Muskegon River Juvenile Steelhead Survival and Production: Quantifying Seasonal Thermal Stress

Presidential Grant Proposal

Grand Valley State University

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Rainbow trout (*Oncorhynchus mykiss*) is a species of salmonid native to the tributaries of the Pacific Ocean. They display highly variable life history strategies in the habitats they spend the majority of their lives in. The two major life history strategies that rainbow trout display are resident and anadromous forms (Raleigh 1984). The anadromous form of rainbow trout are commonly known as steelhead. 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. The first documented introduction of steelhead came in the late 1800s (MacCrimmon 1972). Following their introduction, weirs were installed at the mouth of several tributaries including the Little Manistee River to collect the eggs of returning fish. Since their introduction, steelhead have become an important component in the Great Lakes sport fishery, which is valued at approximately two billion dollars (Southwick Associates 2007). 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). In Lake Michigan, approximately 20-30% of adult steelhead caught in the sport fishery are of natural origin (Rand et al. 1993). 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 its 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.

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 at least influenced by temperature. It is this reason that temperature is such a vital factor in determining habitat quality. 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). Heat shock proteins are a group of highly conserved cellular proteins that function as molecular chaperones and are present in all 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 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 (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 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. I propose that heat shock proteins can provide a means to quantify the thermal stress juvenile steelhead (*Oncorhynchus mykiss*) 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 has on survival, the goal of this project is to quantify the level of thermal stress juvenile steelhead are experiencing in the Muskegon River. In doing so, one of the project objectives is to investigate current natural juvenile steelhead abundance, survival, and production in the Muskegon River and compare that with findings by Godby et al. (2007). In addition, I would like to determine if juvenile steelhead in the Muskegon River are currently experiencing thermal

stress during their early life stages, and whether heat shock proteins can be used as an indicator of the thermal habitat quality in the Muskegon River. These data can be used to determine the level of heat stress juvenile steelhead are experiencing and help managers determine the appropriate levels of effort required to manage stream temperature for juvenile steelhead.

Study Area

This study will be conducted on the Muskegon River and Bigelow Creek, Michigan. 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, Rogers, and Hardy Dams. Croton Dam serves as the upstream barrier for migration of adfluvial salmonids. Six of the eight sampling locations for this study will be on the Muskegon River. My study reach will include the primary spawning and nursery habitats for salmonids, which extended approximately 22.5 km from Croton Dam downstream to Newaygo, Michigan.

Two sample sites will also be located in Bigelow Creek, which will serve as the experimental control for this study. Bigelow Creek is a small, free-flowing cold water tributary of the Muskegon River, which enters upstream of 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).

Methods

Heat Shock Protein

Temperature Data for heat shock proteins

Stream temperature will be monitored using Hobo and Stowaway temperature loggers set to record temperature (^{0}C) each hour at the eight study locations. Temperature loggers will be deployed in the early spring and allowed to record temperature data until sampling is completed in the fall.

Fish sampling for heat shock proteins

Sampling began during August and October of 2011 and will continue in the spring, summer, and fall of 2012 in the Muskegon River and Bigelow Creek. Ten fish were collected from each of eight different study locations in 2011 and will again be collected in 2012. Fish will be collected by electrofishing shoreline segments using a Smith-Root Model LR-24 backpack electrofisher and 250-300 V DC stream electrofishing unit and held in flow through tubs. Following collection, fish will be removed and euthanized with a lethal solution of tricaine methanesulfonate (MS-222). Fish will then be measured for fork length (mm) and weight (g), and visually examined for signs of parasites and diseases.

For each individual fish, tissues will be removed and immediately frozen on dry ice. Liver tissue and caudal fin tissue will be quickly removed and placed in individually labeled centrifuge tubes. Fish

carcasses will also be individually labeled and stored in plastic tubes and frozen on dry ice. Following sampling the fish carcasses and tissues will be transported back to Grand Valley State University (Allendale, MI) and stored at -80°C for later analysis of heat shock proteins.

Tissue sample preparation for hsp70 determination

Fin and liver tissue will be prepared following methods described in Feldhaus et al. (2008 and 2010). Fish tissue will be weighed and placed in individually labeled eppendorf tubes. Following this, fin tissue will be frozen with liquid nitrogen and crushed into small pieces with a pestle. Lysis buffer and protease inhibitors will then be added at equal proportions to the mass of the fin tissue and further homogenized manually to help break down the cell. Following homogenization, fin tissue will be centrifuged and the resulting supernatant will be aliquoted and stored at -80°C. Liver samples will be broken down by adding protease inhibitors and lysis buffer. Liver tissue will then be homogenized, centrifuged, and the resulting supernatant will be aliquoted and stored at -80°C. Protein concentrations in lysates will be assayed with the bicinchoninic acid (BCA) protein assay method for both fin and liver tissue (Smith et al. 1985).

Hsp protein analysis

Hsp70 in fin and liver tissue will be analyzed using western blotting analysis following methods described by Towbin et al. (1979) and Feldhaus et al. (2008 and 2010). Protein samples will be diluted with equal amounts of buffer and heated for 3-5 minutes. Then equal amounts of protein (25 µg) will be placed in each lane and separated by running the samples on 8% tri-glycine gels for 2 hours at 125 V. In order to determine molecular weight and blotting efficiency, a calibrated molecular weight marker and a recombinant hsp70 protein will be applied to each gel as internal standards for determining molecular weight and blotting efficiency. Proteins will then be transferred to polyvinylidene difluoride membrane at 100 V for 1 hour, and then blocked overnight at 48 °C in blocking solution. The membranes will then be incubated and probed for hsp70 for 1 hour at room temperature. Blots will then be allowed to develop for 15 minutes and then the reaction will be stopped by rinsing with distilled water for 10 minutes. The relative hsp70 band density will then be quantified using densitometry software.

Data analysis

Data will be analyzed using regression techniques, general linear models, and one and two-way ANOVA. Paired t-tests will be used to compare fin and liver hsp70 levels. In addition, differences in hsp70 levels will be tested among different locations during a particular sampling season using one-way ANOVA. One-way ANOVA will also be used to analyze for differences in hsp70 levels at a location during different sampling times of the year. We will also be using two-sample t-tests to compare mean hsp70 between the Muskegon River sites and Bigelow Creek. Finally, the induction temperature for hsp70 will be estimated by using sigmoid dose-response curve. Significance will be determined at $P \leq 0.05$.

Management Implications

Information gathered from this study can provide valuable information to fishery managers and biologists about natural steelhead recruitment in the Muskegon River. The information gathered from this project can be used in evaluating the thermal habitat quality in the Muskegon River and help with management decisions regarding the need for hatchery supplementation and additional watershed restoration projects. There is potential that if this study shows a significant increase in natural steelhead recruitment since Godby et. al (2007) study, that the Michigan Department of Natural Resources could reduce hatchery supplementation efforts of steelhead in the Muskegon River. In addition, it will provide information on the effectiveness of the diffuser at alleviating high summer water temperatures and its potential effectiveness and use on other impounded rivers.

Dissemination of Information

The results from this study will be presented at several conferences including Michigan Chapter of the American Fisheries Society and International Association of Great Lakes Research conferences. In addition, manuscripts will be generated for publication in peer-reviewed journals such as North American Journal of Fisheries Management and Environmental Biology of Fish.

Institutional Animal Care and Use Committee

The Muskegon River Juvenile Steelhead Survival and Production study has been approved by the Institutional Animal Care and Use Committee on June 20, 2011 under project number 11-11-A.

Time Table

Spring 2012

- Deploy temperature loggers
- Collect spring samples of fin tissue from juvenile steelhead in May

Summer 2012

- Collect summer samples of fin tissue from juvenile steelhead in July
- Learn western blotting techniques

Fall 2012

- Collect fall samples of fin tissue from juvenile steelhead in October
- Analyze fin tissue for heat shock proteins in the lab

Winter 2013

- Present data at Michigan Chapter of the American Fisheries Society conference.
- Write and defend thesis
- Prepare manuscripts for publication

Budget

Total amount requested for this proposal is $1,000.00. A detailed budget of how this money will be used is provided below.

Presidential Grant: 2012			
Item	Quantity	Price per unit	Total
Bicinchoninic Acid (BCA) Protein Assay (G-Biosciences 786-570)	500 assays	$91.00	$91.00
8% Tris-Glycine Gels (Invitrogen Corporation)	10 gels	$113.57	$113.57
Calibrated Molecular Weight Marker (Biorad 116-0376)	250 µl	$156.00	$156.00
Alkaline Phosphate Conjugated Goat-Anti Rabbit IgG (StressGen SAB-301)	1 ml	$105.00	$105.00
Calibrated Chinook Salmon HSP70 Standard (StressGen SPP-763)	50 µg	$199.00	$199.00
Alkaline Phosphate Conjugated Substrate Kit (Biorad 170-6432)	1 kit	$136.00	$136.00
Polyclonal HSP70 Antibody (StressGen SPA-758)	100 µl	$226.00	$226.00
Total for Lab Supplies			**$1,026.57**

Departmental support will sought after for any expenses occurred over $1,000.00.

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