CHAPTER III

EXTENDED REVIEW OF LITERATURE

Bioenergetics has been considered an important concept in aquatic systems since Lindeman's (1942) investigation of energy transfer between trophic levels in Cedar Bog Lake (Benke et al. 1988). The term "bioenergetics" involves energy in relation to biological aspects of the stream such as production and feeding of organisms. Types of energy input into streams include autochthonous (i.e. within the stream) and allochthonous (i.e. outside the stream) sources; these sources have been extensively studied for their contribution towards freshwater stream energy (Benke et al. 1988). Autochthonous energy sources can be algae or organism fecal matter within a stream that provides energy to consumers (Minshall 1978). Allochthonous energy sources can be insects falling into the stream or leaf litter from riparian vegetation (Baxter et al. 2004). An additional energy source to a freshwater stream can come from migrating fish that enter streams to spawn (Bilby et al. 1996, Flecker et al. 2010, Ivan et al. 2011).

Migration is the movement of organisms from one location to another, usually at an expected time. Fish migrate for several reasons, such as feeding, reproduction, or refuge from harsh temperatures (Flecker et al. 2010). Migration can be defined as either diadromous, or the movement between saltwater and freshwater, or potadromous, that involves movement between freshwaters only (Flecker et al. 2010). Diadromous migration can be broken down into three major categories, but the most commonly studied of the three is anadromous. Pacific salmon are the model species of anadromous behavior. Many studies have focused on Pacific salmon, particularly in the Pacific Northwest, and the effect of their migration on recipient streams (Janetski et al. 2009).

Pacific salmon spend a majority of their lives growing in the ocean before returning to their natal freshwater streams for spawning. Salmon individuals at spawning grounds can reach up to the millions (Gende et al. 2002). After spawning, the salmon will die (i.e. semelparous species), and contribute nutrients to freshwater streams and energy as organic material (i.e. eggs, carcass). Considering the magnitude of salmon individuals at spawning, the amount of nutrients and energy contributed is massive.

Earliest data on fish migration has focused more on nutrients than energy, starting with Juday's et al. (1932) limnological study of Karluk Lake in Alaska. Juday et al. (1932) found that spawning sockeye salmon affected both lake and stream chemistry by depositing on average 2 million kg of organic matter and 5,000 kg of the important nutrient, phosphorus. Recent studies have quantified salmon marine-derived nutrients (SDN) and found that these nutrients have a generally positive effect on streams and the surrounding terrestrial ecosystem, and that SDN can supplement the stream for long periods of time (Naiman et al. 2002, Rinella et al. 2013). The dispersal of nutrients from salmon can take a few pathways: direct consumption of carcass or egg, consumption of organisms enriched with SDN, and recycling by processes such as excretion, leaching, and decomposition of carcass (Gende et al. 2002). Stable isotope analysis (SIA) is generally used to track nutrients through trophic levels or identify organisms enriched with SDN (Mantel et al. 2004). Salmon have heavy carbon and nitrogen isotopes, which makes SIA a useful candidate when investigating nutrient dispersal in streams from salmon spawners (Bilby et al. 1996).

Primarily, epilithic biofilm, terrestrial vegetation, and other primary producers are directly affected by recycling of SDN from decomposing carcass (Ben-David et al. 1998, Wipfli et al. 1999, Helfield and Naiman 2001, Johnston et al. 2004, Mitchell and Lamberti 2005,

Claeson et al. 2006, Cak et al. 2008, Tiegs et al. 2009, Tiegs et al. 2011). Epilithic biofilm or standing stock tend to be enriched with carbon and nitrogen following salmon runs, resulting in increased growth rates and biomass (Cak et al. 2008, Kohler et al. 2008, Tiegs et al. 2009, Tiegs et al. 2011). However, epilithic response to SDN can be highly variable depending on salmon density and stream environment (Johnston et al. 2004). Terrestrial vegetation experience enrichment in nitrogen, meaning salmon could be natural fertilizers (Bilby et al. 1996, Helfield and Naiman 2001, Rüegg et al. 2011), but Ben-David et al. (1998) suggested that is largely dependent on if the vegetation has limited access to nitrogen.

SDN distribution to macroinvertebrates is usually through macroinvertebrates consuming primary producers that were supplemented by SDN (Bilby et al. 1996, Cederholm et al. 1999). Macroinvertebrates can be enriched by SDN indirectly by feeding on primary producers and microbes supplemented by SDN (Gende et al. 2002, Marcarelli et al. 2014). Several macroinvertebrate functional groups can be enriched by carbon and nitrogen, such as scrapers consuming SDN enriched biofilm and collectors and shredders consuming SDN enriched fine and coarse particulate matter, stimulating secondary production (Chaloner and Wipfli 2002, Claeson et al. 2006, Kohler et al. 2008). but this tends to be variable amongst taxa (Claeson et al. 2006). Generally, secondary production is stimulated in response to SDN in some mayfly species, but chironomids respond the most to SDN (Chaloner et al. 2004, Monaghan and Milner 2008; Lessard et al. 2009). Chironomids are hypothesized to be successful in terms of production because they respond well to SDN, but there is also the possibility that salmon spawners reduce competitors (i.e. disturbance), allowing chironomids to survive. Chironomids are a very important energy source for predators, such as juvenile salmonids (Chaloner et al. 2004). Assuming that chironomid (and possibly other macroinvertebrates) secondary production is a

response to SDN, then SDN indirectly affects fish in addition to macroinvertebrates (Bilby et al. 1996).

It is difficult to differentiate if fish enriched with SDN from salmon were supplemented indirectly by consuming macroinvertebrates enriched by SDN (e.g. salmonids consuming chironomids), or by directly consuming carcass or egg from salmon; this is because SIA can only measure isotopic signatures but not reveal the mode of transport. Regardless, resident stream fish can be enriched with SDN when exposed to a salmon run. For example, Bilby et al. (1996) observed that several adult salmonids (i.e. Coho, steelhead, cutthroat trout) had increased carbon and nitrogen isotopes when salmon spawners moved into their habitat. It is important to note that the amount of nutrients incorporated into stream resident fish is dependent on spawner biomass (Rinella et al. 2012).

In addition to nutrients, salmon provide energy to the stream with organic material derived from eggs and carcass after spawning and death, respectively. Some studies have found that macroinvertebrates have increased growth rates in response to salmon spawners. The general consensus is that the incorporation of energy into macroinvertebrates is dependent on feeding ecology or species (Chaloner and Wipfli 2002). For example, Chaloner and Wipfli (2002) observed salmon flesh in microcosms and natural runs increased growth rates of shredder and collector functional groups only. Another example is Minkawa et al.'s (2002) observation that salmon meat was nutritionally important for some specific species of caddisfly *Asynarchus pacificus* and *Ecclisomyia conspersa*, but *E. conspersa* growth rate responded significantly greater than *A. pacificus* to salmon tissue. Consumption of salmon tissue can increase lipid content of some macroinvertebrates such as chironomids and stoneflies, which assists in overwintering (Heintz et al. 2010). Larger body size and fatness of macroinvertebrates in

combination with increased secondary production (e.g. chironomids) from nutrients and energy creates a favorable prey environment for resident stream fish.

Resident stream fish can also consume energy directly by eating eggs or carcass flesh rather than indirectly by eating supplemented prey. Consumption of salmon carcasses and eggs can increase the lipid content of juvenile fish; this energy can be allocated into storage or enhance growth of juveniles (Heintz et al. 2004, Heintz et al. 2010). Densities, biomass, and growth rates of some resident stream fish such as brown trout, dolly varden, and sculpin increase in streams with the influx of salmon spawners, where diets are composed mostly of salmon eggs, an energy rich source (Ivan et al. 2011, Rinella et al. 2012, Koshino et al. 2013, Swain et al. 2014). Moreover, fish consuming tissue and eggs can maintain their body mass overwinter, indicating SDE acts as an important subsidy for overwintering, such as the case with cutthroat trout and dolly varden exposed to Pink salmon carcass treatments (Wipfli et al. 2003). Increased body mass from SDE implies the ability for fish to have higher chances of reproductive success and survival (Wipfli et al. 2003, Wipfli et al. 2004).

Potadromous salmon have received less attention than their anadromous counterparts, especially in the Laurentian Great Lakes region. Pacific salmon were introduced in the late 1960's for sport and commercial fishing, retaining their migratory behavior. There have been a limited number of studies examining how Pacific salmon energy and nutrients affect stream energy budgets in Michigan coastal streams. Schuldt and Hershey (1995) found salmon carcass were an important nutrient source in oligotrophic tributaries of Superior, while Ivan et al. (2011) concluded that more nutrient rich areas will be relatively unaffected by salmon-derived nutrients. Ivan et al. (2011) also documented that Pacific salmon could be a strong contender as an energy source in lower peninsula streams, where eggs increased brown trout density and energy content.

Hildebrand (1971) and Collins et al. (2011) documented negative and positive effects from Pacific salmon; spawning tends to disturb benthic communities but can increase the availability of prey to the drift.

There is less information on migratory fish other than Pacific salmon in their natal ranges, and even more so in the Great Lakes region (Flecker et al. 2010). The Great Lakes is home to several other migrants, from introduced to native. Introduced migrants, other than Pacific salmon, include the steelhead. There has been one study that has documented steelhead as a potential energy source, where stream resident brown trout consumed steelhead eggs in spring (Ivan et al. 2011). Native migrants, such as suckers, have not been studied from an energy perspective but rather from a nutrient perspective. Longnose and white suckers have been found to stimulate productivity in some oligotrophic Great Lakes coastal streams (Burtner 2009, Childress et al. 2014, Childress and McIntyre 2015).

Because there are a limited number of studies on migratory fish as energy subsidies in the Great lakes region, it is not clear what the importance of these fish is to stream production or energy budgets. This is particularly alarming with the volatility of Great Lakes stream and lake communities. Dams have been implemented or removed altering fish passage, and there have been declines of migratory fish returns for both introduced and natives. These events can potentially disrupt the energy and nutrient contribution from migratory fish to stream communities. There needs to be more information on the effects of migrants on stream energy budgets to predict the potential impacts from recent events.

EXTENDED METHODOLOGY

Tissue Collection

Chinook eggs, Chinook muscle, and steelhead eggs were obtained from the Little Manistee River Weir in September 2013 and May 2015. Trichoptera and larval white suckers were collected with D-nets placed 3km downstream from Tippy Dam in June 2014. Additional trichoptera were captured from Muskegon River at the Pine street river access with bug nets in June 2015. *Hexagenia limbata* were collected with bug nets in July 2015 with bug nets near CCC Bridge Campground. All tissues were frozen and sent to the lab for analysis.

Tissue Weights

Tissues were thawed after removed from the freezer. Tissues were weighed to the nearest 0.00001 grams using a Mettler XS Excellence scale. Initial wet weights were recorded for each tissue to get an average wet weight for each (Chinook egg n = 50, steelhead egg n=100, trichoptera = 100, *H. limbata* = 100). Larval white suckers could not be weighed because of evaporation variability. Tissues were placed on pre-weighed tinfoil, weighed, then placed in a drying oven at 50 degrees Centigrade for 48 hours, except for Chinook eggs, which were placed in microcentrifuge tubes. Pre-weighed tin foil or tube were subtracted from the tissue to get an individual tissue wet weight. When removed from the oven, each tissue including the tinfoil or tube were weighed to get an average dry weight. Pre-weighed tin foil or tubes were subtracted from the tissue to get an individual tissue dry weight. Chinook female and male muscle wet weight was based on the average of two filets taken from fish collected at the LMRW in 2015 (G. Parks, Andy's Tackle Box, personal communication). Dry weight for salmon muscle was calculated using equation (1).

1. (1) $$M_w = (W_w/(W_w+D_w)$$

M_w is the percent moisture on a wet basis (75%, Peters et al. 2007), W_w is the wet weight, and D_w is the dry weight.

Pellet Creation

After tissues were weighed, each tissue was combined until ~0.025g was achieved. Then, the tissue was placed in the crucible of a Parr Pellet Press model Z 4027. A lever was pressed downward, where a hammer pressed the tissue into a compact pellet. This process was repeated to make a total of 20 pellets for each tissue, except for Chinook egg, Chinook male muscle, and Chinook female muscle, which were 15, 15, and 14 pellets made respectively.

Semimicrobomb Model

A 1425 Parr Semimicrobomb Calorimeter was used for finding energy density, or the heat of combustion (cal/g) of tissue. An attached 1672 Parr Thermometer was connected to a semimicrobomb for computing precise temperature measurements. Instructions on proper bomb use were followed from the Parr 1425 Semimicrobomb Calorimeter Operating Instruction Manual. Below is a synopsis of that process.

I. *Standardization of the semimicrobomb*

Benzoic acid tablets (6318 cal/g) were used to calculate the energy equivalent, W. A series of tests were run till a consistent W value was obtained. W was calculated as:

$$W = ((m)*6{,}318)+ f)/T$$

Where m = mass of the sample in grams, f = correction of fuse in calories, and T = temperature rise, given by the thermometer. Sample weight "m" is described in "II." Fuse correction "f" is described in "i." The value "W", "m", and "f" were used in the true heat of combustion calculation (ii) of the organism samples.

II. *Sample Pellet and Fuse Weight Before Bombing*

After pellets were created, each individual pellet was weighed to the nearest 0.00001 grams and recorded ("m"). A 10 cm piece of wire (1,400 cal/g) was cut, weighed, and recorded. Protocol proceeded as indicated in the operating manual.

III. *Calculating the Heat of Combustion*

The purpose of the thermometer was for calculation of gross heat of combustion, but calculations were also done by hand to ensure accurate calculation. Below is listed the calculations that were performed to obtain the "true" gross heat of combustion.

i. *Fuse Correction*

After the bomb was run, fuse wire was removed with tweezers, weighed and recorded. 1,400 (cal/g) was the heat of combustion of the wire. The fuse correction "f" was calculated as:

$$f = (\text{Wire weight beginning} - \text{Wire weight end})*1{,}400$$

ii. True Heat of Combustion

True heat of combustion was calculated as:

$$((W*T)-f)/m$$

Where W is the standard value from the benzoic acid trials, T from the temperature rise, f as fuse correction and m for mass of sample. The true heat of combustion is expressed in calories per gram (cal/g).

IV. *Calculating Energy Density*

The average of the dry weights (dw) were multiplied by the averaged values of the true heat of combustion. This gave an average energy content for tissue entering a stream. The calculations were performed as:

Average dw of larval sucker (g) * (average larval sucker cal/g dw)

Average dw of trichoptera (g) * (average trichoptera cal/g dw)

Average dw of ephemeroptera (g) * (average ephemeroptera cal/g dw)

Average dw of Chinook salmon egg (g) * (average Chinook salmon egg cal/g dw)

Average dw of female Chinook salmon muscle (g) * (Average female Chinook salmon muscle cal/g dw)

Average dw of male Chinook salmon muscle (g) * (Average male Chinook salmon muscle cal/g dw)

Average dw of steelhead egg (g) * (Average steelhead egg cal/g dw)

Statistical Analyses

Shapiro-Wilk normality were run on the energy density (n = 20 larval white sucker, n = 20 Trichoptera, n = 20 *H. limbata*, n = 12 Chinook salmon egg, n = 12 Chinook female muscle, n = 15 Chinook male muscle, n = 20 steelhead eggs). Data were normal for all energy densities. Trichoptera were collected in two different years and locations. We tested for differences between years and location with a t-test. Because there was no significant difference, we pooled trichoptera data together for the one-way ANOVA. Chinook eggs were taken from mothers whose muscle was also taken. Thus, the energy densities of the eggs and female muscle were analyzed with Pearson's correlation to find if both tissue energy densities can be used in the one-way ANOVA. Energy densities were tested for homoscedasticity with Levene's test to meet the assumptions of the one-way ANOVA. A Welch's correction (McDonald 2009) was applied due

to lack of homoscedasticity. After the one-way ANOVA, multiple comparison Holm was run as a post-hoc.