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SEASONAL CHANGES IN GROWTH AND THYROID HORMONES IN LAKE SUPERIOR BROOK TROUT

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SEASONAL CHANGES IN GROWTH AND THYROID HORMONES IN LAKE SUPERIOR BROOK TROUT

By

Carla A. Serfas

THESIS

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ABSTRACT

SEASONAL CHANGES IN GROWTH AND THYROID HORMONES IN LAKE SUPERIOR BROOK TROUT

By

Carla A. Serfas

Coaster brook trout (*Salvelinus fontinalis*) are a unique life history variant that were once common in Lake Superior’s waters, but abundance has diminished due to human activities such as overfishing and habitat alteration. Coaster research is ongoing to better understand differences between coaster and resident brook trout. Compared to residents, coasters may have increased overall size, higher growth rates, and differences in concentrations of thyroid hormones (TH) which are key in growth, metabolism, and migration. In addition, differences in these parameters may also be present between types of coasters (migratory vs. non-migratory). The goal of this study was to investigate these traits under controlled laboratory conditions. Three coaster strains and one resident strain of juvenile brook trout were reared under controlled laboratory conditions with subsets of fish sampled monthly. Morphological measurements were used to calculate growth parameters and blood plasma was used to quantify concentrations of TH using enzyme immunoassay. Growth rate did not differ between coaster and resident strains; however, condition and relative weight were higher among some coasters, corresponding to increases in levels of TH before spring and fall. No differences were found in growth or TH between coaster strains. The data suggest that coaster brook trout show increased overall TH, likely linked to metabolism, as compared to residents, but there is little difference amongst coasters.
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LIST OF ABBREVIATIONS

ANCOVA: Analysis of covariance
EIA: Enzyme immunoassay
FL: Fork length
IRV: Iron River strain
K: Condition factor
NIP: Nipigon strain
RGR: Relative growth rate
SIS: Siskiwit strain
T₃: triiodothyronine
T₄: tetraiodothyronine or thyroxine
TBH: Tobin Harbor strain
TH: Thyroid hormones
TL: Total length
TSH: Thyroid stimulating hormone
Wᵢ: Relative weight
Y₁: Sampling Year 1
Y₂: Sampling Year 2
Brook Trout Biology

Brook trout (*Salvelinus fontinalis*), also called brook charr, display morphological and ecological variability depending on their habitat, feeding and life history strategies (Power 1980). Many types of trout have a caudal fin with a deep angular fork, but brook trout have a squared tail with only a slight fork, which is why another common name for this fish is the “square-tailed trout”. This type of tail has a low aspect ratio and was not designed for high speed swimming (Naiman et al. 1987). It is apparently a good adaptation for stream and lake dwelling fish because it helps them turn well in confined spaces (Power 1980).

Brook trout are generally a dark green or grey color with yellowish vermiculations (wavy, pale markings) on their dorsal surface. On the lateral surface, the markings are interspersed with small red dots that are surrounded by blue halos. Ventral to the lateral line in sexually mature brook trout, the color of the body surface can range from pale yellow to a bright orange. Dorsal and caudal fins of these fish have dark spots, while the pectoral, pelvic and anal fins have a reddish-orange appearance with both a black and a white stripe along the leading edge (Behnke 2002). Brook trout that migrate are often more silvery than those that do not. All brook trout have terminal mouths on large heads, which front a streamlined, moderately laterally compressed body (Becker 1983). Generally in smaller streams, adult brook trout can be as small as 13 cm and 43 g or as large as 30 cm and 227 g (Behnke 2002), but on average stream fish are 15-20 cm
(Becker 1983). In larger rivers and/or lakes, brook trout can get as large as 70 cm and 4-4.5 kg with an official world record weight at 6.6 kg (Behnke 2002).

Brook trout are native to the eastern part of North America. Many populations are found in Canadian waters and northeastern parts of the United States. Populations also occur in headwater streams as far south as the Appalachian Mountains (Power 1980). This species has also been introduced to western parts of North America near the Rocky Mountains. The Great Lakes Region has many native populations of brook trout. One reason these locations are good for brook trout is the water quality. Brook trout need cold, clean, clear water to survive. They are usually found in headwater springs, ponds, and spring-fed streams where the temperature is 20ºC or colder (Becker 1983).

Depending on the oxygen demands and spawning requirements, the trout may need cooler or warmer water to swim upstream or maintain egg viability (Power 1980).

Temperature is not the only reason that brook trout inhabit these particular areas. Water flow and substrate composition at near shore areas is also vital to the survival of the species. Based on behavioral observations that showed higher densities of brook trout and competition for cold groundwater sites, Biro (1998) suggests these habitats could be a limiting factor for juvenile brook trout. Brook trout often build nests and spawn in flowing streams and the downstream ends of pools where the water flows through a gravel substrate, particularly riffles which are essentially raised areas of gravel or rock (Power 1980, Becker 1983). There is increased oxygen in these regions and this ensures transport of oxygen and other nutrients to the eggs buried in the nests (Becker 1983). Not only is the substrate type a factor, but according to Borwick et al. (2006), the specific temperature of the substrate can effect location for spawning and habitat use.
Spawning generally occurs in the fall (mid-late October) in the Lake Superior region (Becker 1983), but can be as late as December in the more southern regions of the United States (Power 1980). Non-anadromous brook trout generally spawn annually after they have reached maturity (about 1+ or 2+ years). Age at maturation can vary between males and females because the male fish tend to mature early due to more rapid growth (Power 1980, Naiman et al. 1987) and because for males the investment in reproduction is small. The eggs produced by a female are quite large in size, 3-5 mm in diameter, depending on the number of eggs produced. Generally, a female will produce 300-400 eggs, but the total number depends on the fish size and the size of each egg will vary depending on the total number of eggs. According to Hutchings (2006), female brook trout utilize a common biological tradeoff mechanism by producing eggs at a proportion of their body size (15-20%). In good conditions, 80-90% of eggs will survive (Becker 1983), but more commonly, only 56-61% actually develop (Power 1980) and according to Marschall and Crowder (1996) this is due to differences in substrate size, pH and calcium content of the water. The incubation time for the eggs varies with the temperature of the water, but generally the eggs can hatch in a little over a month. The larvae stay buried in the nest until their yolk sac is depleted and then emerge into the water column. This may occur anytime from January to April, depending on when spawning began and the temperature of the water. During this larval stage, the fish are only about 20 mm long and survival is commonly low (Becker 1983). Subsequent growth and survival is limited by the nutrients available and other environmental factors. Finding brook trout over the age of 3 years in stream habitats is generally rare due to competition from introduced species (Taylor and Ferreri 1999), angling mortality (Becker...
1983), and habitat destruction (Newman and DuBois 1996) as well as natural environmental limitations.

Though the basic biology of all brook trout is the same, adaptation to environmental factors has generated differing life history patterns. Depending on the diverse habitats and resources available to them, different life history traits can appear in the same species of fish (Behnke 1980), and even coexist within the same population. For example, in smaller bodies of water both migrant and resident forms of brook trout can be present within the same population. In Lake Superior (Fig. 1), one can find adfluvial brook trout populations (e.g., Salmon Trout River) that migrate between a lake and a stream, as well as lacustrine brook trout populations (e.g., Isle Royale Tobin Harbor or Lake Nipigon) which reside in the lake and spawn on the shallow shoals (Becker 1983). The Lake Superior area also contains many permanently stream-resident fish (e.g., Iron River population) that never leave the stream in which they were initially hatched (Power 1980). Some brook trout also utilize the anadromous life history pattern that many other salmon and trout commonly demonstrate, though none are found in Lake Superior because anadromous salmonids are born in freshwater streams, migrate to saltwater as juveniles to grow, and ultimately return to freshwater as adults to spawn (McCormick et al. 1985, Boula et al. 2002). These anadromous brook trout are often called ‘salters’ in Massachusetts and ‘sea trout’ in Canada. It has been suggested that migration circumvents resource limitations by providing more living space and less competition (Naiman et al. 1987). Hendry and Stearns (2004) proposed that poor food sources and increased competition in natal streams can lead to a migratory pattern in salmonids. Morinville and Rasmussen (2006) suggested that there is wider habitat
occupancy when more than one metabolic form (migratory vs. non-migratory) of fish is present. Other studies agree and show that temporary movement can greatly enhance the ability for growth by utilizing habitats that are rich in food sources and poor in competing species (Power 1980, Rose 1986). A specific advantage of this strategy is that migrant fish are generally larger than mature stream residents and benefit from higher specific fecundity (Morinville and Rasmussen 2003, Northcote 1997).

It is clear that there are environmental determinants of these different brook trout life history strategies and recent evidence showed that there is no genetic difference between these types of brook trout, including a unique life history variant of brook trout called coasters (D’Amelio and Wilson 2008), although this is still debated. The “coaster” brook trout were given this name because they have a tendency to occupy near-shore areas in Lake Superior (the coast) for all or part of their lives (Becker 1983); the term includes both the adfluvial and lacustrine brook trout discussed earlier. It is partially due to this near-shore occupancy, as well as their larger size, that by the 1860’s anglers noticed that in certain areas of Lake Superior catches of these fish were diminished and by the early 1900’s coasters had been eliminated from much of their range (Newman and Dubois 1996, Newman et al. 1999). These fish are avid feeders and this attribute combined with the easy access to the fish congregated near the mouths of rivers and streams led to large catches from Lake Superior’s waters (Newman and Dubois 1996). Increased logging, mining and damming of rivers helped to decrease the numbers of coasters as well by changing the specific habitat in which they fed and spawned (Newman and Dubois 1996). Also, introduced exotic species such as rainbow trout (Oncorhynchus mykiss) (Rose 1986) and Pacific salmon (O. tshawytscha) (Taylor and
Ferreri 1999) may have led to competition for resources. In addition, parasitic species such as the sea lamprey (*Petromyzon marinus*) have also had an effect on not just the coasters, but on many other fish in Lake Superior (Curtis 1990). Currently, under the direction of several government agencies and the close watch of the Great Lakes Fishery Commission, the remaining coaster populations are being protected and restocked (Newman et al. 2003). Hatcheries breed coasters from broodstocks taken from the Lake Superior basin that include two lacustrine strains, Nipigon (Lake Nipigon, ON) and Tobin Harbor (Isle Royale), and the adfluvial Siskiwit strain (Isle Royale). The adfluvial Siskiwit fish are bred from a known migratory population (Newman et al. 2003), however, currently, the migratory habits and physiology of the lacustrine coasters are still not understood in detail.

**Migratory Behavior, Physiology and Growth**

The behavioral patterns and physiology before, during and after migration have been studied in depth in many salmonid fishes. However, most research goes into the more commercially important, anadromous fish such as Atlantic salmon (*Salmo salar*) (McCormick et al. 1998), Arctic charr (*Salvelinus alpinus*) (Damsgård 1991) and many Pacific salmon (*Oncorhynchus* sp.) (Beacham et al. 1996, Withler et al. 1995). For these salmonids and other anadromous fishes, particular changes must be made before they can make the journey into a saltwater system. The preparation for downstream migration is referred to as smolting or the parr-smolt transformation and is the basis for the migration models in salmonid species. Essentially, smolting is the physiological, behavioral and morphological suite of changes that occur prior to downstream migration of juvenile
anadromous salmonids and is regulated by hormonal (Folmar and Dickhoff 1980) and environmental (Whalen et al. 1999) interactions.

One such change is in the outward appearance of the fish. Before becoming a ‘smolt’ the young fish are known as ‘parr.’ This term is used to characterize the fish because vertical stripes called parr marks develop on their sides. During the transformation process, these marks fade and the fish becomes more silvery in color as a result of an increase in guanine crystals and the deposition of hypoxanthine in the scales (Boula et al. 2002). This coloration is better camouflage for life in the open sea because light reflects on the scales, which in turn better reflects the open water. This light reflection helps to break up the outline of the fish, making it less visible to predators and prey.

Before moving to seawater, other physiological systems in the body are also altered by the smoltification process. For instance, during the smolting period, the olfactory bulb of the fish is imprinted with odors that help spawning fish return to their natal streams. The olfactory bulb, or rosette, is a conglomeration of folded epithelial cells in the snout of the fish. During the imprinting process, physiological changes in the rosette epithelium allow the fish to detect the specific ‘odor’ or chemical make-up of the water in the stream and retain it as an ‘odor memory’. The detectable chemical composition can include many types of molecules from amino acids, amines, and nucleotides, to minerals, plants, and pheromones (Derby and Sorensen 2008). Due to environmental variations, every stream has its own distinct smell, so imprinting allows salmonid fish to discriminate amongst streams to detect combinations of molecules that are particular to their natal stream (Hasler et al. 1978). This leads mature migratory adult
fish back to their original hatching area for spawning (Lema et al. 2004, Morin et al. 1989). This is a good strategy for survival because it gives the fish a chance to spawn on grounds that have already proven suitable for successful reproduction.

For anadromous species, smolting also requires alterations in osmoregulatory capacities that are essential for relocation to the sea. Gill Na\(^+/K^+\) ATPase activity increases in salmonids during this period to help develop hypoosmoregulatory capacity (Aas-Hansen et al. 2003). Upregulation of Na\(^+/K^+\) ATPase activity has been linked to hormonal changes in Pacific salmon and Atlantic salmon (Iwata et al. 2003), with a peak hypoosmoregulatory capacity during May through June in Arctic charr (Aas-Hansen et al. 2003). Furthermore, McCormick et al. (1985) found that male brook trout have the lowest salinity tolerance in the fall, presumably related to sexual maturation since spawning is restricted to freshwater. They also noted that female brook trout have a greater ability to hypoosmoregulate and ionoregulate when subjected to saltwater, but there was no difference in these capabilities or the activity of gill Na\(^+/K^+\) ATPase in freshwater fish. In studies of ‘salters’ in Quebec, Canada, McCormick et al. (1985) found no difference in gill Na\(^+/K^+\) ATPase activity between migratory and non-anadromous brook trout; however, Boula et al. (2002) found higher gill Na\(^+/K^+\) ATPase in anadromous brook trout. In addition, Claireaux and Audet (2000) found that freshwater brook trout exposed to saltwater in May/June had higher osmoregulatory abilities. Since coaster brook trout are strictly a freshwater migrant, it is not certain how they fit into this analysis.

Other smolting modifications are behavioral although they are physiologically controlled. Reversals of phototaxis (light orientation) and rheotaxis (current orientation),
and decreased aggression are three such changes. When juvenile salmonids are in streams, they tend to prefer shaded areas (negative phototaxis), presumably to avoid predators and to facilitate their own prey capture. This tendency declines during smoltification, and the fish move to more open water, allowing them to begin forming schools (Iwata 1995). In addition, the behavioral tendency to be aggressive and nip at other fish is decreased during smolting, also helping in the formation of schools (Iwata 1995, Hutchison and Iwata 1998). Dann et al. (2003) attributes the behavioral change in rainbow trout (O. mykiss) to the loss of specific photoreceptors (cones) and a change in visual pigments in the retina which are both potentially regulated by thyroid hormones (TH). It has been shown that TH also play a role in the change in rheotaxis. Before smoltification and migration occur, the fish show a positive rheotaxis, meaning that they swim into the current, facing upstream to maintain their position, but with increased levels of TH during smoltification, the fish orient with the current which assists in downstream movement (Iwata 1995, Specker et al. 2000). A decrease in swimming performance and contractile force of muscle tissues has also been observed with surges of TH making resisting the current in the stream more difficult and thus assisting downstream migration (Katzman and Cech 2001). Other physiological changes related to swimming performance include increases in levels of metabolic enzymes such as white muscle phosphofructokinase and lactate dehydrogenase, which are responsible for the regulation of energy generation and growth (Leonard and McCormick 2001).

Changing lipid stores and metabolic processes is essential for energy allocation and growth for smolts. Herbinger and Friars (1991) found correlations between lipid content and condition factor (body shape/stockiness) in Atlantic salmon and suggest that
condition can be used as a suitable non-lethal indicator of energy reserves in juvenile salmonids. Smolting salmon generally show an increased growth rate with a simultaneous decrease in condition factor. This indicates an increase in length to weight ratio (Johnston and Saunders 1981). Basically the fish become larger, but more streamlined in shape (less stocky) and this new morphology gives them better protection from predators and an increased capacity for energy storage and usage, as well as better swimming capability in the ocean because of the streamlined shape (Johnston and Saunders 1981).

Fish display indeterminate growth; as long as resources are available, they will continue growing in size. Conditions are not always favorable, however, and there can be a wide range of growth rates within and between populations of fish (Weatherly and Gill 1987). There can be many environmental factors that influence the physiological aspects of growth rate and condition in brook trout such as time of year (Hutchings 2006), length of growing season, stocking densities, food supply, and angling (Power 1980). Intrinsic factors such as genetic strain differences (Power 1980) and sex of the fish (Hutchings 2006) can also affect the growth rate of brook trout. It has been shown that larger, more aggressive salmonids can create both intraspecific (Rose 1986) and interspecific competition (Johnsson et al. 1996, Cutts et al. 1998, Gilmour et al. 2005, Sloman and Armstrong 2002, Biro et al. 2006) that can lead to specialized niche adaptations (Skulason and Smith 1995) and migratory life history strategies (Power 2002). Fish that utilize a migratory life history strategy tend to (eventually) be larger because they are moving temporarily to find better food sources, therefore, increasing their growth rate and size (Naiman et al. 1987, Hendry and Stearns 2004). Yet, while the
migratory lifestyle does have its benefits, there is at least one major risk involved: the chance of becoming prey is greatly increased. In addition, Hutchings (2006) suggests that faster growing individuals are often at greater risk for high mortality because their increased foraging efforts put them at risk to become prey, but if they do survive, they reap the benefits of a larger body size and therefore lessen their chances of becoming prey.

Since some coaster brook trout in Lake Superior have migratory life history patterns they may possess some smolt-like characteristics. Certain traits such as silvery color change and increased size are general exterior markers of smolting salmonids and can be seen in non-anadromous or coaster brook trout, but these changes are more prominent in anadromous brook trout (McCormick et al. 1985). Changes specifically related to migration such as the imprinting process, decreased condition factor, decreased muscle contraction, as well as phototaxis and rheotaxis reversals would benefit these migratory brook trout. One would expect the freshwater brook trout to have a surge in TH during this period similar to their anadromous counterparts to support these alterations. However, it is currently undetermined if such differences are present between resident and coaster strains of brook trout.

**Thyroid Hormones in Salmonid Fishes**

Intrinsic factors and environmental conditions affect the outward appearance and growth rate of brook trout, but are mediated through changes in the endocrine system. A particular family of hormones that are linked to growth and metabolism are thyroid hormones (TH). All vertebrates have the same primary TH. There are two different
forms: 5',3,5-L-triiodothyronine (T₃) and 3',5',3,5-L-tetraiodothyronine (thyroxine; T₄).

These hormones are derived from the amino acid tyrosine. Both forms are lipophilic, meaning that they will dissolve readily in a lipid environment, so they commonly travel through the blood bound to plasma proteins. Thyroid follicles do not generally secrete large amounts of T₃, but moderate amounts can be secreted from the liver (Leatherland 1994). Thyroxine (T₄) is directly produced by thyroid follicles as a response to hormonal signals from the hypothalamus and pituitary gland and is controlled by a negative feedback loop. Thyroid stimulating hormone (TSH) is released from the pituitary and causes the release of T₄ from the thyroid follicles that are scattered near the ventral aorta and into the branchial arches (Hoar et al. 1979). Thyroxine is then converted to T₃ outside the follicles by deiodinase in tissues such as the liver, kidney and gills (Leatherland 1994). Both forms of TH serve as active hormones throughout the body, but T₃ tends to be more active due to its greater affinity for receptor sites (Arcand-Hoy and Benson 1998, Leatherland 1994). Also, as a result of increased deiodinase activity in the brain at critical points in development (such as changes in migration characteristics), T₃ may play a regulatory role in the brain (Specker et al. 2000) as a negative feedback mechanism for the hypothalamic-pituitary-thyroid axis (Kawakami et al. 2003).

Nuclear receptors for TH form a heterodimer with RXR, a receptor for 9-cis retinoic acid. In the absence of TH, this heterodimer binds to a section of DNA called the thyroid response element (TRE) and represses transcription of more TH receptors and other linked genes. In fish, these receptors can bind both T₃ and T₄, but similar to mammals, there is a greater affinity for T₃ (Marchand et al. 2001). When TH are present, they bind to the receptor and release co-repressors, stimulating the transcription and
production of more receptors and/or proteins which have many physiological effects that are reviewed below. There are two forms of thyroid hormone receptors, TRα and TRβ, which can each have multiple isoforms (Marchand et al. 2001). Marchand et al. (2001) found that TRβ is less variable than TRα across teleost species, concluding that TRβ appears to be slower at accumulating mutations over time. This indicates that TRβ is more evolutionarily stable and in fact, the genes for TRβ follow standard fish phylogeny, while phylogenetic information on TRα is not yet clear. In addition, Power et al. (2001) and Marchand et al. (2001) both proposed that T₃ binds to TRβ with greater affinity than T₄.

In humans the functions of bound TH are well documented and include major roles in growth and metabolism (Tata 2007). Thyroid hormones demonstrate some of the same effects in fishes, but they are not as clearly understood. For instance, TH trigger eye relocation during metamorphosis and pigmentation of flatfish (Tagawa and Aritaki 2005, Hamre et al. 2005), but the mechanism is not completely understood. Part of the problem in determining the effects of TH is that there are multiple factors which may affect levels of the hormones, such as seasonal changes (Leonard et al. 2002, White and Henderson 1977), energy content (Cameron et al. 2002, Eales and Shostak 1985) and availability of food (Lema and Nevitt 2006), amount of iodide present (Smith and Eales 1971, Mustafa and MacKinnon 1999) and temperature (Eales and Shostak 1986, Aas-Hansen et al. 2003). Pollutants such as PCBs may also alter TH levels (Brown et al. 2004) and possibly impair the regulation of these hormones (Jørgensen et al. 2004).

Although the complete functions and mechanisms of TH in fish are not yet fully understood, much evidence supports that they are a main factor in proper development
and regulation of growth and tissues. In salmonids, TH have been shown to have an effect on cartilage and bone growth in juvenile rainbow trout (Qureshi 1976) and deficiencies in TH can restrict the process of bone formation (LaRoche et al. 1966). On the metabolic side, TH have been shown to have both acute and chronic lipolytic effects in coho salmon (Sheridan 1986) and rainbow trout (LaRoche et al. 1966) in addition to anabolic and catabolic functions dealing with proteins (controlling the amount of nitrogen incorporated into muscle tissue) (Leatherland 1982, Higgs et al. 1979).

There is also strong evidence of the involvement of $T_3$ and $T_4$ in the migration of many types of fish including eels (Edeline et al. 2005), Atlantic cod (Comeau et al. 2001) and salmonids (Tagawa and Iwata 1991, Leonard et al. 2002). In salmonids, TH also help develop olfactory tissues (Morin et al. 1989) and muscle tissue during smoltification (Lema et al. 2004), and they are generally considered one of the triggers of migratory behavior (Tagawa and Iwata 1991, Fujioka et al. 1990). For instance, during imprinting, epithelial cells in the olfactory rosette have an increased number of TH receptors which actively respond to thyroid hormone surges that occur during smoltification (Lema et al. 2004, Morin et al. 1989). Lema et al. (2004) tested this by elevating $T_3$ levels through the implantation of a hormone filled pellet underneath the skin of coho salmon ($O. kisutch$) in the juvenile parr form. By using an enzyme-linked immuno-absorbent assay (ELISA) and a cell birth dating technique (BrdU) they examined levels of TH and found that the pellets not only increased circulating $T_3$ levels but also increased olfactory cell proliferation. Though the link between increased plasma TH levels and olfactory imprinting is not definitive, substantial evidence suggests that olfactory learning is associated with increased thyroid hormone activity (Scholz et al. 1976, Morin et al. 1989,
Nevitt et al. 1994, Dittman and Quinn 1996, Lema et al. 2004). For instance, coho salmon are unsuccessful at homing to artificial odorants when exposed at early life stages when thyroid activity is comparatively low (Nevitt et al. 1994). Yet, similar to Lema et al. (2004), artificially increasing T$_4$ to smolting levels has been shown to stimulate imprinting, even in early life history stages (Nevitt et al. 1994).

A significant rise in T$_4$ levels occurs during the parr-smolt transformation that takes place prior to downstream migration as well as during the upstream migration back to the spawning grounds (Boula et al. 2002, Tagawa and Iwata 1991, Iwata and Tagawa 1991, Høgåsen and Prunet 1997). When juvenile coho salmon (*O. kisutch*) in the lab were implanted with T$_3$ pellets under their skin, the increased hormone concentration caused a significant decrease in the swimming performance as well as in the contractile forces of the muscles of the fish, making resisting current more difficult (Katzman and Cech 2001). Studies of salmonids conducted in natural habitats also show a significant increase of T$_4$ before and during the beginning of migration (Fujioka et al. 1990, Iwata et al. 2003).

Osmoregulatory changes for moving from freshwater to saltwater are also associated with surges of TH. When concentrations of TH increase during smoltification, gill Na$^+$/K$^+$ ATPase also increases in the gills. However, TH are not the primary mechanism for this upregulation, rather they work in conjunction with other hormones involved in growth such as growth hormone (GH) and insulin-like growth factor (IGF) (McCormick 2001).
The main hormones involved in the change in rheotaxis and phototaxis described earlier are TH. When chum fry were administered high levels of T₃ (Iwata 1995), their orientation changed to swimming head first downstream. Specker et al. (2000) also noted increased levels of T₄ in Atlantic salmon correlated with a change in rheotaxis. For phototaxis, Iwata et al. (1989) demonstrated that chum salmon and coho salmon smolts that received T₄ and T₃ treatments, respectively, preferred open water rather than shaded areas. Both of these behaviors promote schooling behavior in the migrating fish, but in order for groups of fish to do this, there must also be a decrease in aggressive behavior. As smolting progresses and levels of TH increase, territorial aggression decreases not only within the species, but among multiple species (Iwata 1995, Hutchison and Iwata 1998).

Migration, Growth, TH, and Coasters: The Connections

At present, little is known about the migratory patterns of coaster brook trout. Multiple studies have physically tracked them as they move (Kusnierz 2008, Stimmell 2006, Huckins and Baker 2008), but it is unknown what physiological changes occur in this unique fish during these migratory periods and what processes trigger the movement. Previous studies show that in other migratory salmonids thyroid hormones are a major component of the growth, smolting and migratory processes. Even though migratory coasters do not travel to the sea, some still migrate out of streams into very large bodies of water (e.g., Lake Superior) (Becker 1983). Evidence of fall juvenile migration has been documented in both potamodromous (Kusnierz 2008, Stimmell 2006) and anadromous (Smith and Saunders 1958, Curry et al. 2002, Lenormand et al. 2004) brook trout. Due to this migratory life history pattern, it is possible that coasters may show
greater growth accompanied by decreased condition and increased TH during periods of
preparation for migration and spawning. These changes may be important for these
potamodromous migrants in the same way they are important for anadromous brook trout
(i.e., homing, downstream migration, etc.). If these changes are present they should be
most noticeable in the fall because of the metabolic changes needed for spawning and fall
juvenile migration. If coaster brook trout are similar to their anadromous counterparts,
changes may also be evident during the spring preparation for migration.

Investigation of growth parameters and levels of TH in coaster brook trout may
lead to a better comprehension of the process of migration in a facultative, partial
migrant. In addition to understanding the basic mechanisms involved with this
expression of life history variation, studying growth and hormone levels in these fish may
be helpful in the rehabilitation effort of coasters. If growth and TH are different in the
coaster strains of brook trout compared to the resident strain, hatchery managers may be
able to use this to their advantage when breeding coasters to be stocked into Lake
Superior. Given the associations between TH, growth, reproduction, migration,
development, triggering and imprinting, hatchery managers could potentially use TH as a
supplemental treatment to better enhance the fitness of the coaster brook trout strains that
they produce. Increasing levels of TH during critical periods may secure the return of the
coaster brook trout to the habitat in which they are released and increase the reproductive
potential and survival of these endemic, unique life history variants of the Lake Superior
community.
FIG 1. Four existing populations of brook trout in Lake Superior. Iron River brook trout are a resident strain. Tobin Harbor and Nipigon brook trout are lacustrine strains. Siskiwit River brook trout are an adfluvial strain.
Chapter Summary

Four strains of brook trout were reared under controlled laboratory conditions to study changes in growth and thyroid hormone concentrations to determine if there are differences between ‘coaster’ and resident strains as well as between coasters types (adfluvial vs. lacustrine). No difference in growth rate was observed over the study period, but both relative weight and condition factor showed peaks during the summer (Jun-Jul) and fall (Oct) following increases of TH. All strains followed similar seasonal patterns, however, all coaster strains were observed to have higher levels of thyroid hormones compared to the resident strain, and in some cases they had higher condition and relative weight. This suggests that coaster brook trout may demonstrate an increased metabolic rate compared to residents although its impact on growth is not clear. In the artificial laboratory environment, all coaster strains followed similar seasonal patterns in growth parameters and concentration of TH. This was somewhat unexpected because adfluvial coasters and lacustrine coasters come from different populations with different life history strategies. This may be due to the artificial environment in which the fish were reared since natural environmental factors may play a role in whether or not a coaster is migratory.
INTRODUCTION

In Lake Superior multiple populations of brook trout are present with diverse life histories. Some are stream residents that are hatched in tributary streams and stay there for their entire lifetime. Others are adfluvial migrants that move from the stream into Lake Superior and return to the stream later, while others are lacustrine that hatch and spend their lives on the shoals of the lake. Both the adfluvial and lacustrine strains are locally called coasters because they spend some or all of their lifetime in the near shore areas of the Lake Superior (Becker 1983). Coasters tend to be larger as adults than resident brook trout and it is to some extent due to this trait that by the 1860’s anglers noticed diminished catches and by the early 1900’s coasters had been eliminated from much of their range (Newman and Dubois 1996, Newman et al. 1999). In addition to overfishing, increased logging, mining and damming of rivers likely contributed to decreased abundance by changing the type and amount of feeding and spawning habitat available (Newman and Dubois 1996). Competition from non-native species such as rainbow trout (*Oncorhynchus mykiss*) (Rose 1986) and Pacific salmon (e.g., *O. tshawytscha*) (Taylor and Ferreri 1999) may have also led to competition for resources and habitat. Efforts to rehabilitate coasters in Lake Superior include restocking using broodstock taken from the Lake Superior basin and protection from angling (Newman et al. 2003).

Several studies have tracked coaster movements, but physiological changes occurring in this unique fish during migration in the adfluvial form or what may trigger
movements is still unknown. The long held hypothesis has been that since coasters show migratory traits, coasters are physiologically similar to other migratory salmonids. Other migratory salmonids show differences in growth, condition, and hormone profiles, including thyroid hormones (TH), associated with the onset of migration (Aas-Hansen et al. 2003, Boula et al. 2002, Folmar and Dickhoff 1980, Hoar et al. 1979, Weatherly and Gill 1987). It is clear that thyroid hormones are a major component of growth and migratory processes (Iwata 1995, Leatherland 1994, Leatherland 1982, White and Henderson 1977) and that these two larger processes are closely intertwined. Given this information, I hypothesized that compared to resident brook trout, coaster brook trout demonstrate greater seasonal growth and these growth changes are correlated to shifts in TH. I also hypothesized that the adfluvial coasters would exhibit patterns in growth and TH similar to salmonid species with migratory life history strategies, while the non-migratory lacustrine coasters would not. If this hypothesis is correct, changes in relative growth and concentrations of TH should be observable in the spring and fall in preparation for migration. To this end, juvenile brook trout were reared under controlled laboratory conditions, from strains derived from hatchery produced populations that express different life history traits.
METHODS

Fish and culture conditions

The procedure described below was performed over two separate years with fish sampled from November 2005 to July 2006 (Y1) and then again from January 2007 to October 2007 (Y2). Fish were obtained from either the Iron River National Fish Hatchery, Iron River, WI (Siskiwit River [SIS] and Tobin Harbor [TBH] strains) or the Marquette State Fish Hatchery, Marquette, MI (Nipigon [NIP] and Iron River [IRV] strains) at the end of their first year of life (0+). The four strains of brook trout were chosen based on their life history strategies and availability: two lacustrine coasters (NIP: Lake Nipigon, Ontario, CA and TBH: Isle Royale, MI, USA), one adfluvial coaster (SIS: Isle Royale, MI, USA), and one stream-resident strain of brook trout (IRV: Iron River, MI, USA) as a reference strain. During Y1, all four strains were included. Due to the availability of the fish, Y2 did not include TBH. During both sampling years, each strain of fish was housed in its own 460L tank connected to the others through a recirculating system that included a particulate filter, biological filter, and UV sterilizer. For Y1, there was one set of four tanks (~200 fish each; one strain per tank). For Y2, the fish were split up into two independent systems, each containing three tanks (~100 fish each; one strain per tank; systems were replicates). The temperature of the water in the tanks was chilled to ~12°C ±1°C, which is similar to late May and late September locally (Stimmell 2006). A natural photoperiod for Marquette, MI (U.S. Naval Observatory website) was simulated using two timed fluorescent overhead lights. The fish were fed a commercial trout diet (BioOregon) ad libitum twice a day as appropriate for their size. Fish were
monitored, fed, and water quality assessed daily under NMU IACUC # 31 and #45 (Appendix). Fish began the experiment at 79-144 mm and tank densities varied from 1.0-18 g/L depending on strain and year (Table 1).

**Sampling setup**

Once a month (~28 days), 5-8 fish per strain were sampled between 1600 – 1630h. The randomly chosen fish were removed and placed in a 75.7 L (20 gal) freshwater tank (dechlorinated tap water) for 24 hours because they also served as a control for a 24 hour saltwater challenge (data not shown). The experimental tanks contained a particulate and a biological filter to circulate the water which was cooled to ~12°C by a small drop-in chiller. During Y2 of sampling, the smaller experimental tank was divided into two chambers by mesh netting in order to divide the duplicate sets of fish. Due to the increased size of the fish, from June 2007 to October 2007 they were separated into two identical 75.7 L (20 gal) tanks without mesh netting, leaving each tank as one replication.

**Sampling Procedure**

After 24 hours in the experimental tanks, the fish were anesthetized by placing them in a solution of 100ppm tricaine methanesulfonate. Lengths (standard, fork, and total) and weights were recorded for each fish as well as other morphological measurements. Depending on the size of the fish, blood samples were collected from the caudal vein using one of two methods: (i) small fish (< 120 mm) were bled by removing the tail with a razor blade and collecting blood into heparinized capillary tubes; (ii) larger fish (> 120 mm) were bled using a heparinized 25 gauge needle and 1 mL syringe. Blood samples were kept on ice until they could be centrifuged at 3000g for 5 minutes to
separate the plasma from the blood cells. Plasma was transferred into a labeled tube and stored in a -80°C freezer for later analysis. Other samples such as white muscle, liver, and gill filaments are also removed from the brook trout for use in the enzymatic analyses of an ongoing related experiment (data not shown).

Sample Analysis

Growth and Condition

Overall increases in total length (TL), fork length (FL), and weight (W) were calculated as percentages using the general equation below where \( X_i \) represents the initial measurement of TL, FL, or W and \( X_f \) represents the final measurement of TL, FL, or W:

\[
\% \text{ increase} = \left( \frac{X_f - X_i}{X_i} \right) \times 100
\]

Relative weight (\( W_r \)) was calculated by dividing the weight of the sampled fish by the standard weight (\( \log_{10} W_s = -5.186 + 3.103 \log_{10} \text{TL} \)) for brook trout (Hyatt and Hubert 2001). The equation used is shown below where \( W \) is weight in grams:

\[
W_r = \frac{W}{W_s}
\]

Relative growth rate (RGR) was calculated as the difference in weight over each growth interval (sampling month). The equation used is shown below where \( TL_2 \) is mean total length at final measurement and \( TL_1 \) is the mean total length at initial measurement in mm. The number of days in the interval was calculated as the number of days in between sampling events.

\[
RGR = \left( \frac{TL_2 - TL_1}{TL_1 \times \# \text{ days in interval}} \right)
\]

This basic equation was also used to calculate relative growth rate based on fork length (FL in mm) as well as weight (W in g) by replacing \( TL_x \) with the preceding variables,
however no differences were found between RGRs calculated with the other measurements so weight based RGR was used for the statistical analysis.

Condition factor (K) was calculated as shown below where again, W is weight in g and TL is total length of the fish in mm (Weatherly and Gill 1987):

\[ K = \left( \frac{W}{T L^2} \right) \times 10^8 \]

*Thyroid Hormones*

Blood plasma was assessed for both total T₃ and total T₄ using enzyme immunoassay (EIA) kits available through MP Biomedicals (Solon, OH). The assays for both were conducted in a 96-well microplate with a limited number of antibody binding sites immobilized on the wells. The T₄ plates were coated with sheep anti-T₄ and the T₃ plates were covered with goat anti-mouse antibody. For both assays there were 6 standards: T₄ ranging from 0-25 µg/dL and T₃ ranging from 0-10 ng/mL. These were used to optimize the assays for fish plasma. By systematically lowering the volume used in each well it was found that T₄ concentrations were detectable to 0.04±0.01 ng/mL and T₃ concentrations were detectable to 0.09±0.01 ng/mL. Based on this information T₄ assays used 5µL of sample and T₃ assays used 20µL of sample. Each standard and sample was tested in triplicate on the plate.

In order to quantify the amount of bound enzyme, and ultimately the amount of plasma TH, absorbance (450nm) was measured with a microplate spectrophotometer. Color intensity was based on the amount of bound conjugate and was inversely related to the actual amount of TH present (Fig. 2). Concentrations of TH were determined using dose response curves based on the standards.
Statistical analysis

Using SPSS 16.0.2 (SPSS 2008), ANCOVA were run to test the following variables: TL, Wr, RGR, K, T4, T3, and TH ratio (T3/T4). In order to compare across strains, fork length (FL) was used as a fixed factor (blocked covariate) to correct for size. In all cases, sampling month and strain were used as fixed factors, while year was considered a random factor. The interaction between strain and sampling month was also included in the model. ANCOVA tests for overlapping sampling months (Jan-Jul/Aug) were run as a custom model using a type IV sum of squares to account for the missing strain in Y2. This type of assessment was also performed within individual years using a type I sum of squares model. Differences between groups were assessed using the conservative Scheffé’s post hoc assessment. The significance level for all analyses was α = 0.05. For some analyses, the Levene’s test for equality of variances was significant, however the results of the ANCOVA were used because the statistical power of the comparisons was very high indicating a decreased chance of Type II error (failing to find a difference when there is one).
RESULTS

Over the entire study there were 13 sampling months spanning from Nov-Oct, however only the Jan-Jul/Aug months overlapped between Y1 and Y2. For concentrations of TH during the Y2 Jul/Aug sampling month, there are no data for SIS and only one fish each for NIP and IRV strains because the fish did not survive the experimental challenge tank. Since Y2 included 2 separate tank systems, differences across systems were tested and because no differences were found (p=0.874), tank sets were pooled for all further analyses.

Fish size

The highest percent weight gain was SIS in Y1, with about a 300% increase in weight (Table 2). The lowest percent weight gain was NIP from Y2 with approximately 97% increase. In addition, these same NIP fish had the lowest overall percent gain in both TL and FL (Table 2).

When testing TL, sampling month for both Y1 (F(9,250) = 80.535, p<0.001, Power =1.000) and Y2 (F(10,169) = 47.277, p<0.001, Power = 1.000) were significant. Both years displayed a seasonal pattern with all fish steadily increasing in length over the study period (Fig. 3). Both years also showed a significant difference among strains (Y1: (F(3,250) = 46.072, p<0.001, Power =1.000), Y2: (F(2,169) = 72.592, p<0.001, Power = 1.000). For Y1, IRV and TBH were not different (p=0.970) and NIP had larger TLs than all other strains (p<0.001) (Fig. 3). All strains in Y2 were different; NIP had the highest TL values compared to all of the other strains (p<0.001) and IRV had higher TL overall than SIS (p<0.001) (Fig. 3).
For the Jan-Jul/Aug sampling months across years, TL differed with both sampling month ($F_{(7,348)} = 66.318$, $p<0.001$, Power = 1.000) and strain ($F_{(3,348)} = 61.290$, $p<0.001$, Power = 1.000). Again, the fish showed steady increases in TL over the study period (Fig. 4). All strains were different from each other in TL except for IRV and TBH ($p=0.738$). NIP had a larger TLs than all other strains ($p<0.001$) (Fig. 4).

**Relative weight ($W_r$)**

For $W_r$, Y1 showed a significant difference in the covariate block (FL) ($F_{(3,236)} = 1.889$, $p=0.003$, Power = 0.999) indicating that length is important such that $W_r$ varies with size. A significant difference among strains was found ($F_{(3,236)} = 2.953$, $p=0.033$, Power = 0.696) in Y1, however the power was only 69.6% and Scheffé’s post hoc test showed no significant differences (Fig. 5). $W_r$ also differed among months during Y1 ($F_{(9,236)} = 21.033$, $p<0.001$, Power = 1.000), where during the summer months (Jun-Jul/Aug), $W_r$ was approximately 1.2 fold higher than the $W_r$ of winter months (Nov-Dec) ($p<0.05$) with a plateau during the months in between (Jan-May) (Fig. 5).

Strain differences in $W_r$ were also found in Y2 ($F_{(2,147)} = 6.190$, $p=0.003$, Power = 0.887) where NIP had significantly higher $W_r$ than the IRV strain ($p=0.003$) (Fig. 5). Sampling month differences were also found in Y2 ($F_{(10,147)} = 28.807$, $p<0.001$, Power = 1.000). Jan-Jul sampling months showed a plateau until the late summer through fall sampling months (Jul/Aug-Oct) where $W_r$ increased approximately 1.2 fold (all $p<0.001$) (Fig. 5).

For $W_r$ during overlapping sampling months across years (Jan-Jul/Aug), the covariate block (FL) was significant ($F_{(39,306)} = 1.950$, $p=0.001$, Power = 1.000), indicating that length is important and $W_r$ varies with size. $W_r$ was no different among
the four strains of fish studied ($F_{(3,306)} = 1.541$, $p=0.204$, Power = 0.405) (Fig. 6). $W_r$ differed among sampling month ($F_{(7,306)} = 4.417$, $p<0.001$, Power = 0.992) and showed a seasonal pattern where the $W_r$ in summer sampling months (Jun-Jul/Aug) increased about 1.2 fold compared to late winter through spring sampling months (Jan-May) ($p<0.05$) (Fig. 6).

**Relative growth rate (RGR)**

Analysis of separate year RGR showed no differences among strain in either Y1 ($F_{(3,18)} = 0.608$, $p=0.618$, Power = 0.152) or Y2 ($F_{(2,5)} = 0.232$, $p=0.081$, Power = 0.071) (Fig. 7) and Levene’s test of equality of variance was significant for Y2 ($F_{(4, 25)} = 27.425$, $p=0.003$). Y1 did show a significant difference in RGR among sampling months ($F_{(8,13)} = 2.796$, $p=0.048$, Power = 0.735), but Scheffé’s post hoc did not find any differences (Fig. 7). Y2 did not have a large enough sample size to run an ANCOVA to detect differences in sampling months. For overlapping sampling months (Jan-Jul/Aug) showed no significant differences in RGR among strains ($F_{(3,27)} = 0.250$, $p=0.860$, Power = 0.091) or sampling months ($F_{(7,23)} = 1.564$, $p=0.196$, Power = 0.516) (Fig. 8), however the Levene’s test for equality was significant for strain ($F_{(12,39)} = 2.830$, $p=0.028$) most likely because of the low sample size.

**Condition factor (K)**

In the analyses of separate sampling years for K, the covariate (FL) was significant (Y1: $F_{(37,336)} = 2.541$, $p<0.001$, Power = 1.000, Y2: $F_{(42,147)} = 1.901$, $p=0.003$, Power = 0.999). In both Y1 ($F_{(3,236)} = 6.945$, $p<0.001$, Power = 0.978) and Y2 ($F_{(2,147)} = 10.069$, $p<0.001$, Power = 0.984) NIP had a higher K than the other strains (Fig. 9). Both Y1 ($F_{(9,236)} = 31.955$, $p<0.001$, Power = 1.000) and Y2 ($F_{(10,147)} = 40.504$, $p<0.001$, Power
showed seasonal differences in K. Winter through spring sampling months (Nov-May) showed a slow, steady incline before a peak in K in the summer months (Jun-Jul/Aug) where K was approximately 1.4 fold higher (Fig. 9). During Y2, there are three main partitions of sampling months that can be seen with some slight overlap. The winter and spring months (Jan-Apr) have the lowest K, while the early to mid-summer months (May-Jul) increase by about 1.1 fold followed by a subsequent increase of approximately 1.2 fold in the late summer through fall months (Jul/Aug-Sept) (p<0.05) (Fig. 9).

For overlapping sampling months (Jan-Jul/Aug), the covariate (FL) was significant (F(39,306) = 2.806, p<0.001, Power = 1.000), indicating that length impacts K. No differences were found between K of strains (F(3,306) = 1.523, p=0.209, Power = 0.401), although the power was very low. Seasonal differences in K were found (F(7,306) = 4.454, p<0.001, Power = 0.992) where the late winter and spring sampling months (Jan-May) showed a slow, steady increase until the summer sampling months (Jun-Jul/Aug) when K increased about 1.1 fold (p<0.05) (Fig. 10).

Thyroid hormones (TH)

$T_4$ – Analysis of Y1 alone showed $T_4$ differences among strains (F(3,158) = 21.059, p<0.001, Power = 1.000) and sampling months (F(9,158) = 31.333, p<0.001, Power = 1.000). In addition, the pattern of the concentrations of $T_4$ over the sampling period differed with strain (F(23,158) = 2.271, p=0.002, Power = 0.996). NIP had higher $T_4$ compared to all other strains (p<0.001). SIS had higher $T_4$ compared to both TBH (p=0.009) and IRV (p<0.001). IRV and TBH were not different (p=0.647) (Fig. 11).
Fish in the winter through early spring months (Nov-Mar) had T\textsubscript{4} concentrations about 1.75 times higher than fish in the late spring through mid-summer months (May-Jul) (Fig. 11).

Analysis of Y2 alone also showed T\textsubscript{4} differences among strains (F\textsubscript{(2,102)} = 8.505, p<0.001, Power = 0.962) and sampling months (F\textsubscript{(10,102)} = 6.567, p<0.001, Power = 1.000). NIP and SIS were not different from each other (p=0.664), but both had higher levels of T\textsubscript{4} than the IRV (Fig. 11). Fish in the late winter sampling months (Jan-Feb) had about 1.5 fold higher concentrations of T\textsubscript{4} as compared to fish in the mid to late fall months (Sept-Oct) months, with a plateau during the months in between (Fig. 11).

For sampling months Jan-Jul/Aug over both years the covariate (FL) was significant (F\textsubscript{(39,255)} = 1.611, p=0.017, Power = 0.996). Differences in T\textsubscript{4} among sampling months (F\textsubscript{(7,255)} = 9.019, p<0.001, Power = 1.000) and strains were found (F\textsubscript{(3,255)} = 15.687, p<0.001, Power = 1.000). Late winter sampling months (Jan-Feb) were more similar to each other with T\textsubscript{4} concentrations approximately 2 fold higher than early summer months (Jun-Jul) (Fig. 12). NIP had higher concentrations of T\textsubscript{4} than either IRV or TBH (p<0.05). IRV also had significantly lower concentrations than SIS (p<0.001) (Fig. 12).

T\textsubscript{3} – Y1 fish showed T\textsubscript{3} differences among strains (F\textsubscript{(3,149)} = 8.105, p<0.001, Power = 0.990) and sampling months (F\textsubscript{(9,149)} = 14.080, p<0.001, Power = 1.000). In addition, the pattern of the concentrations of T\textsubscript{3} over the sampling period differed with strain (F\textsubscript{(23,149)} = 1.882, p=0.013, Power = 0.982). SIS did not differ from the other strains (p>0.05) and NIP had significantly higher concentrations of T\textsubscript{3} than both the IRV and TBH (p<0.05) (Fig. 13). Summer months (Jun-Jul) had approximately 2 times lower
T₃ concentrations as compared to late winter months (Jan-Feb) (p<0.05). Summer months (Jun-Jul) had the lowest T₃ concentrations over the study period, followed by an approximate 1.25 fold increase in concentrations in Jul/Aug (Fig. 13).

Y2 fish showed T₃ differences among strains (F(2,102) = 8.486, p<0.001, Power = 0.962) and sampling months (F(10,102) = 3.705, p<0.001, Power = 0.993), however the Levene’s test of equality of variance was significant (F(26, 149) = 2.334, p=0.007). IRV had significantly lower concentrations of T₃ than both NIP and SIS (p<0.05), while NIP and SIS were not different (p>0.05) (Fig. 13). The only difference in T₃ for Y2 was between the Feb and Oct months where Feb showed an approximate 2 fold increase in concentrations of T₃ (p=0.001) with a statistical plateau in between these sampling months (Fig. 13).

For sampling months Jan-Jul/Aug over both years, T₃ differences among strains (F(3,250) = 7.154, p<0.001, Power = 0.981) and sampling months (F(7,250) = 6.449, p<0.001, Power = 1.000) were found. NIP and SIS, though not different from each other (p=0.579), had significantly higher concentrations of T₃ compared to TBH and IRV (p<0.001) (Fig. 14). Fish in the late winter/early spring months (Jan-Mar) were more similar to fish in the mid-summer months (Jun-Jul/Aug) (p>0.05) in concentrations of T₃ with a plateau between these periods (Fig. 14). Fish in late spring/early summer months (May and Jun) had approximately 0.6 times lower T₃ concentrations than fish in the late winter sampling months (Jan-Feb) (p<0.05).

**Ratio of T₃/T₄** – Both Y1 (F(3,149) = 1.193, p=0.315, Power = 0.315) and Y2 (F(2,102) = 0.877, p=0.419, Power = 0.197) showed no significant difference in T₃/T₄ ratio among strains (Fig. 15). For sampling months, the analysis of Y1 showed differences
(F_{(9,149)} = 5.934, \ p<0.001, \ Power = 1.000) and in particular the Jul sampling month was approximately 0.4 fold higher than all other sampling months except for Feb (p<0.05) with a plateau in between (Fig. 15). In addition, the pattern of the ratio of TH over the sampling period differed with strain (F_{(23,149)} = 2.349, \ p=0.001, \ Power = 0.997). In Y2, T_3/T_4 ratio differences among sampling months (F_{(10,102)} = 2.128, \ p<0.029, \ Power = 0.881) were detected, however the conservative Scheffé’s post hoc test failed to find any differences (Fig. 15).

For overlapping sampling months (Jan-Jul/Aug) there was no T_3/T_4 ratio differences among strains (F_{(3,250)} = 3.641, \ p=0.101, \ Power = 0.533), but differences among sampling months were found (F_{(7,250)} = 2.113, \ p=0.043, \ Power = 0.801). Overall, the winter through spring months (Jan-May) had approximately 1.2 fold lower TH ratio than summer months Jun and Jul. In particular, Jul had higher TH ratio than all other sampling months except for Jun (Fig. 16). In addition, the pattern of the ratio of TH over the sampling period differed with strain (F_{(20,250)} = 2.167, \ p=0.003, \ Power = 0.991).
DISCUSSION

Growth

All fish in the study gradually increased $W_r$ and $K$ from late fall until mid-summer when they showed a dramatic increase. Both maturing Atlantic salmon (Kadri et al. 1996, Berg and Bremset 1998) and brown trout (*Salmo trutta*) (Berg and Bremset 1998) have shown a similar pattern in $K$ and lipid content with a slow increase over winter months, a spike in summer and subsequent decrease in fall. In other species, growth slowed or stopped during the winter due to appetite changes even though food availability was not limited (Metcalfe and Thorpe 1992, Pottinger et al. 2003). In this study, the coaster NIP strain demonstrated higher growth capacity ($W_r$, $K$) despite a lack of difference in RGR. NIP had larger initial weights and lengths than all other strains (Table 1), and Volkman et al. (2004) also found that age-0 NIP exhibited more growth in length and weight than both SIS and TBH without differences in $W_r$ throughout their 12 week experiment using similar laboratory conditions. In contrast to the present study, Volkman et al. (2004) found a difference in growth rate based on weight where NIP and SIS had higher growth rates than TBH (Volkman et al. 2004). However, the fish in the Volkman et al. (2004) study were younger and reared at colder temperatures compared to the current study.

Thyroid hormones

Strain

In all cases, IRV resident brook trout had significantly lower concentrations of both $T_4$ and $T_3$, supporting the hypothesis that residents have lower levels of TH as
compared to coasters. However, there was no difference in concentrations of TH between the coaster strains (adfluvial vs. lacustrine) despite the differences in life history strategies demonstrated by their parental strains. In addition, McCormick et al. (1985) found seasonal changes in plasma T\textsubscript{4} concentrations in wild anadromous brook trout, but the changes were not different from those observed in wild non-anadromous brook trout.

**Sampling period**

Thyroid hormones have been shown to play a major role in the preparation for migration in salmonids (Iwata 1995, Leatherland 1994) and surges of TH have been documented in anadromous brook trout (McCormick et al. 1985) and other salmonids during the pre-smolting stages before transformation occurs (Folmar and Dickoff 1980, Fujioka et al. 1990, Høgåsen and Prunet 1997, Iwata et al. 2003, Specker et al. 2000).

The data from this study suggest that both T\textsubscript{4} and T\textsubscript{3} concentrations demonstrate a seasonal pattern where large increases of TH precede both spring and fall. Both hormones were highest during the late winter months, particularly February, with decreases throughout the spring, reaching their lowest levels in mid-summer (June). This is followed by a subsequent rise after late summer (Jul/Aug) into early fall (Sept-Oct). These data echo what has been shown in other studies where TH concentrations increase before these growth and migratory periods of salmonid life history (White and Henderson 1977, Morin et al. 1989, Specker et al. 2000, Audet and Claireaux 1992), however most of these fish were also fed *ad libitum*. In addition, these peaks coincide with data found in studies on movement of brook trout in Lake Superior tributaries (Huckins and Baker 2008, Kusnierz 2008, Stimmell 2006) and other similar bodies of water (Smith and Saunders 1958, Morinville and Rasmussen 2003) where most migration starts in late
spring and peaks in mid-fall. However, given that these shifts in concentrations of TH occur in all four strains of brook trout tested (residents and coasters), the changes in TH seem to be more related to growth and energetic resources rather than to migration itself. Additionally, increased T₃ in this study coincides with increases of both Wᵣ and K further suggesting that the increases in T₃ may be required for the growth and metabolism of these fish during these sensitive periods.

*Ratio (T₃/T₄)*

During the mid-summer months (Jun-Jul) there seems to be a relative abundance of T₃ (Fig. 15 and Fig. 16). This pattern is more pronounced in Y1 while Y2 shows the tendency though it is not significant. This may indicate increased conversion of T₄ which could be the result of one or more metabolic processes. Leloup and Lebel (1993) found that in brown trout (*Salmo trutta*) and rainbow trout (*O. mykiss*), T₃ levels and ratio of T₃/T₄ were increased when growth hormone was introduced.

**Conclusions**

In this study there were no differences in the relative growth rates between resident and coaster strains, but there were differences in other growth parameters (TL, Wᵣ, and K) which correlated with clear differences in concentrations of TH. Hutchings (2006) suggests that in a natural environment, growth rate is associated with trade-offs based on habitat and resource availability and in this study those elements were removed. Morinville and Rasmussen (2003) found no differences in growth rate among wild resident and migrant brook trout, however, consumption rates showed that the migrant fish were eating more than the resident fish, yet they had lower growth efficiency. Their findings, in conjunction with the results of this study, indicate that migrants have a higher
metabolic cost than residents, supporting the hypothesis that coaster brook trout demonstrate increased concentrations of TH and that these differences may be linked to increased overall growth and metabolism of these unique fish.

It was also hypothesized that adfluvial/migrant coasters would show patterns of growth and TH similar to other migratory salmonids and lacustrine/non-migrant coasters would not, however the data do not support this proposal. Each coaster strain followed the same seasonal pattern in growth parameters and concentration of TH and there were no differences between coaster strains in this study to indicate that a migrating coaster is different from a permanently lake dwelling coaster as an age 1 juvenile under common rearing conditions. This result was somewhat unexpected because of the different life history strategies practiced by the individual coaster populations used. However, Morinville and Rasmussen (2003) showed that older migrants and residents exhibit differences between age classes where age 2+ migrants had lower growth rates than both age 2+ residents and age 1+ migrants over their first year of life and suggest that there is a trade-off link between metabolic costs and differing life history strategies. Based on these data and the conclusions of Hutchings (2006), the lack of difference between the coaster strains in this study could be attributed to the controlled environment and the short duration of the study period.

One potential limitation of this study is that there was no replication of particular life history traits that are independent of population differences among brook trout. Burnham-Curtis (2000) demonstrated genetic differences in populations of Lake Superior brook trout, but it is unclear how these differences interact with brook trout life history
characteristics. This is clearly an area where broadening the scope of this study to other populations would be beneficial.

By keeping all environmental conditions the same we were able to see differences in these brook trout that may not be obvious in a natural ecosystem. Ultimately, the environment may well play a large role in whether a fish will become a coaster or not. While the distinction in growth parameters and concentrations of TH between migratory/adfluvial and non-migratory/lacustrine coasters is still unclear, the data from this study strongly support the proposal that there indeed is an inherent capacity for coaster brook trout to have increased concentrations of TH compared to resident brook trout which may be linked to metabolism and potential growth.
Table 1. Summary of experimental design variables. Each strain was chosen for its particular life history strategy (C = coaster, L = lacustrine, A = adfluvial, R = resident). S1 and S2 refer to the separate tank systems that were set up during the second study year. The initial size is the mean total length (TL) of the fish when sampling began. Tank densities were calculated as a range because fish were removed throughout the experiment when sampling occurred.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Type</th>
<th>Hatchery Source</th>
<th>Initial Size (TL in mm)</th>
<th>Initial Tank Density (g/L) Year 1</th>
<th>Initial Tank Density (g/L) Year 2</th>
<th>Tank Density (g/L) Year 1</th>
<th>Tank Density (g/L) Year 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nipigon</td>
<td>C-L</td>
<td>Marquette (MI)</td>
<td>116.6</td>
<td>5.6</td>
<td>1.6</td>
<td>5.6 - 15.3</td>
<td>1.0 - 5.3</td>
</tr>
<tr>
<td>Iron River</td>
<td>R</td>
<td>Marquette (MI)</td>
<td>80.3</td>
<td>1.8</td>
<td>3.9</td>
<td>1.8 - 18.0</td>
<td>2.1 - 6.2</td>
</tr>
<tr>
<td>Siskiwit</td>
<td>C-A</td>
<td>Iron River (WI)</td>
<td>69.4</td>
<td>1.2</td>
<td>5.4</td>
<td>1.2 - 7.9</td>
<td>2.2 - 5.8</td>
</tr>
<tr>
<td>Tobin Harbor</td>
<td>C-L</td>
<td>Iron River (WI)</td>
<td>79.2 n/a</td>
<td>1.9</td>
<td>n/a</td>
<td>1.9 - 11.5</td>
<td>n/a</td>
</tr>
</tbody>
</table>
Table 2. Average growth rates (percent) for strains over study period. Both total length (TL) and fork length (FL) were measured in mm. Weight was measured in g. Year 1 (Y1) included all four strains of fish, but for year 2 (Y2) one strain was unavailable; Nipigon (NIP), Iron River (IRV), Siskiwit (SIS), and Tobin Harbor (TBH).

<table>
<thead>
<tr>
<th>Strain</th>
<th>% Increase TL Y1</th>
<th>% Increase FL Y1</th>
<th>% Weight Gain Y1</th>
<th>% Increase TL Y2</th>
<th>% Increase FL Y2</th>
<th>% Weight Gain Y2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIP</td>
<td>102.0</td>
<td>113.4</td>
<td>1171.1</td>
<td>96.2</td>
<td>81.8</td>
<td>973.6</td>
</tr>
<tr>
<td>IRV</td>
<td>141.7</td>
<td>123.3</td>
<td>2124.7</td>
<td>110.7</td>
<td>128.0</td>
<td>1315.2</td>
</tr>
<tr>
<td>SIS</td>
<td>177.3</td>
<td>192.6</td>
<td>3030.2</td>
<td>128.7</td>
<td>114.5</td>
<td>1930.4</td>
</tr>
<tr>
<td>TBH</td>
<td>128.7</td>
<td>145.3</td>
<td>1773.7</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>
FIG. 2. Microplate enzyme immunoassay reaction. (I) Microwell with fixed antibody binding sites (red). Plasma sample or standard containing T<sub>4</sub>/T<sub>3</sub> (orange) and enzyme conjugate (green) added to well. For the T<sub>3</sub> assay, an antibody reagent must also be added (purple). (II) Competition reaction between plasma sample T<sub>3</sub>/T<sub>4</sub> and enzyme conjugate. Incubated for 60 minutes, then liquid is decanted and wells are washed 5 times with distilled water to remove unbound enzyme conjugate and sample. (III) The bound enzyme conjugate reacts with the substrate producing a blue color reaction. When the stop solution is added the liquid in the wells turns yellow and the color concentration is read on a spectrophotometer at 450nm. The amount of T<sub>3</sub> or T<sub>4</sub> present is determined by comparing the result to a standard curve of known concentrations of TH.
FIG. 3. Total length (mm) of brook trout separated by year and organized by strain. These data show the steady growth of all strains of brook trout observed over each study year. Symbols in the plots indicate means with standard error. Statistical differences found using ANCOVA and Scheffé’s post hoc tests are indicated using different letters; strains in capital letters and sampling months in lower case.
FIG. 4. Total length (mm) of brook trout strains sampled monthly between Jan-Jul/Aug over two study years (Y1 and Y2). Symbols in the plots indicate means with standard error. Statistical differences found using ANCOVA and Scheffé’s post hoc tests are indicated using different letters; strains in capital letters and sampling months in lower case.
FIG 5. Relative weight ($W_r$) of brook trout separated by year and organized by strain. These data show the steady growth of all strains of brook trout observed over each study year. Symbols in the plots indicate means with standard error. Statistical differences found using ANCOVA and Scheffé’s post hoc tests are indicated using different letters; strains in capital letters and sampling months in lower case.
FIG. 6. Relative weight ($W_r$) of brook trout strains for sampling months Jan-Jul/Aug during two study years (Y1 and Y2). Symbols in the plots indicate means with standard error. Statistical differences found using ANCOVA and Scheffé’s post hoc tests are indicated using different letters; strains in capital letters and sampling months in lower case.
FIG. 7. Relative growth rate (RGR) of brook trout separated by year and organized by strain. These data show the change in RGR of all strains of brook trout observed over each study year. Symbols in the plots indicate means. ANCOVA found no significant differences for both strain and sampling month for both years.
FIG. 8. Relative growth rate (RGR) of brook trout strains sampled monthly between Jan-Jul/Aug of two study years (Y1 and Y2). Symbols in the plots indicate means. ANCOVA analysis found no significant differences for both strain and sampling month.
FIG. 9. Condition factor (K) of brook trout separated by year and organized by strain. These data show the change in K in all strains of brook trout observed over each study year. Symbols in the plots indicate means with standard error. Statistical differences found using ANCOVA and Scheffé’s post hoc tests are indicated using different letters; strains in capital letters and sampling months in lower case.
FIG. 10. Condition factor (K) of brook trout strains sampled monthly between Jan-Jul/Aug during two study years (Y1 and Y2). Symbols in the plots indicate means with standard error. Statistical differences found in sampling months using ANCOVA and Scheffé’s post hoc tests are indicated using different letters above the plots.
FIG 11. Concentration of T₄ (ng/mL) separated by year and organized by strain. These data show T₄ concentrations in all brook trout strains observed over each study year. Symbols in the plots indicate means with standard error. There are no data for Siskiwit fish in the Y2 Jul/Aug sampling month because the fish did not survive the experimental challenge tank. Statistical differences found using ANCOVA and Scheffé’s post hoc tests are indicated using different letters; strains in capital letters and sampling months in lower case.
Sampling Month
Jan Feb Mar Apr May Jun Jul Jul/Aug
Concentration of T₄ (ng/mL)
0.5 1.0 1.5 2.0 2.5 3.0
Nipigon (Y1)
Iron River (Y1)
Siskiwit (Y1)
Tobin Harbor (Y1)
Nipigon (Y2)
Iron River (Y2)
Siskiwit (Y2)

FIG. 12. Concentration of T₄ (ng/mL) of brook trout strains sampled monthly between Jan-Jul/Aug during two study years (Y1 and Y2). Symbols in the plots indicate means with standard error. There is no data for Siskiwit fish in the Y2 Jul/Aug sampling month because the fish did not survive the experimental challenge tank. Statistical differences found using ANCOVA and Scheffé’s post hoc tests are indicated using different letters; strains in capital letters and sampling months in lower case.
FIG. 13. Concentration of T₃ (ng/mL) separated by year and organized by strain. These data show the concentrations of T₃ in all strains of brook trout observed over each study year. Symbols in the plots indicate means with standard error. There are no data for Siskiwit fish in the Y2 Jul/Aug sampling month because the fish did not survive the experimental challenge tank. Statistical differences found using ANCOVA and Scheffé’s post hoc tests are indicated using different letters; strains in capital letters and sampling months in lower case. Overall, both years show an increase over winter months, decrease over spring in Y1 than in Y2 where concentrations remain steady.
FIG. 14. Concentration of T₃ (ng/mL) of brook trout strains sampled monthly between Jan-Jul/Aug during two study years (Y1 and Y2). Symbols in the plots indicate means with standard error. There are no data for Siskiwit fish in the Y2 Jul/Aug sampling month because the fish did not survive the experimental challenge tank. Statistical differences found using ANCOVA and Scheffé’s post hoc tests are indicated using different letters; strains in capital letters and sampling months in lower case.
FIG. 15. Ratio of $T_3/T_4$ (ng/mL) separated by year and organized by strain. Symbols in the plots indicate means with standard error. These data show the $T_3/T_4$ ratio in all strains of brook trout observed over each study year. There are no data for Siskiwit fish in the Y2 Jul/Aug sampling month because the fish did not survive the experimental challenge tank. Statistical differences found using ANCOVA and Scheffé’s post hoc tests are indicated using different letters above the plots. ANCOVA found no significant ratio differences among strains or sampling months in Y2.
FIG. 16. Ratio of T₃/T₄ (ng/mL) of brook trout strains sampled monthly between Jan-Jul/Aug during two study years (Y1 and Y2). Symbols in the plots indicate means with standard error. Statistical differences found in sampling month using ANCOVA and Scheffé’s post hoc tests are indicated with different letters above the plots. There are no data for SIS fish in the Y2 Jul/Aug sampling month because the fish did not survive the experimental challenge tank.


Kusnierz, P. 2008. The age structure, length, condition, and movement of resident and coaster brook trout (Salvelinus fontinalis) in Pictured Rocks National Lakeshore, Michigan. M.Sc. thesis, Department of Biology, Northern Michigan University, Marquette, MI.


Leloup, J., and Lebel, J-M. 1993. Triiodothyronine is necessary for the action of growth hormone in acclimation to seawater of brown (Salmo trutta) and rainbow trout (Oncorhynchus mykiss). Fish Physiol. Biochem. 11: 165-173. doi: 10.1007/BF00004563.


SPSS. 2008. SPSS for Windows, Rel. 16.0.2. Chicago, IL: SPSS Inc.


October 20, 2005

TO: Jill Leonard  
   Biology Department

FROM: Cynthia A. Prosen, Ph.D.  
   Dean of Graduate Studies & Research

RE: Application to use Vertebrate Animals  
    Application # IACUC 031  
    Approval Period: October 20, 2005 – December 31, 2006

The Institutional Animal Care and Use Committee has approved your project to use vertebrate animals in research entitled “Evaluation of Growth and Smolt Characters in Four Brook Trout Strains.”

If you have any questions, please contact me.

ljh

cc: Biology Department
November 20, 2006

TO: Dr. Jill Leonard  
    Biology Department

FROM: Cynthia A. Prosen, Ph.D.  
      Dean of Graduate Studies & Research

RE: Renewal Application to use Vertebrate Animals  
    Application # IACUC 45  
    Approval Period: October 1, 2006-December 31, 2007

The Institutional Animal Care and Use Committee have approved your renewal for your project to use vertebrate animals in research entitled “Evaluation of growth and smolt characters in four brook trout strains.”

If you have any questions, please contact me.

kjm

cc: Biology Department