water temperature influences the metabolism, growth (Beer and Anderson 2011), and

microhabitat selection of fish (Baltz et al. 1987). Therefore, by examining the cellular response to temperature within my study animal I may be better able to understand how environmental processes (e.g., temperature) affect cellular processes which are linked to organismal responses (e.g., growth, movement) to the environment that ultimately culminate in a measurable population level response. Therefore, monitoring and managing for stream temperature can be an essential component in stream restoration projects and protecting salmonid populations.

A recent technique that has been developed as an indicator of thermal habitat quality is quantifying heat shock proteins to measure thermal stress (Lund et al 2002; Werner et al. 2005). Heat shock proteins are a group of highly conserved cellular proteins that function as molecular chaperones and are present in most organisms that have been examined, including fish (Feder and Hofmann 1999). There are three major families of heat shock proteins; Hsp90 (85-90kDa), Hsp70 (68-73kDa), and low molecular weight heat shock proteins (16-47kDA), with Hsp70 being the most intensively studied in model organisms and in natural occurring populations (Basu et al. 2002; Sorensen et al. 2002). In unstressed cells, these proteins are involved in a variety of functions including the repair and destruction of altered or denatured proteins (Sorensen et al. 2002). However, it is under stressful conditions that heat shock proteins get their title as a molecular chaperone. In stressful conditions, heat shock proteins function to help an organism cope with environmental, physical, or biological stressors by binding to the denatured proteins (Iwama et al. 1999). In doing so, they minimize the occurrence of proteins interacting inappropriately with one another (Feder and Hofmann 1999). Heat shock proteins can be synthesized constitutively or in response to a stressor (Hochachka and Somero 2002). At the cellular level, heat shock proteins play an important role in responding to a variety of stressful and damaging conditions which make them important in the recovery and survival of organisms.

Heat shock protein expression can be correlated with resistance to stress and thresholds for stress such that higher levels of heat shock proteins translate into increased resistance and higher thermal tolerance (Werner et al. 2005). One type of stressor that has been studied in fish is exposure to elevated water temperatures (Feldhaus et al. 2010; Fowler et al. 2009). After cells or whole organisms are exposed to elevated temperatures, they respond by synthesizing heat shock proteins in order to help protect vital cellular functions (Fader et al. 1994). This reaction has been referred to as the heat shock response (Parsell and Lindquist 1994). While heat shock proteins are synthesized in small amounts during normal conditions, it is under stressful conditions that the level of hsp induction increases (Ashburner 1982; Lindquist 1986). This increase in hsp induction during cellular stress makes it possible to quantify heat shock proteins when fish are exposed to seasonal variation in water temperatures (Fader et al. 1994). Quantifying heat shock proteins is a technique that has been used to measure thermal stress in salmonids in laboratory and natural conditions (Feldhaus et al. 2010; Lund et al. 2003). Given the impact temperature can have on growth, metabolism, behavior, and ultimately survival of fish populations (Beer and Anderson 2011; Sauter and Connolly 2010), it is plausible that physiological indicators such as thermal stress could be used as an indicator of thermal habitat quality. I propose that heat shock proteins can provide a means to quantify the thermal stress juvenile steelhead experience in the Muskegon River during seasonal variation in stream temperatures and provide an indicator of thermal habitat quality of the Muskegon River during that time.

Given the impact temperature can ultimately have on survival, the primary goal of this project was to evaluate the success of the diffuser at alleviating high summer water temperatures and low dissolved oxygen levels below Croton Dam that were found to be limiting natural steelhead recruitment in the Muskegon River. In doing so, one project objective was to

investigate current naturally reproduced juvenile steelhead abundance, survival, and production in the Muskegon River and compare that with the findings by Godby et al. (2007) to see if there has been a population level response to the installation of the diffuser. In addition, I used heat shock protein analysis to help bridge the gap between what is statistically significant and biologically meaningful, because while the diffuser may result in a significant drop in summer water temperatures it may not be biologically meaningful in increasing natural steelhead recruitment. Quantifying heat shock proteins will help determine if juvenile steelhead experience thermal stress during warm summer water temperatures.

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Chapter 2

Trends in Water Temperature Before and After the Installation of an "Upwelling" System at

Croton Dam on the Muskegon River, Michigan

ABSTRACT

Hydroelectric dams are an important factor that has contributed to the decrease in

salmonid populations throughout their native and introduced ranges. In addition to fragmenting

spawning and nursery habitat, top withdrawal dams have helped alter the thermal habitat quality,

which in turn impacts the fish communities downstream of those impoundments. The goal of this

project was to evaluate the success of the "upwelling" system at alleviating high summer water

temperatures on the Muskegon River below Croton Dam that are believed to be limiting natural

steelhead (*Oncorhynchus mykiss*) production. Water temperature data from the USGS gauging

station on the Muskegon River from 2006-2008 and 2010-2012 were used to compare stream

temperatures before and after the installation of the diffuser. Based on the summary of the mean

monthly average temperature, temperatures in August remained similar before and after the

installation of the diffuser even though temperatures in July 2010-2012 were $0.8^{o}C$ higher than

July 2006-2008. In addition, temperatures appeared to increase more quickly in July and decline

more rapidly after the installation according to trend analysis, which correspond to the operation

of the diffuser. Although there is some evidence to suggest that the diffuser system is functioning

properly, the results from this study indicate that stream temperatures in the Muskegon River do

not appear to have improved since the installation of the diffuser and additional efforts are

needed in order to alleviate high summer water temperatures.

INTRODUCTION

Water temperature is arguably the most important physical property of streams and

rivers. In addition to temperature influencing many of the physical and chemical characteristics

of water, water temperature is an important factor influencing fish populations, especially

salmonids (Torgersen et al. 1999). At the individual level, water temperature influences the

metabolism, growth, and microhabitat selection of a fish (Beer and Anderson 2011; Baltz et al.

1987). At the population level, temperature influences the distribution of a fish in an ecosystem

(Torgersen et al. 1999) and ultimately their viability (Li et al. 1994).

Steelhead (*Oncorhynchus mykiss*) were introduced to the Great Lakes in the late 1800s

and have become an important component in the Great Lakes sport fishery. Although naturally

reproducing steelhead populations are well established in some of the Great Lakes tributaries

(Seelbach 1993; Woldt and Rutherford 2002), the general lack of suitable spawning and nursery

habitat leaves the steelhead fishery dependent on hatchery supplementation (Rand et al. 1993).

Similar to their native range, natural steelhead recruitment in Great Lakes tributaries may be

limited by hydroelectric dams. In addition to fragmenting spawning and nursery habitats, many

dams in the Great Lakes region are top withdrawal dams and pass warm surface water from the

reservoirs to downstream reaches which can result in increased summer water temperatures and

decreased juvenile steelhead survival (Godby et al. 2007; Lessard and Hayes 2003; Woldt and

Rutherford 2002).

Questions about natural steelhead recruitment lead to a study that was conducted from

1998 to 2001 by Godby et al. (2007) on the Muskegon River, a tributary to Lake Michigan. The

purpose of their study was to investigate factors regulating age-0 steelhead production in a large

regulated river and a connecting tributary creek that is characteristic of many Great Lakes

watersheds. Results of this study indicated that high summer temperatures caused by Croton

Dam were severely limiting natural steelhead production. Subsequently, an "upwelling" system

was designed and installed during the summer of 2008 on Croton Dam as part of a $1.75 million

commitment by Consumers Energy to address temperature and dissolved oxygen issues

associated with hydropower dams on the Manistee, Muskegon, and Au Sable Rivers. By

installing the "upwelling" system, Consumers Energy hoped to address those concerns by

upwelling colder bottom water behind the dam with oxygen to help alleviate elevated water

temperatures and low dissolved oxygen levels downstream of hydropower dams. Given the

impact water temperature can ultimately have on the survival and distribution of stream fishes

like juvenile steelhead, the goal of this project was to evaluate the success of the "upwelling"

system at alleviating high summer water temperatures on the Muskegon River below Croton

Dam.

METHODS

Study Area

The Muskegon River is one of the largest tributaries to Lake Michigan with a

contributing watershed of over 5,900 km^2 (O'Neal, 1997). The Muskegon River has a moderate

gradient of 2-5 m/km and mixed substrate that is primarily composed of gravel, cobble, and sand

(Ichthyological Associates, 1991). There are three major impoundments on the Muskegon River:

Croton, Hardy and Rogers dams. Croton Dam serves as the upstream barrier for migration of

adfluvial salmonids, effectively limiting steelhead spawning and rearing habitat 72 rkm below

the dam.

Croton Dam creates a 489-hectare reservoir with a maximum depth of 12.192 meters (40

feet). The Croton upwelling system has two large diffusers, which can be operated separately or

in tandem. The first diffuser is located in relatively shallow water near the turbine inlets and is

designed to aid in upwelling the cooler water into the turbines. The second diffuser is located

further from the dam in deeper water and is designed to upwell the colder water from the deeper

reaches of Croton Pond into the shallower area near the turbine inlets. The diffusers were

installed in the fall of 2008 with initial testing beginning in July 2009 (Consumers Energy 2010).

Temperature and Diffuser Analysis

This study is based on the statistical analysis and direct observations of the hourly water

temperature data that were available from the U.S Geological Survey (USGS) for the Muskegon

River directly below Croton Dam. Stream temperatures from 2006 to 2008 were used to evaluate

water temperatures prior to the installation of the diffuser, while stream temperatures from 2010

to 2012 were used to evaluate water temperatures after the diffuser was installed and fully

operational. Water temperature data from 2008-2009 were not included in this study because the diffuser was still being installed and tested. In addition to the water temperature data, air temperature data from the Roben-Hood Airport located approximately 40km from Newaygo, Michigan in Big Rapids was used to visualize whether there were any differences in air temperature before and after the installation of the diffuser.

The impact of the Croton Dam diffuser at alleviating high summer water temperatures was evaluated by first visualizing and summarizing temperature before and after the installation of the diffuser and then by a Seasonal Kendall test. The visual and summary temperature metrics incorporated these temperature metrics: mean monthly average temperature (MMAT), mean monthly maximum temperature (MMT_{max}), mean monthly minimum temperature (MMT_{min}), mean daily temperature range (MDTR), monthly maximum temperature (Max), and monthly minimum temperature (Min). The second technique used was the use of the Seasonal Kendall test, which was developed by the USGS in the 1980s to analyze trends in surface-water quality data throughout the United States (Helsel et al. 2005). While this technique is not intended for exploring the hypothesis that change has occurred at some predetermined time, it is used for detecting monotonic trends or change (gradual or sudden) during some interval of time (Hirsch et al. 1962). In this study, the Seasonal Kendall test was used to visualize the monotonic trends in mean, maximum, minimum, and temperature ranges from 2006- 2008 (i.e., pre-diffuser installation) and 2010-2012 in July and August to determine whether there were significant trends in water temperature and if those trends differed prior to and after the installation of the diffuser. All Seasonal Kendall tests were done using the program available from USGS (http://pubs.usge.gov/sir/2005/5275/downloads/). Significance was determined at $P \leq 0.05$.

RESULTS

Mean air temperature data in Big Rapids, Michigan from July of 2006-2008 and 2010-2012 in July ranged from 19.5^0C to 21.5^0C and 22.0^0C to 23.4^0C, respectively (Table 2.1). In August, mean air temperatures ranged from 19.5^0C to 20.8^0C and 19.4^0C to 21.4^0C before and after the installation of the diffuser, respectively.

Stream temperatures in the Muskegon River in the months of July and August from 2006-2008 and 2010-2012 ranged from 19.1^0C to 24.9^0C and 18.6^0C to 25.2^0C, respectively (Table 2.2). The MMAT, MMT_{max}, MMT_{min}, and MDTR in July were higher from 2010 to 2012 than from 2006 to 2008 (Figure 2.1). Despite this difference in the July stream temperatures measurements, MMAT, MMT_{max}, MMT_{min}, and MDTR, the same temperature metrics from 2006-2008 and 2010-2012 did not differ by more than 0.1^0C from the same measurements in 2006-2008 measurements (Table 2.2).

Results from the seasonal Kendall test (Table 2.3) indicate that trends in water temperature were not uniform in 2006-2008 and 2010-2012, except for the range in the daily water temperature. Significant increases in mean, maximum, and minimum daily water temperatures were detected during July both prior to and after the installation of the Croton Dam diffuser. Prior to installation of the diffuser, the slope of the trend for mean, maximum, and minimum water temperatures in July were 0.4215, 0.4218, and 0.4377, respectively compared to 0.6438, 0.7068, and 0.6234 from 2010 to 2012. In August, a significant decrease in mean, maximum, and minimum daily water temperatures were detected from 2006-2008 and 2010-2012. From 2006-2008, the slope of the trend for mean, maximum, and minimum daily

temperatures were -0.3182, -0.2778, and -0.3046, respectively, compared to -0.5274, -0.5208, and -0.5618, respectively from 2010-2012.

Table 2.1. Air temperature data (^{0}C) recorded at the Roben-Hood Airport in Big Rapids, Michigan (#14864)

Location	Year	Month	Mean	Max	Min
Prior to Installation of the Diffuser					
Big Rapids	2006	July	21.5	34.0	7.0
	2006	August	19.5	34.0	5.0
	2007	July	19.6	34.0	5.0
	2007	August	19.9	34.0	3.0
	2008	July	20.8	32.0	5.0
	2008	August	19.5	31.0	5.0
Average		July	20.6	33.3	5.7
		August	19.6	33.0	4.3
After Installation of the Diffuser					
Big Rapids	2010	July	22.0	31.0	4.0
	2010	August	21.4	32.0	6.0
	2011	July	22.2	33.0	7.0
	2011	August	19.4	32.0	6.0
	2012	July	23.4	36.6	10.8
	2012	August	19.5	32.6	6.5
Average		July	22.5	33.5	7.3
		August	20.1	32.2	6.2

Table 2.2. Muskegon River temperature data (^{0}C) recorded at the USGS gauging station near Croton Dam (Gauge #04121970).

Location	Year	Month	MMAT	MMT$_{max}$	MMT$_{min}$	MDTR	Max	Min
Prior to Diffuser Installation								
Croton	2006	July	21.2	21.9	20.6	1.3	23.3	21.9
	2006	August	22.7	23.2	22.2	1	24.9	23.2
	2007	July	21.6	22.3	21	1.3	23.9	20.1
	2007	August	22.2	22.7	21.6	1.1	24.6	20
	2008	July	21.4	22.1	20.8	1.3	24.5	19.1
	2008	August	22.4	23	21.8	1.2	24.4	21.2
Average								
		July	21.4	22.1	20.8	1.3	23.9	20.4
		August	22.4	23	21.9	1.1	24.3	21.5
After Diffuser Installation								
Croton	2010	July	21.8	22.6	21.1	1.5	24.5	19.1
	2010	August	22.7	23.3	22.3	1	24.5	21.4
	2011	July	21.9	22.6	21.2	1.4	25	18.6
	2011	August	22.4	23	21.9	1.1	24.6	21
	2012	July	22.9	23.7	22.1	1.6	25.2	20.5
	2012	August	21.9	22.5	21.3	1.2	24.5	20.3
Average								
		July	22.2	23	21.5	1.5	24.9	19.4
		August	22.3	22.9	21.8	1.1	24.5	20.9

MWAT = mean monthly average temperature; *MMTmax* = mean monthly maximum temperature; *MMTmin* = mean monthly minimum temperature; *MDTR* = mean daily temperature range; *Max* = maximum monthly temperature; *Min* = minimum monthly temperature
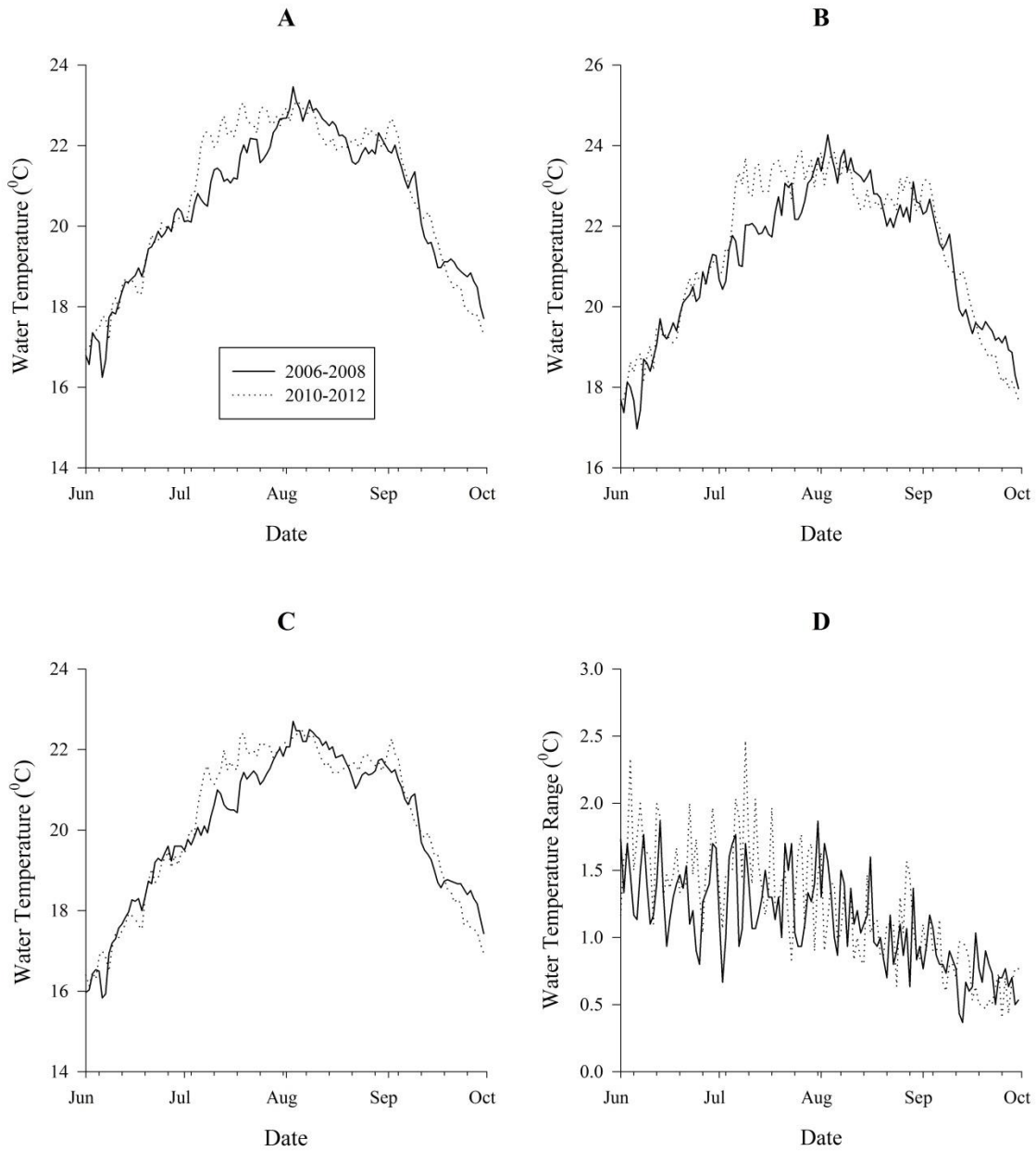
Figure 2.1. Water temperature data (°C) at Croton Dam for 2006-2008 (pre-diffuser installation) and 2010-2012 (post-diffuser installation); (**A**) mean daily temperature, (**B**) mean maximum daily temperature, (**C**) mean minimum daily temperature, and (**D**) mean daily temperature range

Table 2.3. Trends in water temperature in July and August from 2006-2008 and 2010-2012 based on application of the seasonal Kendall test and the slope estimator to daily values.

Month	Measurement	Slope of Trend	T	S Value	Z	Significance of Trend
		Prior to Diffuser Installation				
July	Mean	0.4215	0.236	1011	3.352	0.0008
	Maximum	0.4218	0.196	840	2.787	0.0053
	Minimum	0.4377	0.268	1148	3.813	0.0001
	Range	0.0000	0.024	102	0.337	0.7364
August	Mean	-0.3182	-0.281	-1200	-3.980	0.0001
	Maximum	-0.2778	-0.189	-809	-2.687	0.0072
	Minimum	-0.3046	-0.301	-1287	-4.278	0.0000
	Range	0.0509	0.082	350	1.162	0.2453
		After Diffuser Installation				
July	Mean	0.6438	0.446	1906	6.323	0.0000
	Maximum	0.7068	0.379	1620	5.378	0.0000
	Minimum	0.6234	0.456	1949	6.474	0.0000
	Range	0.0000	0.052	221	0.732	0.4641
August	Mean	-0.5274	-0.328	-1405	-4.660	0.0000
	Maximum	-0.5208	-0.283	-1212	-4.402	0.0001
	Minimum	-0.5618	-0.386	-1651	-5.487	0.0000
	Range	0.0000	0.050	212	0.703	0.4820

DISCUSSION

The objective of this study was to determine the success of the "upwelling" system at alleviating warm summer water temperatures on the Muskegon River below Croton Dam. Based on the results from this study, stream temperatures in the Muskegon River have remained relatively warm since the installation and operation of the diffuser. In fact, based on the summary of the mean monthly average temperature, July temperatures were slightly warmer (0.8°C) during 2010-2012 while temperatures in August remained similar to temperatures for both periods. In addition, trend analysis (Table 2.3) suggests that temperatures appeared to increase more quickly in July following the installation of the diffuser. Thus, it appears that the volume of hypolimnetic water generated by the diffuser was not sufficient to reduce warm summer water temperatures during the period used for analysis.

Several general factors may partially account for the trends I observed in these data. For example, the relatively small data set of three years before and after the installation of the diffuser might not be large enough to take into account the yearly and seasonal variations in temperature. In addition, the warmer mean air temperatures observed in July and August after the diffuser system could have concealed a decrease in water temperature as a result of the diffuser installation. Furthermore, a decrease in July and August stream water temperature might not have been realized as a result of dam operation related to the hydrologic conditions: an increase in the volume of surface water discharged from Croton Dam due to reservoir volumes may nullify the benefits of the diffuser.

Based on the average daily temperature and the trend analysis, July stream temperatures downstream of Croton Dam have not improved since the installation of the diffuser. However,

water temperatures and trend analysis of August data suggest that diffuser operation may be lowering stream temperatures and with modifications could make a more substantial impact on stream temperatures in the downstream reaches. For example, despite higher stream temperatures in July after the diffuser installation, August stream temperatures dropped quickly to temperatures comparable to 2006-2008 and the August trend analysis indicates temperatures declined more rapidly than before the diffuser was installed. While this could be the result of yearly variation in temperature and hydrologic conditions, the more rapid decline in stream temperatures observed after the diffuser was installed corresponds to diffuser operational procedure and could be the result of how frequently the diffuser was in operation. Although these trends are encouraging, future operation criteria may need to consider hydrologic conditions (discharge from the dam) along with stream temperatures to design an operational procedure that will maximize the effectiveness of the diffuser system at alleviating warm summer water temperatures downstream of Croton Dam.

Unlike the diffuser at Hodenpyl Dam on the Manistee River, the diffuser at Croton Dam has a smaller volume of cool water to draw from because the Croton pond is shallower than the Hodenpyl Dam pond (Consumers Energy 2010). As a result of the limited amount of cool water available, the diffuser system at Croton has a different set of activation conditions than the Hodenpyl Dam diffuser. For activation of the Croton Dam diffuser, the average daily outflow temperatures must be 22.2°C (72°F) in the month of July and 21.1°C (70°C) for the month of August (Consumers Energy 2010). The 22.2°C activation temperature meant the diffuser would not be operating for the entire month of July during 2010-2012, which corresponds to higher stream temperatures than observed from 2006-2008. When comparing the August stream temperatures before and after the installation of the diffuser, temperatures were similar despite

warmer July temperatures from 2010-2012. While this could be the result of other factors, it also corresponds to when the diffuser was operating more frequently because of the lower activation temperature.

Despite efforts to alleviate high summer waters temperatures below Croton Dam, summer stream temperatures in the Muskegon River remain similar to stream temperatures documented prior to the diffuser installation and are outside the optimum temperature ranges of 12.8^0C to 15.5^0C for juvenile steelhead and fry (U.S. Fish and Wildlife Service 1995). Although the diffuser has shown the ability to reduce water temperatures by 0.72^0C in initial testing (Consumers Energy 2010), this reduction is not substantial enough to provide the necessary thermal habitat quality needed to support a naturally occurring steelhead population that is not dependent on hatchery supplementation.

In order to decrease stream temperatures so they are suitable for steelhead survival, further efforts may need to be implemented to reduce summer stream temperatures. This could be accomplished by including discharge as an activation criterion, lowering the activation temperature of the diffuser, or by running the diffuser for longer periods. However, extended operation would probably require dredging the reservoir to create a larger volume of colder water that could be used to lower stream temperatures. While these efforts could be substantial enough to make an immediate impact on stream temperatures, additional restoration efforts and monitoring are needed in order to protect and maintain the Muskegon River from climatic change.

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Chapter 3

Growth, Survival, and Production of Juvenile Steelhead in the Muskegon River, Michigan

ABSTRACT

Steelhead (*Oncorhynchus mykiss*) are an important component of the Great Lakes sport fishery. Although significant natural reproduction is occurring in some Lake Michigan tributaries, natural recruitment is often limited by hydropower dam operations. In 2011-2013, I investigated age-0 natural steelhead abundance, survival, and production in the mainstream of the Muskegon River and Bigelow Creek, a free-flowing coldwater tributary of the Muskegon River. The objective of this study was to investigate natural steelhead production in the Muskegon River to determine whether the diffuser system installed on Croton Dam has helped alleviate the high summer water temperatures that were previously found to be severely limiting juvenile steelhead survival. Pass depletion surveys were used to estimate parr survival and growth based on changes in fish weight and density between sampling periods in July and October. Average fall density of parr in Bigelow Creek was 48-fold higher than in the Muskegon River. Average summer daily mortality rate of parr in the Muskegon River was nearly six-fold higher than in Bigelow Creek. High mortality rates in the Muskegon River corresponded to average summer water temperatures exceeding 21^0C. These results were similar to previous work completed on the Muskegon River and suggest that natural steelhead production in the Muskegon River is still severely limited due to high summer water temperatures.

INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is a species of salmonid native to tributaries of the Pacific Ocean. Their native range extends from the Kamchatka Peninsula (Russia) eastward to Alaska and as far south as Mexico. Within this range they display highly variable and plastic life history strategies that include two major life history strategies; resident and anadromous forms (Raleigh 1984). The anadromous form of rainbow trout, commonly known as steelhead, has a life expectancy of 4 to 8 years (Raleigh 1984). The steelhead life cycle begins in freshwater streams where adults will return to spawn after maturing in the ocean. Following spawning, many of the adult steelhead will die, but some return to the ocean and survive to spawn again.

The incubation time for steelhead eggs in the gravel is dependent on temperature, but will generally take 3 to 4 weeks to hatch with temperatures from 10 - 15^{0}C (Moyle 2002). After hatching, the steelhead alevins remain in the gravel for 2 to 3 weeks before emerging. Following emergence, steelhead fry usually live in small schools in the shallow water along stream banks. As the steelhead grow, the schools break up and they establish individual feeding territories. Most steelhead parr will live in riffles zones during their first year, but larger parr will reside in deep pools and runs (U.S. Fish and Wildlife Service 1995). In the river, they will predominantly feed on aquatic and terrestrial insects and other small invertebrates. The optimum temperature range for juvenile steelhead and fry is 12.8^{0}C to 15.5^{0}C, with temperature ranges of 15.6^{0}C to 20^{0}C, 20.1^{0}C to 22.5^{0}C, and greater than 22.5^{0}C corresponding to thermal stress levels of low, medium, and high, respectively (U.S. Fish and Wildlife Service 1995). Juvenile steelhead will reside in freshwater for 1 to 4 years before migrating out to the ocean as smolts.

Although steelhead is an anadromous form of rainbow trout in its native range, they have been successfully introduced to freshwater systems including the Great Lakes for sport fishing. The first documented introduction of steelhead into the Great Lakes occurred in the late 1800s (MacCrimmon 1972). Supplementation efforts continued until the 1920s when naturalized populations became well established around Lake Michigan with most natural reproduction occurring in Michigan tributaries due to the abundance of suitable spawning habitat (MacCrimmon 1972). Additional supplementation efforts in Lake Michigan did not occur again until the 1950s following a decline in the lake-wide steelhead population as a result of overfishing, the population explosion of parasitic sea lampreys (*Petromyzon marinus*), and the unintentionally introduction of alewives (*Alosa pseudoharengus*), which depleted food sources and altered the food web (MacCrimmon 1972). During this time, hatchery supplementation primarily utilized the Michigan winter-run strain that is derived from adults returning to the Little Manistee River. While the Michigan strain historically has been used around the Lake Michigan basin, additional hatchery strains such as the summer-run Skamania strain from the Washougal River in Washington have been used since the mid-1980s to take advantage of strain-specific variation in life history characteristics (i.e., run timing) that are valued by recreational anglers. As a result of their introduction and subsequent hatchery supplementation efforts, steelhead have become an important component in the Great Lakes sport fishery, which is valued at approximately $7 billion annually (American Sportfishing Association 2008).

Although significant effort has been put into supplementing natural reproduction with hatchery produced steelhead, their relative contribution to populations did not significantly increase until 1983 when hatchery management practices changed and began stocking large yearlings (>150 mm) instead of small fall fingerlings (<110 mm)(Seelbach 1987). This change

resulted in an increase in hatchery-raised steelhead surviving to the smolt stage (Rand et al. 1993) and helped increase the contribution of hatchery steelhead to spawning runs from 0-30% (Seelbach and Whelan 1988) to 13-79% (Barton and Scribner 2004) in six Michigan rivers during the 1998-1999 spawning run. Currently, the steelhead fishery is maintained primarily through hatchery supplementation, but significant natural reproduction occurs in some Lake Michigan tributaries with optimal thermal habitat like the Little Manistee River and Pine Creek (Seelbach 1993; Woldt and Rutherford 2002). In Lake Michigan, natural origin steelhead comprise approximately 20-30% of adult steelhead caught in the sport fishery (Rand et al. 1993).

While natural reproduction is occurring, and is important to managing the sport fishery, little is known about the factors regulating natural recruitment in marginal habitats. In a study by Newcomb and Coon (1997) on the Betsie River and its connecting tributaries, they found small tributaries contributed disproportionally to overall production of steelhead parr in the Betsie River watershed. They found that the abundance and survival of steelhead parr in these connecting tributaries was higher than that of the Betsie River due to the more favorable thermal habitat quality. In another study, Woldt and Rutherford (2002) investigated the production and survival of steelhead parr in the large regulated Manistee River and compared those values with those for parr in the adjacent free-flowing Little Manistee River. Results from that study indicated that survival and production of steelhead parr in the Manistee River was significantly lower than in the Little Manistee River as a result of high summer water temperatures.

Questions about natural steelhead recruitment in the Muskegon River led to a study that was conducted from 1998 to 2001 by Godby et al. (2007). The purpose of this study was to investigate factors regulating age-0 steelhead production in a large, regulated river and a connecting tributary creek that is characteristic of many Great Lakes watersheds. Results from

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this study indicated that high summer temperatures caused by surface water release from Croton Dam were severely limiting natural steelhead production. Subsequently, a diffuser system was designed and installed during the summer of 2008 on Croton Dam as part of a $1.75 million commitment by Consumers Energy to address temperature and dissolved oxygen issues associated with hydropower dams on the Manistee, Muskegon, and Au Sable rivers. By installing the diffuser systems, Consumers Energy hoped to address those concerns by upwelling colder bottom water behind the dam with oxygen to help alleviate elevated water temperatures and low dissolved oxygen levels downstream of hydropower dams. Increases in natural reproduction of anadromous salmonids and abundance of age-0 salmonids have been linked to changes in hydropower dam operations in other studies (Raymond 1988). Changes in hydropower dam operations have improved salmonid nursery habitat, increased availability to spawning areas, and subsequently increased natural recruitment and production of salmonids downstream of hydropower dams (Horne et al. 2004; Raymond 1988; Woldt and Rutherford 2002).

This study was initiated to determine current abundance, survival, and production of age-0 steelhead in the Muskegon River and Bigelow Creek, and if there has been a population-level response in juvenile steelhead survival and production since a diffuser system was installed at Croton Dam. I used two methods to assess the response. First, I compared the Muskegon River populations to a reference site (Bigelow Creek). Second, I compared data from this study to a similar study conducted by Godby et al. (2007). In doing so, the primary objectives of this study were to quantify the current abundance, survival, growth, and production of naturally produced juvenile steelhead in the Muskegon River and Bigelow Creek and compare that to previous work conducted on the Muskegon River to assess the impact the diffuser system has had on the juvenile steelhead population since its installation.

METHODS

Study Area

The Muskegon River is one of the largest tributaries to Lake Michigan with a contributing watershed of over 5,900 km^2 (O'Neal, 1997). The Muskegon River has a moderate gradient of 2-5 m/km and mixed substrate that is primarily composed of gravel, cobble, and sand (Ichthyological Associates, 1991). There are three major impoundments on the Muskegon River: Croton, Hardy and Rogers dams. Croton Dam serves as the upstream barrier for migration of adfluvial salmonids. My study reach included the primary spawning and nursery habitats for salmonids, which extended approximately 22.5 km from Croton Dam downstream to Newaygo, Michigan (Figure 3.1).

Sampling sites also were located in Bigelow Creek, which served as reference sites for this study and corresponded to sites used by Godby et al. (2007). Bigelow Creek is a small, free-flowing cold-water tributary of the Muskegon River, which enters upstream from the city of Newaygo. Bigelow Creek is 12.1 km long with an average width of 5.3 m, and has a contributing watershed of 44.9 km^2 (O'Neal 1997). Stream gradient is moderate and substrate is primarily composed of sand and gravel (Godby et al. 2007).
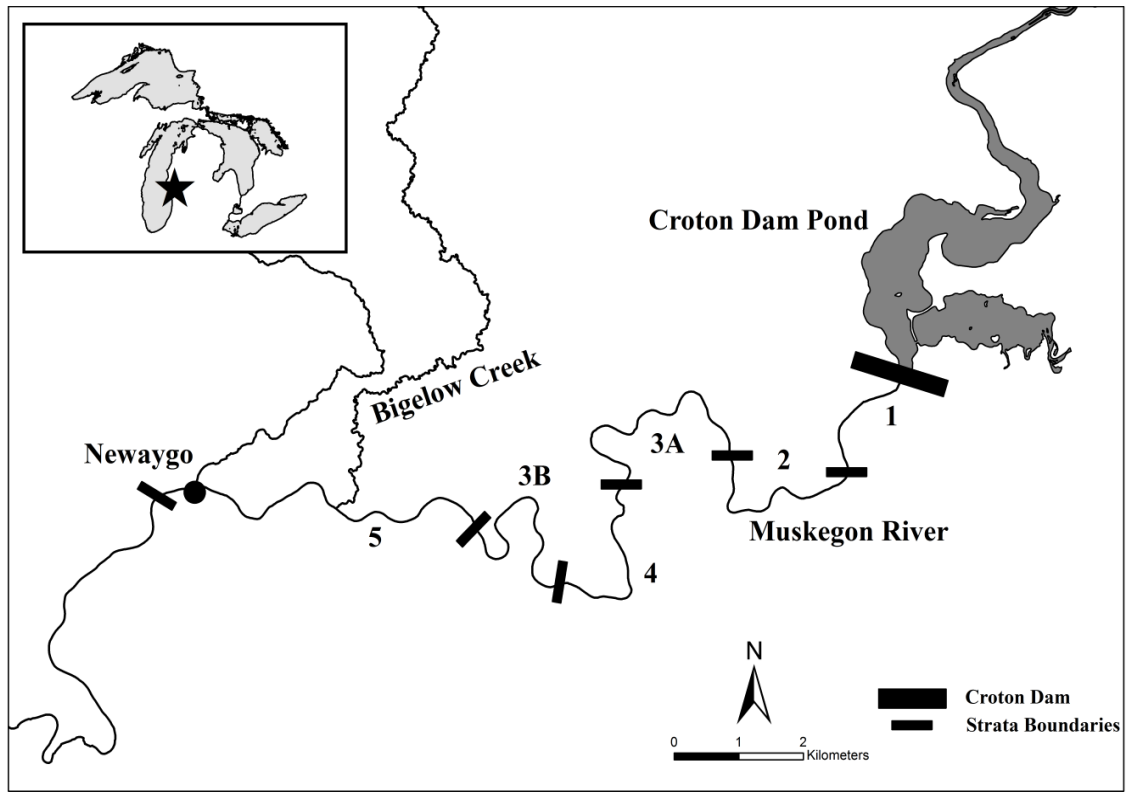
Figure 3.1. Map of the study area in the Muskegon River and Bigelow Creek, Michigan, showing locations of the five strata.

Sampling design

Abundance and density of age-0 steelhead in the Muskegon River were estimated using a stratified random sampling design following the protocol and methodology of Godby et al. (2007). The Muskegon River from Croton Dam downstream to Newaygo was previously divided into five strata based on a multivariate analysis of in-stream substrate composition, hydrology, and riparian zone composition (Figure 3.1; Ichthyological Associates 1991). Each stratum was divided into 100-m long by 3-m wide shoreline segments. In order to be a potential survey site, the site had to be wadeable throughout and be at least 30m away from other sites. Sites were then randomly selected from each of the five strata. A total area of 9,300 m^2 was sampled in the Muskegon River with 12 sites in stratum 1, 9 sites in stratum 2, 4 in stratum 3, 2 from stratum 4, and 4 sites from stratum 5, which corresponded to the number of sites in each stratum used by Godby et al. (2007). The number of sites chosen in each stratum was based on the dominate substrate type present and weighted based on the perceived amount of optimal spawning and rearing habitat for salmonids (Woldt and Rutherford 2002). In addition to the sites in the Muskegon River, two sampling sites were located in Bigelow Creek that corresponded to sites sampled by Godby et al. (2007).

Temperature

Temperature was monitored continously (1-h intervals) in 2011-2013 at one site at the mouth of Bigelow Creek using an Onset stowaway tibit temperature logger. Temperature records from the U.S. Geological Survey gauging station, located just downstream of Croton Dam, were used to monitor temperature on the Muskegon River.

Density, abundance, and survival

Density and abundance of age-0 steelhead parr were estimated for three cohorts in July and October of 2011, 2012, and 2013 for each of the 31 sites in the Muskegon River and the 2 sites in Bigelow Creek. Each site was sampled using a 250-300 volt DC barge electrofishing unit. Parr density was estimated using the Moran-Zippan two-pass depletion method for unblocked 300-m^2 shoreline segments (Everhart and Young 1981); additional passes were made at some sites to achieve approximately two-thirds depletion. These methods assume that effort is constant between passes, the population being sampled is closed (no immigration or emigration), and that the chance of capture is equal for all fish and constant from sample to sample. In an effort to meet those assumptions, sampling effort was kept constant by using the same equipment for the duration of the experiment and immigration and emigration were limited by allowing as little time as possible between passes. Shoreline segments were not blocked off with nets owing to the size of the river being sampled and the desire to repeat the methodology used by Godby et al. (2007) to allow direct comparisons. The abundance for each site was converted to density (number of fish/hectare) and then expanded to mean stratum density and population mean density using a stratified sampling equation (Scheaffer et al. 1996). The capture probability for the pass depletions method averaged 70% for age-0 steelhead over all surveys where steelhead were present. There were no significant differences in capture probability among strata within the main stem (Kruskal-Wallis test: $\chi^2 = 5.89$, $P = 0.117$), or between Bigelow Creek and the Muskegon River (Kruskal-Wallis test: $\chi^2 = 2.87$, $P = 0.090$).

Percent survival of steelhead parr was calculated as $S = N_{t+1}/N_t$, where N_t and N_{t+1} are densities of steelhead at times t and $t+1$, respectively. In addition to percent survival, the instantaneous daily mortality rate (Z) of steelhead parr was calculated using the equation $Z=ln(S)/d$, where d represents the number of days between sampling. Changes in parr density over the course of sampling a cohort from summer to fall represent a loss in the number of steelhead due to immigration, emigration, and mortality. While my survival and mortality estimates include immigration and emigration, I assume that immigration and emigration rates were low among the different strata and streams between summer and fall sampling periods based on other studies in the Great Lakes that focused on movement and density of age-0 steelhead parr (Sheppard and Johnson 1985; Woldt and Rutherford 2002).

Growth, Condition, and Production

Growth was estimated from changes in length and weight of the cohort between sampling periods. Length (mm) and wet weight (g) were determined for up to 30 fish at each site during the summer and fall of each year. For the 2011 cohort, fork length (FL, mm) was used to measure juvenile steelhead length while in 2012 and 2013 total length (TL, mm) was used for length. Due to the different length measurements, regression techniques were used to develop a linear equation between FL and TL measurements using 112 juvenile steelhead kept for diet analysis throughout the study (Ramseyer 1995). The FL to TL conversion equation for age-0 steelhead parr was *TL = -0.84 + 1.07(FL)*. Based on the high regression coefficient obtained (r^2 > 0.99), I am confident that my estimates of total length from fork length measurements in 2011 are reliable when this linear equation is used. Instantaneous growth rate in weight (G) was

estimated by $G = (lnW_{t+1} - lnW_t)/d$, where d is the number of days between samples and W_t and $W_{t+}1$ represent the mean weight at times t and $t+1$, respectively.

Condition of juvenile steelhead were estimated during different sampling periods using length-weight relationships of up to 30 fish that were measured for length (mm) and wet weight (g) at each site. This was done by fitting a linear regression model to the logarithmically transformed data and using the regression coefficient as an indicator of the population condition (Guy and Brown 2007). A slope of three indicates isometric growth and values less than or greater than three indicate fish are in better or worse condition, respectively. Due to the different length measurements in 2011, I used the linear equation to convert FL to TL in 2011 in order to make comparisons based on condition between years.

Production estimates were made using the Allen (1971) method and by also calculating the $G{:}Z$ ratio. The $G{:}Z$ ratio is a relative index of production and is calculated by dividing the instantaneous growth rate (G) by the instantaneous mortality rate (Z). The Allen (1971) method involves using the relationship between cohort biomass and cohort density over time. For this method the $\log_e$ transformed steelhead density was regressed against the average wet weight of individuals, and production (area under the curve) was calculated using the formula (Pitcher and Hart 1996);

$$P = \int_{w0}^{wt} D_i dw,$$

where P is production, w_0 is average weight at time 0, w_i is average weight at time t, D_i is density at time i, and dw is the derivative of average weight. The area under the curve of cohort biomass

and population size represented total production in grams per hectare, which was then converted to grams per square meter.

Statistical analyses

Parametric tests were used to compare differences in mean parr density, and fish length and weight among streams and years when samples were normally distributed and had equal variances. I choose not to compare differences among strata in the Muskegon River as a result of low parr abundance in the lower strata. Parr density was averaged over all sites within strata and these estimates were combined using a weighted approach for the Muskegon River estimates to account for the difference in sampling effort between strata (Scheaffer et al. 1996). The annual point estimates of survival, growth, instantaneous mortality, and production for each year were averaged for each river. Paired comparisons of density, length, and weight between the Muskegon River and Bigelow Creek were calculated using paired *t*-tests. The nonparametric Wilcoxon signed-rank test was used when assumptions of normality were not met. All significance was determined at $P < 0.05$.

RESULTS

Temperature

Average summer water temperatures were higher in the Muskegon River than in Bigelow Creek from 2011-2013 (Table 3.1). Average daily temperatures in the Muskegon River in 2011-2013 ranged from 18.6°C to 25.2°C in July and declined to 10.3-18.9°C in October. Over the same period, temperatures in Bigelow Creek declined from 16.3-23.3°C in July to 5.3-14.9°C in October. In addition warmer stream temperatures, average temperatures remained above 20^0C for much longer periods of time in the Muskegon River than in Bigelow Creek. In 2012, average daily temperatures exceeded 20.1^0C for 88 consecutive days in the summer on the Muskegon River (June 21st to September 17th) compared to only 4 consecutive days in Bigelow Creek (July 4th to July 7th). Similar trends in elevated temperatures also were observed in the Muskegon River in 2011 and 2013, with average daily temperatures exceeding 20.1^0C for 74 and 71 consecutive days, respectively.

Table 3.1. Monthly temperature data (^{0}C) for the Muskegon River and Bigelow Creek from

2011-2013.

Year		2011			2012			2013		
	Month	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
Muskegon	June	18.3	20.4	15.9	19.0	22.7	15.7	18.6	22.7	15.5
River	July	21.9	25.0	18.6	22.9	25.2	20.5	21.8	24.7	19.7
	August	22.4	24.6	21.0	21.9	24.5	20.3	21.2	23.5	19.7
	September	19.6	22.9	16.3	20.0	24.0	16.9	20.7	22.8	17.8
	October	14.4	17.7	10.3	13.9	18.2	10.3	14.3	18.9	11.2
Bigelow	June	-	-	-	16.6	21.5	11.1	-	-	-
Creek	July	19.7[a]	22.7[a]	16.3[a]	19.4	23.3	16.3	15.7	19.0	12.8
	August	17.2	21.5	14.0	16.6	20.8	12.8	16.0	19.3	12.0
	September	13.9	19.3	9.4	13.5	18.4	8.5	14.0	18.7	9.7
	October	10.0	13.7	5.3	9.8	14.9	6.5	10.3	15.5	5.6

[a]Temperature measurements only include temperature data from July 19-31due to the temperature logger not being deployed until the 19th.
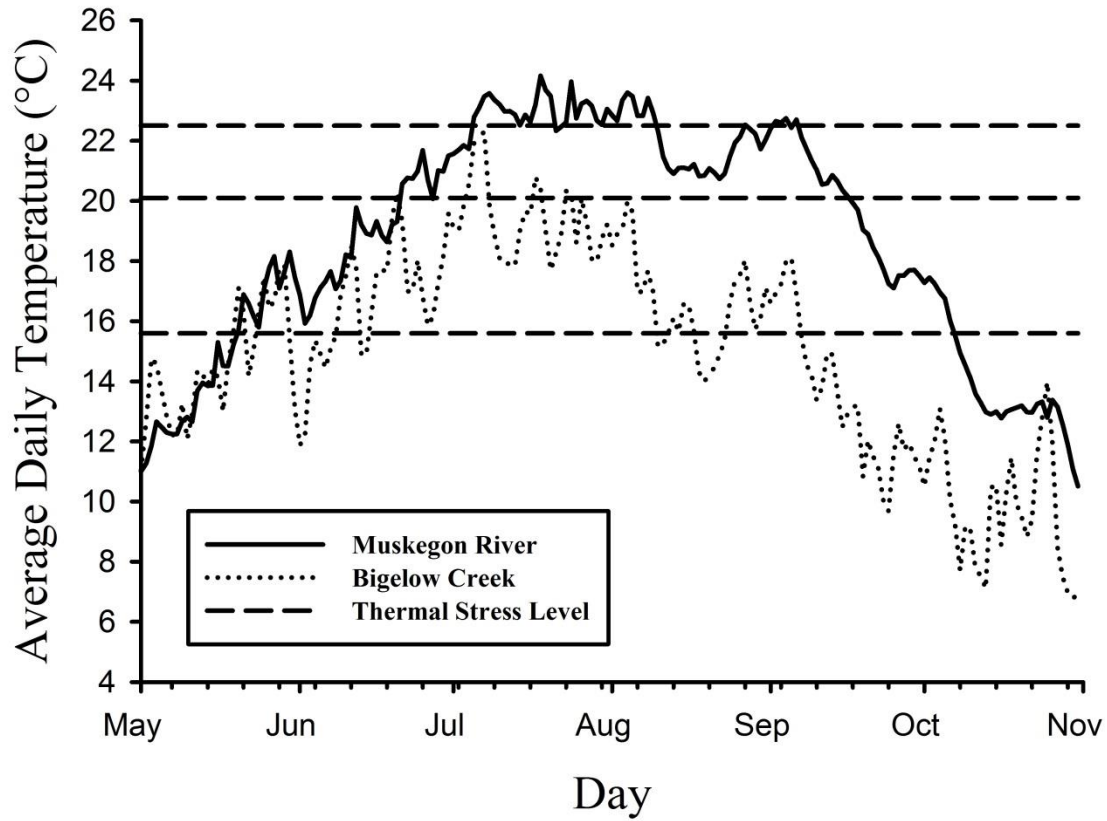
Figure 3.2. Mean daily temperature in the Muskegon River and Bigelow Creek, Michigan during 2012. Horizontal lines delineate the temperature ranges of 15.6^0C to 20^0C, 20.1^0C to 22.5^0C, and greater than 22.5^0C, which correspond to thermal stress levels of low, medium, and high for juvenile steelhead determined by the US Fish and Wildlife Service (1995).

Density

Mean densities of age-0 steelhead parr were significantly lower in the Muskegon River than Bigelow Creek in 2011, 2012, and 2013 during all sampling periods (t = -4.19, df = 5, P = 0.01) (Table 3.2). Density of steelhead parr declined from an average of 1,094 ± 470 parr/ha (mean ± 2 SE) in July to 47 ± 19 parr/ha by October in the Muskegon River. In Bigelow Creek, the density of parr declined from an average of 4,518 ± 3,625 parr/ha in July to 2,253 ± 269 parr/ha by October. Average parr density was 4.1-fold higher in Bigelow Creek than in the Muskegon River in July and 48-fold higher by October. Spatial trends in parr density were relatively consistent during all three years with parr densities being higher in strata 1 and 2 in the Muskegon River (Table 3.2). In Bigelow Creek, the site near the mouth had the highest parr densities for the river.

Table 3.2. Average (±95% CI) population density (fish/ha) of age-0 steelhead parr in the Muskegon River and Bigelow Creek, Michigan for 2011-2013. Estimates were based on pass depletion techniques. Number of sites sampled in the Muskegon River ($n = 31$) and Bigelow Creek ($n = 2$) were across seasons and years.

Year	Month	Muskegon River Density	Bigelow Creek Density
2011	July	1,307 ± 1,903	1,940 ± 3,080
	October	42 ± 46	2,522 ± 4,368
2012	July	1,350 ± 574	8,014 ± 14,961
	October	65 ± 53	2,123 ± 4,179
2013	July	624 ± 319	3,600 ± 7,200
	October	34 ± 17	2,114 ± 3,627

Table 3.3. Average (±2SE) strata density (fish/ha) of age-0 steelhead parr in the Muskegon

River, Michigan. Number of sites sampled were constant within strata and across season (stratum

1, n = 12; stratum 2, n = 9; stratum 3, n = 4; stratum 4, n = 2; stratum 5, n = 4).

Year and Stratum	July-August	October
2011		
1	3,100 ± 4,908	89 ± 116
2	366 ± 387	22 ± 29
3	0 ± 0	0 ± 0
4	0 ± 0	17 ± 33
5	8 ± 17	0 ± 0
2012		
1	2,601 ± 1,324	130 ± 135
2	1,100 ± 885	48 ± 37
3	0 ± 0	0 ± 0
4	317 ± 500	17 ± 33
5	25 ± 17	0 ± 0
2013		
1	858 ± 534	81 ± 86
2	897 ± 821	4 ± 7
3	0 ± 0	0 ± 0
4	106 ± 144	0 ± 0
5	193 ± 341	17 ± 33

Survival and Mortality

Survival of juvenile steelhead during the summer was lower in the Muskegon River than in Bigelow Creek (Table 3.4). Parr survival was on average 16 times higher in Bigelow Creek than the Muskegon River from 2011 to 2013. Average instantaneous daily mortality rates of parr during the summer were higher in the Muskegon River than in Bigelow Creek (Table 3.4).

Table 3.4. Vital rates and production of age-0 steelhead parr in the Muskegon River and Bigelow Creek, Michigan, during summer (July-October) of 2011 - 2013. Vital Rates are instantaneous daily growth rate in weight (G; day^{-1}), fraction surviving (S), instantaneous daily mortality rate (Z; day^{-1}), unit less G:Z ratio, and production (g/m^2).

Year	G	S	Z	G:Z	Production
		Muskegon River			
2011	0.029	0.032	0.041	0.70	0.48
2012	0.021	0.048	0.036	0.58	0.40
2013	0.028	0.054	0.033	0.85	0.16
Average	0.026	0.045	0.037	0.71	0.34
		Bigelow Creek			
2011	0.027	1.300	-0.003	7.76	1.95
2012	0.015	0.265	0.014	1.02	2.17
2013	0.019	0.587	0.006	3.32	1.50
Average	0.020	0.717	0.006	4.03	1.87

Growth and Condition

Summer lengths and weights of parr were not significantly different between Bigelow Creek and Muskegon River in either 2011 or 2012 (Figure 3.3, 3.4). However, in 2013 summer lengths and weights of parr were significantly higher in Bigelow Creek than the Muskegon River ($t = 3.92$, df $= 33.4$, $P < 0.001$; $t = 3.28$, df $= 32.3$, $P = 0.002$, respectively (Figure 3.3, 3.4). In the fall of 2011, lengths and weights were higher in the Muskegon River than Bigelow Creek, but only statistically significant for lengths ($t = -2.24$, df $= 75.3$, $P = 0.028$; $t = -1.73$, df $= 75.6$, $P = 0.088$, respectively). Both lengths and weights were found to be significantly greater in the Muskegon River than Bigelow Creek ($t = -5.04$, df $= 64.2$, $P < 0.001$; $t = -2.91$, df $= 60.6$, $P = 0.005$, respectively) in the fall of 2012. In the fall of 2013, the average parr lengths and weights were 7.3 mm and 2.1 grams greater in the Muskegon River than Bigelow Creek, but this difference was not significant ($t = -1.60$, df $= 41.6$, $P = 0.12$; $t = -1.65$, df $= 40.4$, $P = 0.11$, respectively).

Comparing the overall parr weights in both streams between years, I found that summer parr weights were significantly different between years with parr weighing the most in 2012 and the least in 2013 (ANOVA: $F_{2,1099} = 119.14$, $P < 0.001$; post hoc Tukey's tests: all $P < 0.001$). While parr weighed more in the summer of 2012, fall parr weights tended to be higher in 2011 than 2012 and 2013, but only statistically significant for 2013 (ANOVA: $F_{2,225} = 5.9891$, $P = 0.003$; post hoc Tukey's tests between 2011-2012, 2011-2013, and 2012-2013 were $P = 0.136$, $P = 0.002$, and $P = 0.170$, respectively). Average instantaneous daily growth rate of parr did not appear to differ between the Muskegon River ($G = 0.026 \pm 0.005$ per day) and Bigelow Creek ($G = 0.020 \pm 0.007$ per day; Table 3.4).

In addition to comparing the lengths and weights of steelhead parr, weight-length relationships were also used to examine the annual variation in steelhead parr in the Muskegon River and Bigelow Creek. The estimated slope and intercept for $\log_{10}$-transformed weight-length relationship from Bigelow Creek in July 2011 were 2.825 and -4.722 ($r^2 = 0.92$) compared with 3.388 and -5.710 ($r^2 = 0.98$) in the Muskegon River (Figure 3.5A). In July 2012, the estimated slope and intercept for Bigelow Creek was 3.275 and -5.525 ($r^2 = 0.95$), respectively, compared to 3.109 and -5.220 ($r^2 = 0.97$) in the Muskegon River (Figure 3.5C). In July 2013, the estimated slope and intercept for Bigelow Creek was 2.834 and -4.797 ($r^2 = 0.91$), respectively, compared to 3.1608 and -5.349 ($r^2 = 0.89$) in the Muskegon River (Figure 3.5E). These equations suggest that an average parr of 50mm during July 2011, 2012, and 2013 would weigh 1.19g, 1.10g, and 1.04g in Bigelow Creek, respectively, compared to 1.11g, 1.16g, and 1.05g in the Muskegon River. Based on the regression results and analysis of covariance (Table 3.5), Bigelow Creek and Muskegon River had similar slopes but different intercepts, which indicate that there are similar trends in the summer weight (condition) relative to length and the weight of steelhead parr relative to a given length depends both on the stream and year (i.e. neither Bigelow Creek or Muskegon River steelhead parr are consistently heavier at a given length over all three years)

In October of 2011, the estimated slope and intercept for $\log_{10}$-transformed weight-length relationship from Bigelow Creek are 3.328 and -5.692 ($r^2 = 0.98$) compared with 3.118 and -5.243 ($r^2 = 0.98$) in the Muskegon River (Figure 3.5B). In October 2012, the estimated slope and intercept for Bigelow Creek was 3.063 and -5.086($r^2 = 0.92$), respectively, compared to 3.123 and -5.261($r^2 = 0.99$) in the Muskegon River (Figure 3.5D). In October of 2013, the estimated slope and intercept for transformed weight-length data from Bigelow Creek are 3.003 and -5.024 ($r^2 = 0.98$) compared with 3.118 and -5.247 ($r^2 = 0.99$) in the Muskegon River (Figure 3.5F).

These equations suggest that an average fish of 100mm during October 2011, 2012, and 2013 would weigh 9.19g, 10.96g, and 9.59g in Bigelow Creek, respectively, compared to 9.85g, 9.68g, and 9.75g in the Muskegon River. Based on the regression results and analysis of covariance (Table 3.5), data on juvenile steelhead length-weight relationships showed that fish in Bigelow Creek and Muskegon River had similar slopes but different intercepts, which indicate that the trends in the fall weight (condition) relative to length and the weight of steelhead parr relative to a given length depends both on the stream and year (i.e. neither Bigelow Creek or Muskegon River steelhead parr are consistently heavier at a given length over all three years).
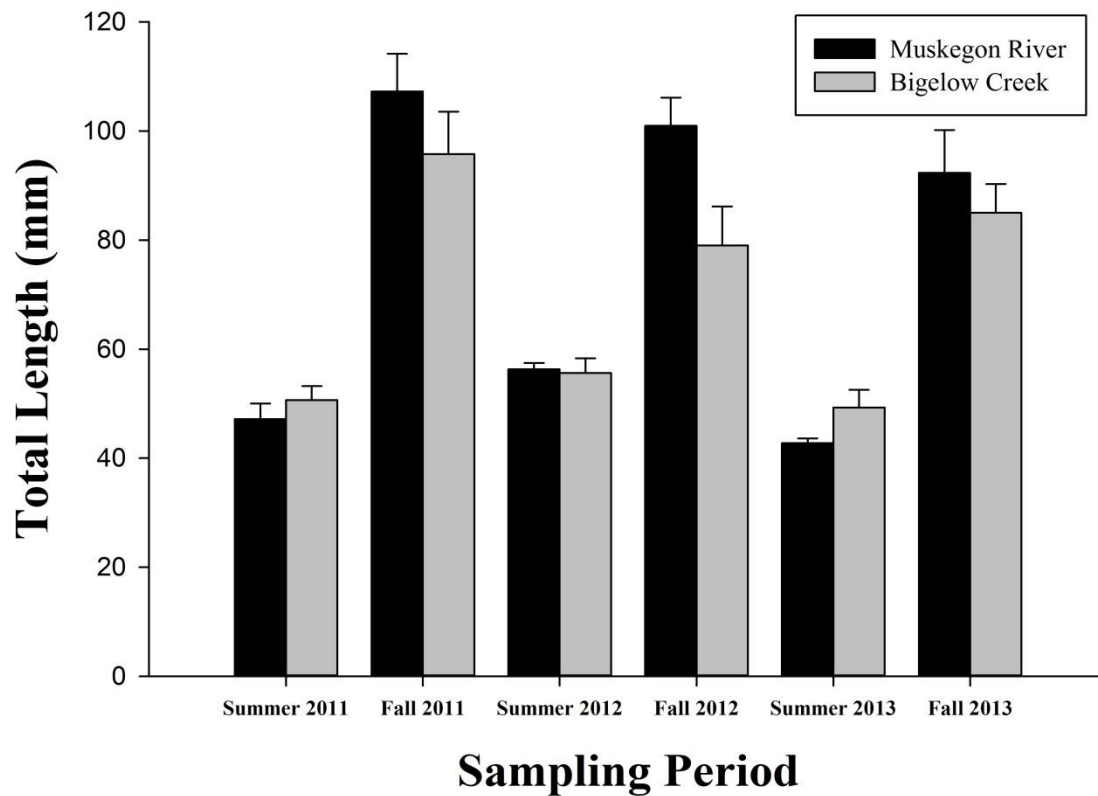
Figure 3.3. Mean (+95% CI) total length (mm) of age-0 steelhead parr in the Muskegon River and Bigelow Creek, Michigan. See text for length calculations. Number of parr measured in the Muskegon River was 262 in July 2011; 38 in October 2011; 417 in July 2012; 60 in October 2012; 312 in July 2013; 23 in October 2013. In Bigelow Creek, the number of fish measured ranged from 30 to 42 fish/season for each year.
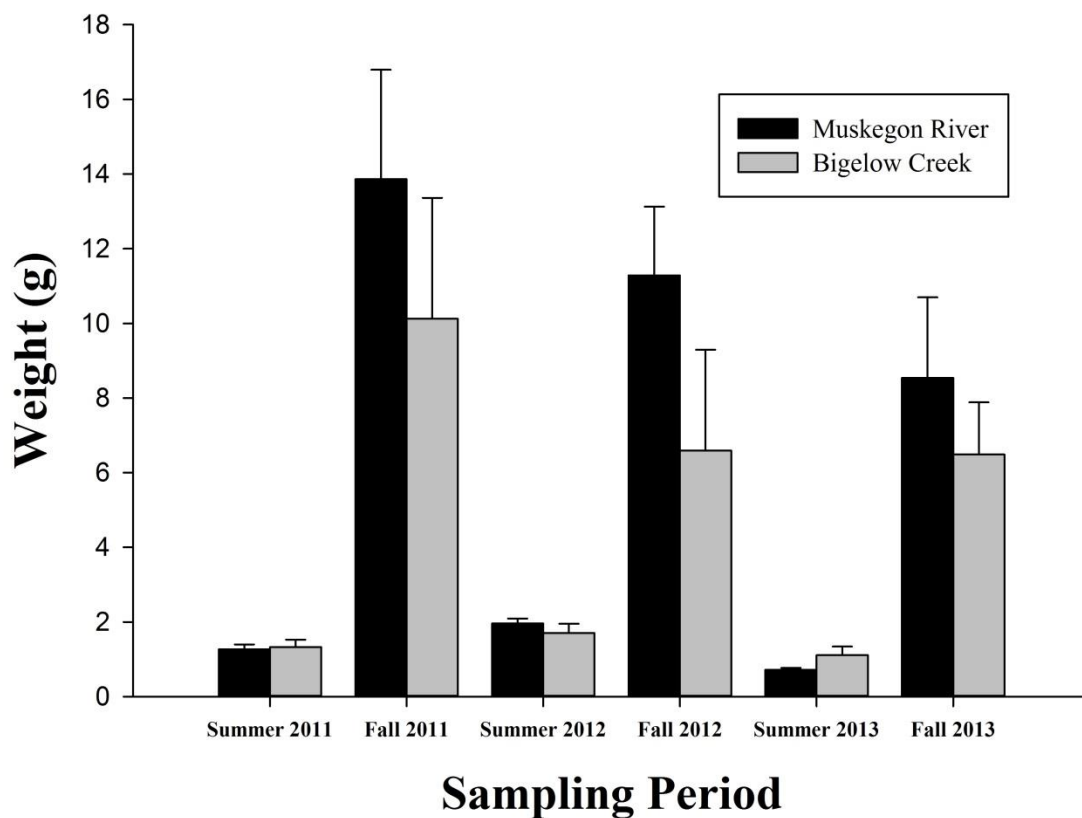
Figure 3.4. Mean (+95% CI) weight (g) of age-0 steelhead parr in the Muskegon River and Bigelow Creek, Michigan. Number of parr weighed in the Muskegon River was 262 in July 2011; 38 in October 2011; 417 in July 2012; 60 in October 2012; 312 in July 2013; 23 in October 2013. In Bigelow Creek, the number of fish weighed ranged from 30 to 42 fish/season for each year.
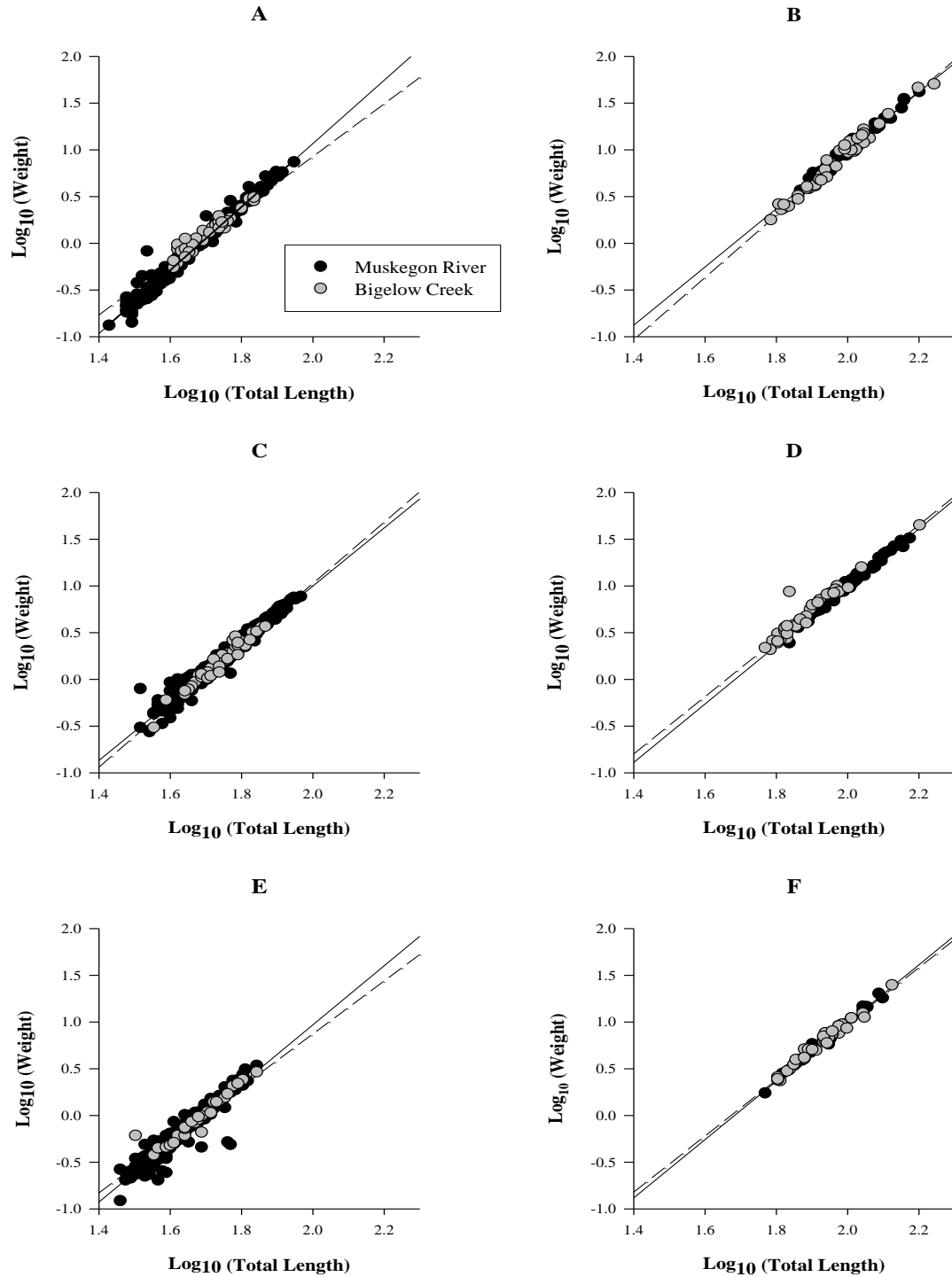
Figure 3.5. Length-weight relationship for steelhead parr in the Muskegon River (solid line) and
Bigelow Creek (dashed line) during the summer and fall of 2011-2013; (**A**) July 2011 (**B**)
October 2011 (**C**) July 2012 (**D**) October 2012 (**E**) July 2013(**F**) October 2013.

Table 3.5. Summary of ANCOVA results for July samples with log-transformed weight as the dependent variable with stream (Bigelow Creek or Muskegon River) and year as factors and log-transformed total length as the covariate.

	Df	Sum of Squares	Mean Square	F Value	p-value
Stream	1	0.74	0.74	187.56	<0.001
Year	1	5.83	5.83	1481.88	<0.001
Length	1	113.04	113.04	28741.07	<0.001
Stream*Year	1	0.02	0.02	4.43	0.035
Stream*Length	1	0.04	0.04	10.71	0.001
Year*Length	1	0.03	0.03	7.91	0.005
Stream*Year*Length	1	0.00	0.00	0.574	0.449
Residuals	1094	4.30	0.00		

Table 3.6. Summary of ANCOVA results for October samples with log-transformed weight as the dependent variable with stream (Bigelow Creek or Muskegon River) and year as factors and log-transformed total length as the covariate.

	Df	Sum of Squares	Mean Square	F Value	p-value
Stream	1	2.39	2.39	803.69	<0.001
Year	1	0.80	0.80	287.73	<0.001
Length	1	17.30	17.30	6257.78	<0.001
Stream*Year	1	0.02	0.02	5.37	0.021
Stream*Length	1	0.00	0.00	0.35	0.556
Year*Length	1	0.01	0.01	2.94	0.088
Stream*Year*Length	1	0.01	0.01	1.80	0.181
Residuals	220	0.61	0.00		

Production

Average production rates of age-0 steelhead parr in Bigelow Creek were higher than those in the

Muskegon River. The average *G:Z* ratio of steelhead parr from July to October was 4.03 ± 3.96

for Bigelow Creek and 0.71 ± 0.16 for the Muskegon River (Table 3.4). The Allen (1971)

estimate of production from July to October averaged 1.87 ± 0.39 g/m^2 for Bigelow Creek and

0.34 ± 0.19 g/m^2 for the Muskegon River.

DISCUSSION

This study was conducted to obtain quantitative estimates of parr survival and mortality in the Muskegon River and Bigelow Creek to better understand whether stream temperature was still limiting natural recruitment in the Muskegon River. Results from this study suggest there was higher survival and lower mortality rates of age-0 steelhead parr in Bigelow Creek than in the Muskegon River. One explanation for this difference in juvenile survival is temperature. In July and August the average monthly water temperature was 3.9 and 5.2°C, respectively, colder in Bigelow Creek than in the Muskegon River. Although the survival and mortality estimates include immigration and emigration, I suspect that immigration and emigration rates were low between summer and fall sampling periods based on other studies in the Great Lakes (Sheppard and Johnson 1985; Woldt and Rutherford 2002) and spatial trends in steelhead abundance observed throughout this study. In the Muskegon River, spatial trends remained consistent between summer and fall sampling with the highest parr densities occurring in strata 1 and 2, with very few parr being found in the strata farther downstream (i.e., 3, 4, and 5). In addition, there was no apparent increase in steelhead abundance from summer to fall in lower sections of the Muskegon River or in sampling sites that had obvious coldwater inputs, which suggest that downstream migration and emigration into thermal refugia is negligible. In Bigelow Creek, spatial trends in steelhead densities remained consistent between summer and fall sampling periods. While survival estimates greater than one in 2011 suggest that parr may have been emigrating into Bigelow Creek from the Muskegon River, spatial trends and site-specific observations in the Muskegon River suggest that parr were not emigrating into Bigelow Creek in search of thermal refugia. It is possible that the high survival in 2011 was the result of a high discharge event in early August that may have pushed parr down from sections directly upstream

of my sample site in Bigelow Creek. To a lesser extent, slight differences in the sampling effort or experience between July and October may have contributed to these differences.

Based on spatial trends in steelhead densities, migration and emigration into and out of the studies did not appear to influence survival or mortality rates to a great extent in the Muskegon River or Bigelow Creek. Parr survival was higher in Bigelow Creek than in the Muskegon, which was associated with more optimal temperatures ranges in Bigelow Creek than the Muskegon River. These results are supported by a laboratory study by Hokanson et al. (1977), which found that survival of age-0 steelhead parr begin to decline dramatically at temperatures exceeding 20^0C with an upper incipient lethal temperature of 25.6^0C. In the Muskegon River, daily temperatures exceeded 20^0C for over 70 consecutive days in 2011, 2012, and 2013 from late June to the middle of September with maximum temperatures reaching over 25.2C, compared to less than 10 consecutive days in July with temperatures exceeding 20^0C in Bigelow Creek from 2011 to 2013.

In addition to temperature contributing directly to mortality, the warmer stream temperatures in the Muskegon River also may indirectly increase steelhead mortality through predation. The warm temperature regime of Muskegon River may indirectly increase steelhead parr mortality by providing suitable thermal habitat for warm-water predators. Currently the Muskegon River supports a good population of smallmouth bass (*Micropterus dolomieu*), which is a potential predator of steelhead parr. To further exacerbate the situation, warmer water temperatures have been shown to increase metabolic rates and activity, thereby increasing the vulnerability of steelhead parr to predators (Mangel and Stamps 2001).

Growth rates in the Muskegon River were marginally higher than Bigelow Creek. In general, the average length and weight of age-0 steelhead was higher in the Muskegon River than in Bigelow Creek (Figures 3.3 and 3.4). While juvenile steelhead in the Muskegon River generally grew faster and had a larger overall body size, the body condition of age-0 steelhead showed no clear indication that fish in either stream was in any better condition. In the summer, the slopes (condition) of the length-weight regression were similar but had different intercepts, which indicates that steelhead parr had similar weights at a given length and that neither Bigelow Creek nor Muskegon River steelhead parr were consistently heavier at a given length during all three years. Therefore, the variability in condition between the Muskegon River and Bigelow Creek steelhead is likely due to annual variations in stream temperatures which influence the spawning and emergence of adult and juvenile steelhead in their perspective stream or slight differences in when each stream was sampled during the course of this study. In the fall, the slope (condition) of the length-weight regression were similar, suggesting that despite steelhead parr in the Muskegon River being larger in the fall, the condition of age-0 steelhead in the Muskegon River and Bigelow Creek are similar at a given length.

The lack of a clear difference in age-0 steelhead growth was unexpected considering the differences in stream temperatures. Mean temperatures in the Muskegon River ranged from 22.9^0C in July to 13.9^0C in October. In contrast, temperatures in Bigelow Creek ranged from 19.7^0C to 9.8^0C over the same time periods, with the optimum temperature range for juvenile steelhead growth of $15-17^0$C (Hokanson 1977). As a result of this, parr in Bigelow Creek could consume fewer prey items to meet their metabolic demands and achieve similar growth rates as Muskegon River parr. That is, a possible explanation for the marginally higher growth rates in the Muskegon River compared to Bigelow Creek is that there could be higher prey densities in

the Muskegon River, which could allow for faster growth despite higher metabolic costs due to the higher than optimal temperatures in the Muskegon River. Warm temperatures have been shown to decrease growth rates in salmonid growth models (Brett 1971; Elliott 1981). Thus, the absence of a clear difference in growth rate between streams could be due to several factors.

In addition to evaluating the current steelhead production in the Muskegon River and its tributary, another objective of this study was to determine if there has been a population-level response in juvenile steelhead survival since the installation of the diffuser system on Croton Dam by making direct comparisons to the Godby et al. (2007) study. In the Muskegon river, the densities of juvenile steelhead did not change in the summer or fall between studies ($W = 8$, $P = 0.2$, $W = 6$, $P = 0.7$, respectively). In addition, the survival of steelhead parr in the Muskegon River did not significantly improve, with parr survival averaging 0.07 and 0.04 before and after the installation of the diffuser, respectively($W = 4$, $P = 1$). Although substantial effort has been invested into the Muskegon River to improve nursery habitat for steelhead, fall densities of steelhead parr in the Muskegon River still remain similiar to steelhead parr densities found in marginal thermal habitat compared to more optimal thermal habitat found in streams like Bigelow Creek. As a result of this, temperature still appears to be limiting natural steelhead recruitment in mainstem of the Muskegon River, which is evident from low parr survival and high summer water temperatures that continue to exceed 20^0C throughout the summer.

Despite efforts to alleviate the high summer water temperatures believed to be limiting natural steelhead production, fall parr densities in the Muskegon River remained similiar to steelhead parr densities (120- 1,120 parr/ha) found in the Manistee and Betsie rivers with marginal thermal habitat (Newcomb and Coon 1997; Woldt and Rutherford 2002). Fall densities in the mainstream of the Muskegon River remain well below Lake Michigan tributaries with

more optimal thermal habitats such as Bigelow Creek, Little Manistee River, and Pine Creek with fall densities ranging from 1,500 to 2,470 parr/ha (Seelbach 1993; Rutherford 2002). Densities of steelhead parr were higher in Bigelow Creek than the Muskegon River during all sampling periods, suggesting that natural recruitment in adjacent coldwater tributaries continue to contribute disproportionally to the watershed steelhead abundance as compared to the marginal thermal habitat found in the mainstem of this large impounded river.

Management Implications

Management of the Muskegon River has already taken several strides to improve habitat conditions below Croton Dam. These improvements have included changing the flow regime from peaking to run-of-river to stabilize flows and habitat conditions for many aquatic organisms and the installation of a diffuser system in 2008 to alleviate high summer water temperatures and low dissolved oxygen levels below the dam. Although management efforts were intended to improve nursery habitat and survival of steelhead in the Muskegon River, this study indicates that more work may be needed in order to establish a self-sustaining steelhead population in the mainstream of the Muskegon River that is less reliant on hatchery supplementation efforts.

One area that could be improved upon to help the survival of juvenile steelhead is increasing the thermal relief generated by the diffuser system on Croton Dam. Currently, the diffuser is designed to upwell the cooler water from the deeper reaches of Croton Pond from midnight to 6 a.m. after being activated by average daily outflow temperatures of 22.2^0C in July or 21.1^0C in August as a result of the limited cool water available in the reservoir (Consumers Energy 2010). While the diffuser has shown to reduce water temperatures by 0.72^0C (Consumers

Energy 2010), this reduction does not appear to be biologically meaningful since average stream temperatures remain above 20^0C for extended periods of time (>70 days). In order to produce biologically meaningful results like an increase in steelhead survival, the operation of the diffuser may need to be modified in such a way that increases the amount of thermal relief. This could be done by changing the current operation hours of the diffuser to times when stream temperatures are the greatest during the day, lowering the activation temperature, or by running the diffuser for longer periods, which may require dredging the reservoir to create a larger volume of colder water that could be used to lower stream temperatures.

While dredging the reservoir would further utilize the economic investment in the diffuser installation, there are other options that could be implemented to increase natural steelhead production in the Muskegon River drainage. The first option is maintain and improve juvenile steelhead nursery habitat in small tributary creeks in the Muskegon River watershed. This can be accomplished by protecting these nursery streams from development and degradation or by improving access to steelhead spawning areas in those tributaries. Another option that exists would be changing the stock in the Muskegon River derived from returning adults to the Little Manistee River (coldwater tributary) to a strain of steelhead that has a life history more suitable for the Muskegon River. This could include stocking a strain of redband rainbow trout (*Oncorhynchus mykiss newberrii*) from Upper Klamath Lake, Oregon, which display an adfluvial life history similar to those in the Great Lakes but have a higher thermal tolerance (Behnk 1992; Hamilton et al. 2005), or introducing a strain of steelhead from the Pacific Northwest that migrate out of their natal streams at age-0 instead of residing there for 1 to 2 years. Although natural recruitment still appears to be limited in the mainstem of the Muskegon River due to high summer water temperatures, several options still remain to improve

steelhead survival and abundance in this stream system, and lessening the need for hatchery

supplementation.

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Chapter 4

Variation in Heat Shock Protein Expression in Juvenile Steelhead in the Muskegon River,

Michigan

ABSTRACT

Steelhead (*Oncorhynchus mykiss*) are an important component of the Great Lakes sport

fishery. Although significant natural reproduction is occurring in some of the Lake Michigan

tributaries, natural recruitment in the Muskegon River has been limited as a result of high

summer water temperatures. The objective of this study was to investigate whether naturally

produced steelhead in the Muskegon River and Bigelow Creek are experiencing thermal stress as

a result of high summer water temperature using fin tissue from juvenile steelhead to conduct

heat shock protein analysis. I found that there were significant differences in fin heat shock

protein 70 (hsp70) levels across sites and seasons. Relative fish hsp70 levels for each site in

August varied significantly from June and October in all of the sites, except for the site in

Bigelow Creek that had lower summer water temperatures. In general, fin tissue samples with the

highest hsp70 levels were collected from fish at the sites in the Muskegon River during the

summer, which corresponded to the highest water temperatures. Collectively, these results

provide support for the hypothesis that juvenile steelhead in the Muskegon River are still

experiencing thermal stress and the physiological consequences of this stress that could be

contributing to the reduced juvenile steelhead survival in the Muskegon River.

INTRODUCTION

Over a billion dollars is spent annually in the United States on watershed restoration and in-stream habitat improvement projects (Bernhardt et al. 2005). Many of these restoration projects are designed to increase in-stream habitat quality, riparian zone integrity, bank stabilization, fish passage, and improve water quality (Bernhardt et al. 2005). These projects are a widely used management strategy to lessen the anthropogenic impact on watersheds by mimicking natural conditions and thereby helping restore aquatic ecosystem integrity. Many of these habitat restoration projects are geared toward lowering stream temperature because of the overall influence it has on all levels of biological organization (Tait et al. 1994: Beitinger el al. 2000). At the cellular level, temperature virtually affects all components of the cellular process, including protein stability and enzymatic rates (Hochachka and Somero 2002). At the individual level, water temperature influences the metabolism, growth (Beer and Anderson 2011), and microhabitat selection of fish (Baltz et al. 1987). At the population level, temperature influences the distribution of fish in an ecosystem (Torgersen et al. 1999) and ultimately their viability (Li et al. 1994). As such, temperature can be considered a master controller in fish distribution, life-history strategies, fecundity, and energy budget. In short, all aspects of fish ecology are influenced by temperature and that is why temperature is such a vital factor in determining habitat quality. Therefore, monitoring and managing for stream temperature can be an essential component in stream restoration projects and protecting salmonid populations.

A recent technique that has been developed as an indicator of thermal habitat quality is quantifying heat shock proteins (hsp) to measure thermal stress (Lund et al 2002; Werner et al. 2005; Feldhaus et al. 2010). Heat shock proteins are a group of highly conserved cellular proteins that function as molecular chaperones and are present in almost all of the organisms that

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have been examined, including fish (Feder and Hofmann 1999). There are three major families

of heat shock proteins; Hsp90 (85-90kDa), Hsp70 (68-73kDa), and low molecular weight heat

shock proteins (16-47kDA), with Hsp70 being the most intensively studied in model organisms

and in natural occurring populations (Basu et al. 2002; Sorensen et al. 2002). In unstressed cells,

these proteins are involved in a variety of functions including the repair and destruction of

altered or denatured proteins (Sorensen et al. 2002). However, it is under stressful conditions that

heat shock proteins get their title as a molecular chaperone. In stressful conditions, heat shock

proteins function to help an organism cope with an environmental, physical, or biological

stressor by binding to the denatured proteins (Iwama et al. 1999). In doing so, they minimize the

occurrence of proteins interacting inappropriately with one another (Feder and Hofmann 1999).

Heat shock proteins can be synthesized constitutively or in response to a stressor (Hochachka

and Somero 2002). At the cellular level, heat shock proteins play an important role in responding

to a variety of stressful and damaging conditions, which make them important in the recovery

and survival of organisms.

Heat shock protein expression can be correlated with resistance to stress and thresholds

for stress such that higher levels of heat shock proteins translate into increased resistance and

higher thermal tolerance (Werner et al. 2005). One type of stressor that has been studied in fish is

exposure to elevated water temperatures (Fowler et al. 2009; Feldhaus et al. 2010). After cells or

whole organisms are exposed to elevated temperatures, they respond by synthesizing heat shock

proteins in order to help protect vital cellular functions (Fader et al. 1994). This reaction has been

referred to as the heat shock response (Parsell and Lindquist 1994). While heat shock proteins

are synthesized in small amounts during normal conditions, it is under stressful conditions that

the level of heat shock protein induction increases (Ashburner 1982; Lindquist 1986). This

increase in heat shock protein induction during stressful conditions makes it possible to quantify heat shock proteins when fish are exposed to seasonal variation in water temperatures (Fader et al. 1994). Quantifying heat shock proteins is a technique that has been used to measure thermal stress in salmonids in laboratory and natural conditions (Feldhaus et al. 2010; Lund et al. 2003). Given the impact temperature can have on growth, metabolism, behavior, and ultimately survival of fish populations (Beer and Anderson 2011; Sauter and Connolly 2010), it is plausible that physiological indicators such as thermal stress could be used as an indicator of thermal habitat quality.

Rainbow trout (*Oncorhynchus mykiss*) is a species of salmonid native to the tributaries of the Pacific Ocean, but have been successfully introduced to other bodies of water including the Great Lakes for sport fishing. Since their introduction, steelhead have become an important component in the Great Lakes both ecologically and economically, and significantly contribute to the Great Lakes sport fishery, which is valued at approximately $7 billion dollars (American Sportfishing Association 2008). Currently, the steelhead fishery is maintained primarily through hatchery supplementation, but significant natural reproduction occurs in some of the Lake Michigan tributaries like the Little Manistee River and Pine Creek (Seelbach 1993; Woldt and Rutherford 2002). While natural reproduction is occurring, little is known about the factors regulating natural recruitment.

From 1998 to 2001, a study was conducted by Godby et al. (2007) on the Muskegon River, a tributary to Lake Michigan, to investigate factors regulating age-0 steelhead production in a large impounded river and a connecting tributary creek that is characteristic of many Great Lakes watersheds. Their study indicated that high summer temperatures caused by Croton Dam were severely limiting natural steelhead production. As a result, a diffuser system was designed

and installed during the summer of 2008 to upwell colder bottom water behind the dam with oxygen to help alleviate elevated water temperatures and low dissolved oxygen levels downstream of Croton Dam.

Given the impact temperature has on survival, the goal of this project was to quantify the level of thermal stress juvenile steelhead are experiencing in the Muskegon River. In addition to determining if juvenile steelhead in the Muskegon River are currently experiencing thermal stress, another goal of this project was to use heat shock protein expression as an indicator of thermal habitat quality to help managers determine the appropriate levels of effort required to manage stream temperature for juvenile steelhead.

METHODS

Study Area

The Muskegon River is one of the largest tributaries to Lake Michigan with a contributing watershed of over 5,900 km^2 (O'Neal, 1997). The Muskegon River has a moderate gradient of 2-5 m/km and mixed substrate that is primarily composed of gravel, cobble, and sand (Ichthyological Associates, 1991). There are three major impoundments on the Muskegon River: Croton, Hardy and Rogers Dams. Croton Dam serves as the upstream barrier for migration of adfluvial salmonids. Sampling occurred in the primary spawning and nursery habitats for salmonids, which extended approximately 22.5 km from Croton Dam downstream to Newaygo, Michigan.

A sampling site was also located in Bigelow Creek, which is a small, free-flowing cold water tributary of the Muskegon River, which enters upstream from the city of Newaygo. Bigelow Creek is 12.1 km long with an average width of 5.3 m, and has a contributing watershed of 44.9 km^2 (O'Neal 1997). Stream gradient is moderate and substrate is primarily composed of sand and gravel (Godby et al. 2007).

Fish Sampling

Fish were collected on three occasions in 2012 on the Muskegon River and Bigelow Creek. Ten fish were collected from each of four different study locations on June 21, August 15, and October 21-25 (Figure 4.1). All fish were collected by electrofishing using a Smith-Root Model LR-24 backpack electrofisher or 250-300V DC stream electrofishing unit. Collected fish

were held in 5 gallon buckets and quickly transferred into flow though tubs. Following

collection, fish were removed and anesthetized with a lethal solution of tricaine

methanesulfonate (MS-222). Fish were then measured for total length (mm) and weight (g), and

visually examined for signs of parasites or diseases. For each individual fish, fin tissue from the

caudal fin was removed, placed in labeled Eppendorf tubes, and immediately frozen on dry ice.

All tissue was then transported to Grand Valley State University (Allendale, MI) and stored at -

80^0C for later analysis of heat shock proteins. This study followed the Institutional Animal Care

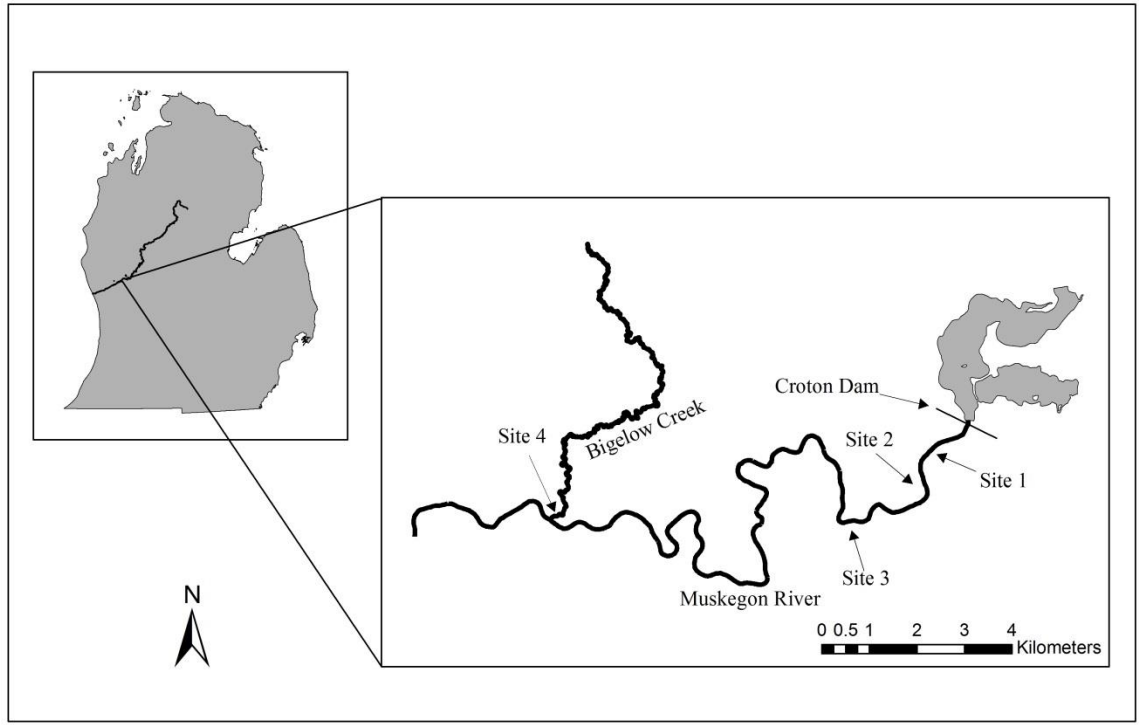and Use Committee guideline under project number 11-11-A.

Figure 4.1. Map of the study area and sample sites in the Muskegon River and Bigelow Creek, Michigan.

Temperature Data

Stream temperature was monitored using Hobo and Stowaway temperature loggers set to record temperature (^{0}C) each hour at the four study locations. Temperatures loggers were deployed in April and allowed to record temperature data until sampling was completed in October. Temperature records from the logger at site 2 (Figure 4.1) were lost due to logger failure. As a result of this, temperature records from the U.S. Geological Survey (USGS) gauging station located just downstream of Croton Dam were used for the temperature records at site 2.

Tissue sample preparation for hsp70 determination

Fin tissue was prepared following methods described in Feldhaus et al. (2010). Fish tissue was weighed on an analytical balance ($\pm$ 0.1 mg) and placed in individually labeled Eppendorf tubes. Following this, fin tissue was frozen with liquid nitrogen and crushed into small pieces with a Teflon pestle. Ice-cold Lysis buffer (50mM Tris Base, 20mM NaCl, 5mM EDTA, pH 7.5) and protease inhibitors (0.5 mg/ml leupeptin, 2mM phenylmethysulfonyl fluoride (PMSF), 1 mg/ml aprotinin, and 0.7 mg/ml pepstatin) were then added at equal proportions to the mass of the fin tissue and further homogenized manually to help break down cells. Following manual homogenization, fin tissue was further broken down using a handheld homogenizer run for 30 seconds (VWR AHS 200). Following homogenization, fin tissue was centrifuged and the resulting supernatant was aliquoted and stored at -80^{0}C. Protein concentrations in lysates were assayed with the bicinchoninic acid (BCA) protein assay method (G-Biosciences, 786-570, 786-571) for fin tissue. Protein concentrations were determined using a GE Healthcare Life Sciences NanoVue.

Hsp protein analysis

Hsp70 in fin tissue was analyzed using western blotting analysis following methods described by Towbin et al. (1979) and Feldhaus et al. (2010). Protein samples were diluted with equal amounts of SDS sample buffer and heated for 3-5 minutes at 95^0C. Then equal amounts of protein (2 µg) were placed in each lane and separated by running the samples on 8% tri-glycine gels (Biorad) for approximately 40 minutes at 200 V. In order to determine molecular weight and blotting efficiency, a calibrated molecular weight marker (Biorad) and 55-ng of recombinant Chinook salmon hsp70 protein (StressGen Biotechnologies Corp., SPP-763) were applied to each gel as internal standards for determining molecular weight and blotting efficiency. Proteins were then transferred to polyvinylidene difluoride membrane (PVDF) at 100 V for 1 hour, and then blocked overnight at 4^0C in blocking solution (5% non-fat dry milk, 20mM Tris buffer, and 0.01% Tween-20). The membranes were then incubated and probed with a polyclonal primary antibody for hsp70 (StressGen, SPA-758) for 1 hour at room temperature at a 1:5000 dilution. Following incubation, membranes were washed three times (10 minutes per wash) in tris-buffered saline solution (TBS), TBS with 0.5% Tween-20 (TBS/Tween), and then TBS again. Blots were then incubated at room temperature for 1 hour with a 1:5000 dilution of alkaline phosphate conjugated goat-anti rabbit IgG (StressGen, SAB-301). Blots were then rinsed and washed as previously described, and the proteins were visualized using an alkaline phosphate conjugate substrate kit (Biorad, 170-6432) according to manufacturer instructions. Blots were developed for 20 minutes and the reaction was stopped by rinsing with distilled water for 15 minutes. The relative hsp70 band density was then quantified using densitometry. Pictures of each blot were taken using a UVP Imaging System and band density was measured using

VisionWorksLS analysis software. Protein band density was expressed by subtracting the

background and dividing by the hsp70 standard band density.

Data analysis

The effects of site and season on fish lengths and weights, and relative hsp70 expression

in fin tissue were analyzed by one or two-way ANOVA, followed by Tukey post-hoc

comparison tests. All statistical analysis was performed in R version 2.15.2. All data were tested

for normality and equality of variance. Significance was determined at $P{\leq}0.05$.

RESULTS

Temperature metrics (Table 4.1) did not appear to vary considerably at the four fish collection sites in June. For example, the 24-hour average temperature prior to fish collection and the mean weekly maximum temperature (MWMT) ranged from 19.3-20.3 and 19.2-20.5°C, respectively. Similarly, when fish were collected in August, temperatures did not appear to vary in the three Muskegon River sites, while the site in Bigelow Creek had a considerably lower temperature. In August, the 24-hour maximum temperature prior to fish collection at the three Muskegon River sites ranged from 21.5-22.4°C compared to 17.2°C in Bigelow Creek. Similar to August, temperatures within the Muskegon River did not appear to vary during October while temperatures in Bigelow Creek were considerably lower.

Fish length and weight increased significantly over the course of the three sampling periods at all sampling sites except for fish weight between sampling in June and August (Figure 4.2). In June, the average total length ranged from 31.6-44mm, with the largest fish being found in Bigelow Creek (Site 4). During the second fish collection in August, average fish lengths between the 4 sites ranged from 60.4-72mm. Lastly, in October, average fish lengths and weights ranged from 75.4-101.2 and 4.5-10.8g, respectively, with the smallest fish being found in Bigelow Creek.

There were significant differences in fin hsp70 levels across sites and seasons. Differences were due to site (2-Way ANOVA: $F_{2,81} = 6.24$, $P < 0.05$), season ($F_{2,81} = 37.77$, $P < 0.01$), and interaction effects ($F_{2,81} = 11.26$, $P < 0.01$). As a result of the significant interaction, the dataset was divided along each level of the independent variables (i.e. treatments) to investigate the simple main effects. In examining for differences between sites in each season,

there was no significant difference in hsp70 levels between sites in June or October (ANOVA: $F_{3,22} = 2.17$, $P = 0.120$, ANOVA: $F_{3,33} = 1.24$, $P < 0.318$, respectively). However, in August there was a significant difference in hsp70 levels between sites (ANOVA: $F_{3,30} = 15.04$, $P < 0.001$). Based on post-hoc analysis, there was no significant difference in hsp70 levels between the three Muskegon River sites (1, 2, and 3). However, the hsp70 levels in fish collected from site 4 (Bigelow Creek) were significantly lower than all three sites in the Muskegon River in August (Figure 4.3). In evaluating the difference in relative hsp70 levels between seasons for each site, there were significant differences in hsp70 levels between seasons for sites 1, 2, and 3 (ANOVA: $F_{2,16} = 38.05$, $P < 0.001$, ANOVA: $F_{2,19} = 35.61$, $P < 0.001$, ANOVA: $F_{2,17} = 15.66$, $P < 0.001$, respectively). Based on post-hoc analysis, relative hsp70 levels were significantly higher in August than June and October at sites 1, 2, and 3 (Table 4.2). Unlike the three sites in the Muskegon River, the relative hsp70 levels for fish collected in Bigelow Creek (site 4) did not differ significantly between seasons (ANOVA: $F_{2,23} = 0.56$, $P = 0.581$; Table 4.2). Overall, the relative fish hsp70 levels were significantly higher in August than June and October, except for site 4 where there was no statistically significant difference in hsp70 levels across seasons (Table 4.2). In addition, fin tissue samples with the highest hsp70 levels corresponded to fish collected from the sites in August with the highest water temperatures.

Table 4.1. Temperature data (^{0}C) for fish collection sites in the Muskegon River and Bigelow Creek in June, August, and October of 2012. Weekly temperature averages are for the 7 days preceding the sampling date. Monthly temperature ranges are from the first of the month to the sampling date.

Site	Sampling date	24 hr Avg	24 hr Min	24 hr Max	Max Temperature ; Date	MWAT	MWMT	MMAT	MMTmax	MDTR
1	21 June	19.7	18.5	20.0	25.0; 10 July	19.1	20.0	18.1	19.1	1.9
1	15 August	20.5	19.7	21.5		21.2	21.9	21.7	22.6	1.7
1	23 October	13.0	12.6	13.5		13.1	13.4	14	14.4	0.7
2	21 June	19.3	18.6	20.0	25.2; 7 July	19.0	19.9	18	18.8	1.6
2	15 August	21.1	20.5	21.7		21.9	22.4	22.5	23.1	1.3
2	23 October	13.0	12.7	13.3		13.0	13.3	14.4	14.7	0.6
3	21 June	20.1	19.0	20.9	25.6; 24 July	19.5	20.5	18.4	19.4	2.0
3	15 August	21.2	20.5	22.4		22.1	22.7	22.6	23.6	1.7
3	25 October	13.6	13.4	14.3		13.3	13.7	14.4	14.8	0.7
4	23 June	20.3	18.7	21.5	23.3; 6 July	17.7	19.2	16.2	17.9	3.0
4	15 August	15.7	14.6	17.2		16.1	17.2	17.3	18.6	2.3
4	21 October	9.5	9.1	10.0		10.1	11.1	9.9	10.8	1.7

The 24 hr average, 24 hr minimum, and 24 hr maximum are for water temperatures during the 24 hours preceding fish collection, pre-visit maximum is the maximum temperature recorded from June 1 to October 31.

MWAT mean weekly average temperature, *MWMT* mean weekly maximum temperature, *MMAT* mean monthly average temperature, *MMTmax* mean monthly maximum temperature, *MDTR* mean daily temperature range.

A

B
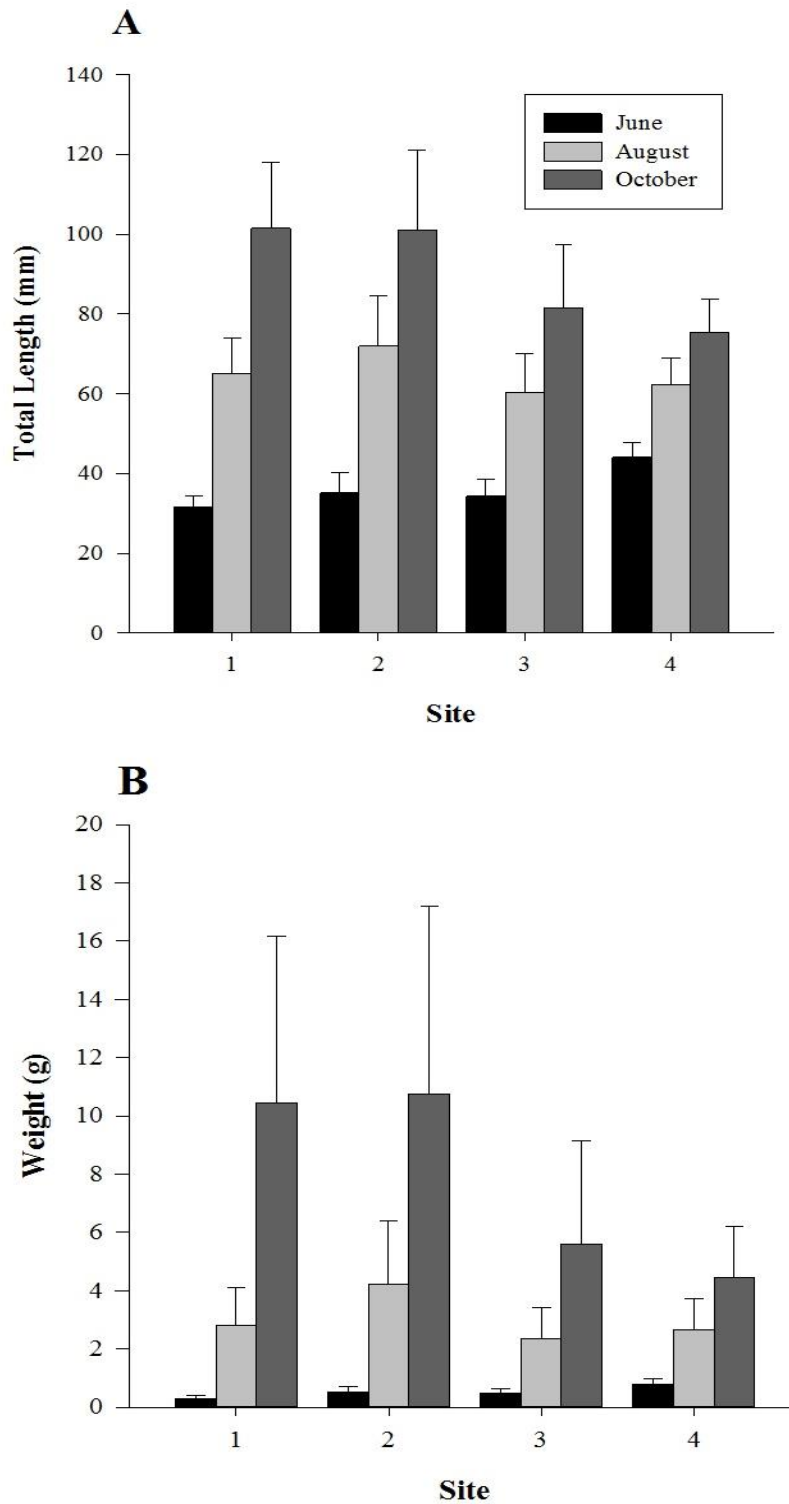
Figure 4.2. Mean (± 95% CI) length (**A**) and weight (**B**) of juvenile steelhead in June, August, and October of 2012.

Table 4.2. Relative fin tissue heat shock protein 70 levels in juvenile steelhead across three

seasons.

Season	June		August		October	
Site	Hsp 70	S.E	Hsp 70	S.E.	Hsp 70	S.E.
1	0.08*	0.04	0.40*	0.08	0.21*	0.07
2	0.15	0.08	0.38*	0.04	0.16	0.06
3	0.15	0.06	0.43*	0.16	0.14	0.01
4	0.17	0.07	0.17	0.05	0.20	0.08

Significant differences, determined by ANOVA ($P<0.05$), between seasons for the same site are indicated by an asterisks (*).

Figure 4.3.Relative heat shock protein 70 band density (mean ± 1 standard error of the mean) in fin tissue from juvenile steelhead collec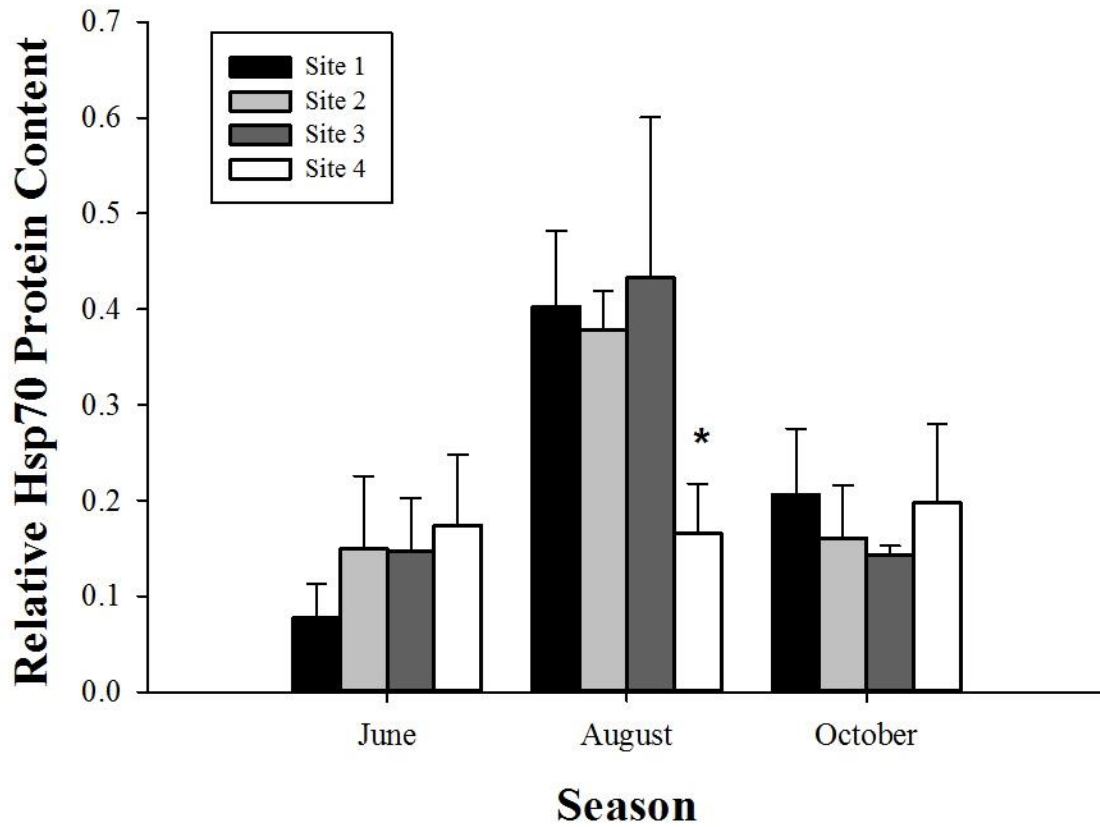ted in June, August, and October of 2012. Significant differences, between sites (ANOVA $P<0.05$) during the same season are indicated by an asterisks (*).

DISCUSSION

Water temperature is an important factor affecting the distribution, abundance, and ultimately the survival of salmonids, thus knowing the thermal tolerances of those species is particularly valuable to resource managers (Beer and Anderson 2011; Torgersen et al. 1999; Li et al. 1994; Baltz et al. 1987). Previous studies (Godby et al. 2007) hypothesized that low survival of juvenile steelhead in the Muskegon River was related to warm summer water temperatures. Following the findings of Godby et al. (2007), this study was conducted to investigated whether juvenile steelhead in the Muskegon River are experiencing thermal stress using heat shock protein expression. Based on the results of this study, it appears that steelhead parr in the Muskegon River do experience thermal stress during the summer due to warm summer water temperatures. Although summer temperatures may not be the only variable that limits juvenile steelhead production (e.g., spawning habitat), it currently appears to be the most critical factor given the overlap between warm temperatures and steelhead life stage.

Like most fish, steelhead are ectotherms and have body temperatures close to the surrounding water temperature (Moyle and Cech 2000), which make expression levels of temperature inducible heat shock proteins excellent monitors of a fish's exposure to elevated stream temperatures (Werner et al. 2005). In stressful conditions like elevated stream temperatures, heat shock proteins function to help an organism cope with the environmental, physical, or biological stressors in order to maintain cellular homeostatsis (Parsell and Lindquist 1993; Iwama et al. 1999). After organisms are exposed to elevated temperatures, they respond by synthesizing heat shock proteins in order to help protect vital cellular functions (Fader et al. 1994). Based on the appearance of elevated levels of inducible hsp70 in fin tissue of juvenile steelhead in the Muskegon River (sites 1-3) during the summer, it appears that these elevated

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levels of inducible heat shock proteins are the result of cellular stress caused by specific thermal conditions that exceed tolerance levels of juvenile steelhead.

Similar to other field-based heat shock protein response studies on fish populations, results from my study indicated that elevated hsp70 levels in juvenile steelhead are the result of seasonal and site specific thermal conditions. In a study by Kammerer and Heppell (2012), the hsp70 levels in wild redband trout (*Oncorhynchus mykiss gairdneri*) were relative low and comparable in the spring and fall, but were significantly higher in the summer, which was explained by seasonal water temperatures. Similar to the results by Kammerer and Heppell (2012), I observed that hsp70 levels in fish at the three sites in the Muskegon River increased significantly in the summer, which corresponded to the warmest observed water temperatures. In addition, I also observed similar (lower) hsp70 levels in juvenile steelhead between June and October when temperatures were lower.

Although I observed significantly higher hsp70 levels in fish from the three Muskegon River sites during August, that trend was not seen in fish from the site in Bigelow Creek, which could be the result of site-specific thermal conditions (Feldhaus et al. 2010; Werner et al. 2005). A study by Werner et al. (2005) determined the threshold temperature for inducible hsp70 in juvenile steelhead to be 18-18.5^{0}C and 20-22.5^{0}C for the monthly average and maximum temperature during the 24 hours preceding fish collection, respectively. While stream temperatures in Bigelow Creek increased in August, hsp70 levels did not significantly increase likely due to the more favorable thermal conditions and stream temperatures not reaching threshold temperatures that are correlated with elevated levels of inducible hsp70 and sublethal consequences of thermal stress (Werner et al. 2005). In Bigelow Creek, the threshold temperatures were not met during August since the monthly average temperature and 24-hour

maximum temperature preceding fish collection were 17.3^0C and 17.2^0C, respectively while temperatures in the Muskegon River sites ranged from $21.7-22.6^0$C and $21.5-22.4^0$C, respectively (Table 4.1). Although elevated heat shock proteins were only observed during August in the Muskegon River, there were other periods in the summer (June and July) that likely would have induced the heat shock response in the Muskegon River and Bigelow Creek (Table 4.1; Figure 4.4). In June, water temperatures in both the Muskegon River and Bigelow Creek fell within the threshold levels described by Werner et al. (2005), however elevated levels of inducible hsp70 were not observed. It is possible that steelhead had not experienced sufficient exposure to induce a heat shock protein response, i.e., sampling occurred prior to the 24 hour time period it can take for inducible hsp70 expression after heat shock (Werner et al. 2006). Juvenile steelhead also may have had access to thermal refugia during that period that could have moderated their exposure to elevated temperatures. Alternatively, this could be the result of intraspecific variation in hsp70 expression (Heredia-Middleton et al. 2008) between the strain of steelhead present in the Muskegon River watershed and those studied by Werner et al. (2005) in California. In addition, sampling was not conducted in July when water temperatures were the highest in the Muskegon River and Bigelow Creek, and would have likely caused elevated levels of inducible hsp70. While juvenile steelhead in both streams likely experience temperatures high enough to induce elevated levels of hsp70 during the summer, steelhead in the Muskegon River are the only fish that showed signs of thermal stress and are exposed to more frequent water temperatures that would result in thermal stress and heat shock.

The thermal tolerance of juvenile steelhead and other fishes are important physiological traits that help define the habitat requirements that are needed for their continued success in that environment (Rodnick et al. 2004). While heat shock protein response is a protective response to

cellular stress, chronic exposure to elevated temperatures and increased heat shock protein expression have been shown to reduce the metabolic condition of juvenile steelhead (Viant et al. 2003). In addition to the energy intensive process of synthesizing proteins as a result of elevated temperatures, a laboratory study by Hokanson et al. (1977) found that survival of age-0 steelhead parr begin to decline dramatically at temperatures exceeding 20^0C with an upper incipient lethal temperature of 25.6^0C. Although juvenile steelhead in the Muskegon River have shown they can tolerate summer temperatures in excess of 23^0C, it is important to recognize that protein synthesis and repair are energy intensive processes (Hofmann and Somero 1995; Somero 2002), and that fish undergoing thermal stress are physiologically compromised and that the continued production of heat shock proteins could ultimately affect their viability (Li et al. 1994; Feder et al. 1992).

Daily temperatures in the Muskegon River sites exceeded 20^0C for 88 consecutive days from late June to the middle of September with a maximum temperature of 25.2^0C, compared to only 8 consecutive days in July with temperatures exceeding 20^0C and a maximum temperature of 23.3^0C in Bigelow Creek (Figure 4.4). Although some juvenile steelhead have shown that they can tolerate high summer water temperatures in the Muskegon River, the physiological consequences of maintaining elevated heat shock protein levels for long periods of time are likely going to leave juvenile steelhead physiological compromised and confer a fitness cost. While substantial effort has been invested into the Muskegon River to improve nursery habitat for steelhead with the installation of an "upwelling" system on Croton Dam, temperature still appears to be limiting natural steelhead recruitment in the Muskegon River. This is evident based on the elevated levels of heat shock proteins found in juvenile steelhead during the summer in

the Muskegon River, the high summer mortality (Chapter 3), and the sublethal effects of

prolonged exposure to elevated temperatures and thermal stress.

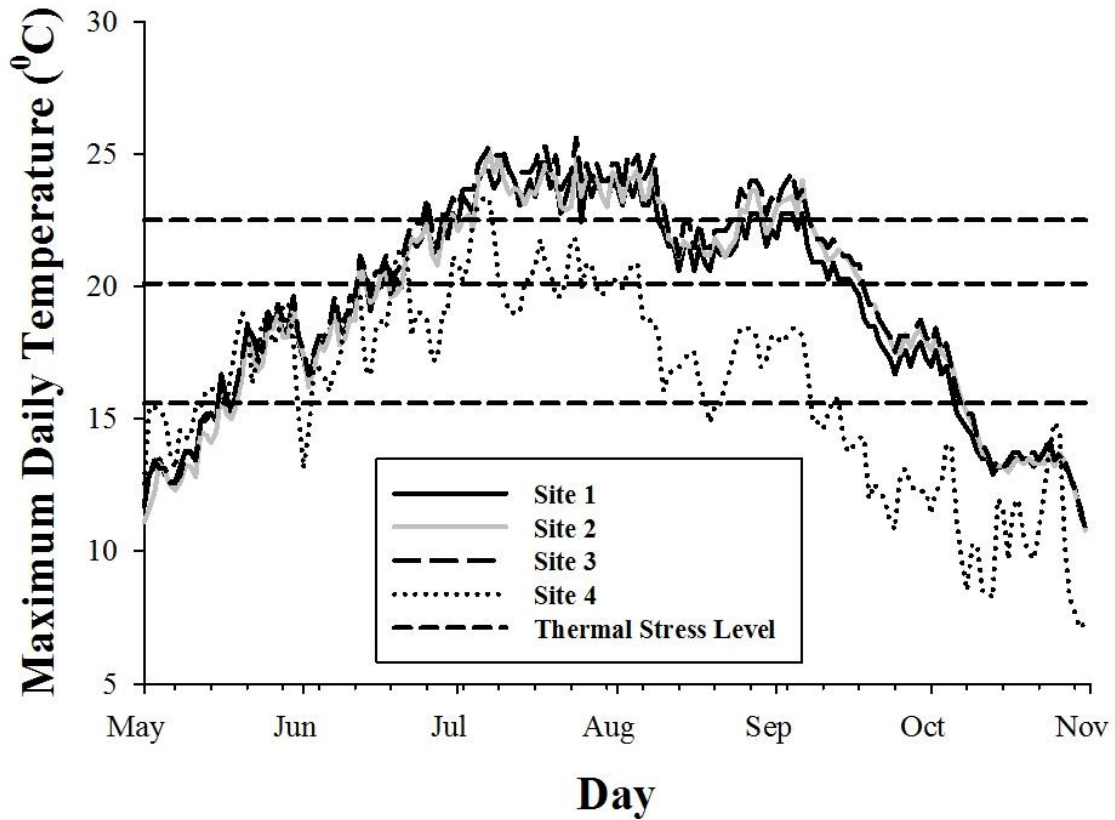Figure 4.4. Maximum daily temperature in the three sites in the Muskegon River (1-3) and
Bigelow Creek (site 4), Michigan during 2012. Horizontal lines signify the temperature ranges of
15.6^{0}C to 20^{0}C, 20.1^{0}C to 22.5^{0}C, and greater than 22.5^{0}C, which correspond to thermal stress
levels of low, medium, and high for juvenile steelhead determined by the US Fish and Wildlife
Service (1995).

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Chapter 5

GENERAL DISCUSSION

In this thesis, I evaluated the success of the diffuser system at alleviating high summer water temperatures that previously was found to be limiting natural steelhead recruitment in the Muskegon River. Given the impact temperature can ultimately have on survival of salmonids, I assessed the success of the diffuser system by evaluating not only its ability to alleviate high summer water temperatures, but also its ability to create a population-level response in natural steelhead abundance and survival since its installation. In addition, I used heat shock protein analysis to determine if juvenile steelhead experience thermal stress during warm summer water temperatures.

Results from the trend analysis in water temperature data (Chapter 2) indicate that stream temperatures in the Muskegon River do not appear to have improved since the installation of the diffuser. Based on the summary of the mean monthly average temperature, temperatures in July were higher after the installation of the diffuser. While July temperatures suggest that the diffuser may not be achieving its goal, trend analysis suggests that patterns in stream temperatures corresponded to the operation of the diffuser. Furthermore, despite warmer air temperatures in July and August after the installation of the diffuser, stream temperatures in August remained similar to those recorded prior to the installation of the diffuser. While there is some evidence to suggest that the diffuser system is functioning properly, the results from this study indicate that stream temperatures in the Muskegon River do not appear to have improved since the installation of the diffuser and that further efforts and alterations to the operation of the diffuser are needed in order to alleviate high summer water temperatures below Croton Dam. For example, operation criteria may need to include discharge as well as temperature.

Despite efforts to alleviate the high summer water temperatures believed to be limiting natural steelhead production, results from Chapter 3 indicate that fall parr densities in the Muskegon River remain well below Lake Michigan tributaries with more optimal thermal habitats such as Bigelow Creek. In addition to higher densities of steelhead parr in Bigelow Creek during all sampling periods, quantitative estimates of parr survival and mortality suggest that the more optimal temperature ranges recorded for Bigelow Creek contributed to the higher survival and lower mortality rates in Bigelow Creek in comparison to the Muskegon River. Although substantial effort has been invested into the Muskegon River to improve nursery habitat for steelhead, fall densities of steelhead parr in the Muskegon River still remain similar to steelhead parr densities found prior to diffuser installation and in marginal thermal habitats. As a result of this, temperature still appears to be limiting natural steelhead recruitment in the mainstem of the Muskegon River, which is evident from low parr survival and high summer water temperatures that continue to exceed 20^0C throughout the summer.

In addition to the low parr survival and high observed summer water temperatures in the Muskegon River, results from heat shock protein analysis (Chapter 4) provides additional support to the argument that natural steelhead recruitment may be limited by high summer water temperatures. Based on heat shock protein analysis, it appears that steelhead parr in the Muskegon River are experiencing heat stress during warm summer temperatures. In addition, based on the presence of elevated levels of inducible heat shock protein 70 in fin tissue from steelhead parr in the Muskegon River compared to Bigelow Creek during the summer, it appears that these elevated levels of inducible heat shock proteins are the result of cellular stress caused by site and season specific thermal conditions.

Water temperature is an important factor affecting the distribution, abundance, and ultimately the survival of salmonids, thus making the thermal tolerances and preferences of those species particularly valuable to resource managers. In addition to prioritizing restoration efforts that moderate warmer stream temperatures, it is also essential to be able to evaluate the success of those restoration efforts. This should include not only evaluating whether restoration efforts lower stream temperatures but also how fish populations respond to those efforts because a species thermal tolerance is an important physiological trait that help define the suitable habitat requirements that are needed for their continued success in that environment.

Although results from this study indicate that some steelhead parr in the Muskegon River can tolerate summer temperatures in excess of 23^0C, it is important to recognize that protein synthesis and repair are energy intensive processes and that fish undergoing thermal stress are physiologically compromised and that the continued production of heat shock proteins over an extended period of time could ultimately affect their viability. While substantial effort has been invested into the Muskegon River to improve nursery habitat for steelhead with the installation of an "upwelling" system on Croton Dam, temperatures still appears to be limiting natural steelhead recruitment in the Muskegon River. This is evident based on the high summer water temperatures observed during 2011-2013 (Chapter 2), the high summer mortality of steelhead parr (Chapter 3), the elevated levels of heat shock proteins found in steelhead parr during the summer (Chapter 4), and other sublethal effects caused by prolonged exposure to elevated temperatures and thermal stress in the Muskegon River.