Swimming performance of Anadromous Atlantic salmon, *Salmo salar* L., during their spawning migration in the Exploits River, Newfoundland, Canada

by

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Abstract

Swimming performance, muscle activity patterns and plasma non-esterified fatty acid profiles were examined in wild Atlantic salmon (*Salmo salar* L.) during their upstream spawning migration and downstream post-spawning migrations. These studies were conducted on the Exploits River, Newfoundland, Canada between June of 1994 and October of 1996. Significant reductions in sustained and prolonged swimming performances were observed during the upstream migration of adult Atlantic salmon. Associated with the reductions in swimming performance, spawning Atlantic salmon demonstrated higher muscle activity indices for any given swimming speed than non-spawning individuals. The greatest loss of swimming performance, and change in muscle activity was observed for females just prior to spawning. Both swimming performance and muscle activity indices were correlated with observed changes in temperature and body cross sectional area. The change in cross sectional area was more pronounced among females and was related to the final stages of ovarian maturation. Sustained, prolonged and burst swimming performance of Atlantic salmon kelts were significantly lower than those of upstream migrating individuals. Smolts were also investigated. Smolts were capable of swimming significantly faster than adults when swimming speeds were expressed relative to body length.

Total plasma non-esterified fatty acid (NEFA) levels declined significantly between freshwater entrance and spawning, and continued to decline during the post-spawning period. Plasma NEFA levels were significantly higher for females but declined to a greater extent during the upstream migration. The rapid decline in plasma NEFAs among females coincided with the largest increase in their gonadosomatic indices. Differences in the circulating levels of polyunsaturated and saturated fatty acids became evident in males and

females just prior to spawning. At spawning, males and females possessed similar amounts of all plasma NEFAs and these did not changes during the post-spawning period.

Decreases in temperature, changes in body morphology and depletion of lipid (i.e. plasma NEFAs) were observed and recorded during the freshwater migration of Atlantic salmon. Collectively, these factors may have resulted in the pronounced changes in swimming capabilities and muscle activity patterns observed in migrating salmon. The observed changes in the swimming performance and the significant loss of plasma NEFAs suggest that Atlantic salmon may become more susceptible to disturbances in their migrations as they approach sexual maturity and prepare to spawn.

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Chapter 1

General Introduction

To swim, a fish must convert the potential energy contained in substrates such as lipid, protein and carbohydrate into kinetic energy in the form of thrust (Azuna 1994, Hochachka 1994). This process starts in the muscle cells where these substrates are converted to adenosine triphosphate (ATP) (Hochachka 1994). ATP supplies the energy required for muscle contraction. Sequential contractions of muscles on either side of the fish's body create a wave of lateral bending that travels along the length of the fish from head to caudal peduncle. Attached to the caudal peduncle is the caudal fin (i.e. tail). The tail is responsible for transferring the power carried in the propulsive wave to the water (Azuna 1994, Videler 1993). This transfer occurs when the side to side motion of the tail converts muscular power to thrust. When the amount of thrust generated by the tail exceeds the resistance forces (i.e. drag) acting on the fish's body, net positive movement occurs. This mode of locomotion is called swimming.

The magnitude of the resistance forces acting on a swimming fish increase in proportion to swimming speed (Azuna 1995). Power requirements for swimming also increase because of a relationship between the amount of power required to produce thrust and the size of the resistance forces acting on the body. The relationship between the amount of power required for swimming and swimming speed is described by a power function that has an exponent between 2 and 3 (Webb 1995).

Fish meet the power requirements of swimming using anatomically separated regions of red and white muscle, (Bone 1978). For most fish, red muscle represents less than 10% of

the total muscle mass and white muscle as much as 80-90% of the total muscle mass (Bone 1978, Hochachka 1994). Red muscle forms a band of muscle that runs along the length of the fish parallel to its lateral line (Bone 1978). In contrast, white muscle extends throughout the body with most of its mass occurring dorsally where it surrounds and supports the spine (Bone 1978). In regions where red muscle and white muscle meet, a third, pink, muscle is sometimes found. Pink muscle is much smaller in size than red and white muscles and many authors believe that it cannot supply significant amounts of power for swimming (Johnston 1982). For this reason, most studies refer to fish muscle in terms of its red and white muscle groups only (as examples: Bone 1978, Rome *et al.* 1993, Hochachka and Somero 1984, Jayne and Lauder 1994).

The red and white muscles of fish differ in their biochemical compositions and metabolic characteristics. Red muscle is an aerobic tissue that produces ATP through the oxidation of lipid and protein (Bone 1978, Hochachka 1994). Characteristics of the red muscle therefore include large quantities of myoglobin, a high concentration of lipid, abundant mitochondria, high oxidative enzyme activities and an extensive blood supply (Bone 1978, Hochachka 1994). White muscle on the other hand, has an anaerobic metabolism and produces ATP via glycogenolysis (Bone 1978, Hochachka 1994). Characteristics of the white muscle include very little myoglobin, few mitochondria, high glycolytic enzyme activities and a large concentration of glycogen (Bone 1978, Hochachka 1994).

In addition to their biochemical and metabolic differences, red and white muscles differ in their ultrastructural characteristics. Red muscle is composed of slow twitch fibres and white muscle is composed of fast twitch fibres (Bone 1978, Hochachka 1994). Slow

twitch fibres are 50% to 80% narrower, significantly shorter and possess less developed sarcotubular systems and lower myosin ATP*ase* activities than fast twitch fibres (Bone 1978, Hochachka 1994). Because the muscle contraction and relaxation depend on the cycling of ATP, ADP and P_i by myosin ATP*ase* and the cycling of calcium by the sarcoplasmic reticulum, the contraction and relaxation velocities of red muscles are much slower than those of the white muscle (Altringham and Johnston 1990, Hochachka 1994). For example, Akster *et al.* (1985), observed that white muscle fibres of perch (*Perca fluviatilis*) reached their peak contraction 6 times faster and relaxed 4 times faster, than red muscle fibres (Akster *et al.* 1985). Results similar to those of Akster *et al.* (1985), have been reported for other teleosts including the common carp (*Cyprinus carpio*) (Rome *et al.* 1990), largemouth bass (Jayne and Lauder 1994) and rainbow trout (Bone 1978). Collectively, these results suggest that differences in red and white muscle characteristics may also occur in salmonids such as the Atlantic salmon.

As a consequence of their different metabolisms, fibre characteristics and contraction-relaxation velocities, red and white muscles generate different amounts of power (Akster *et al.* 1985). Isolated red muscle fibres, for example, produce 5-7 times less power than isolated white muscle fibres (Altringham and Johnston 1990, Rome 1994). Intact red muscle is smaller than white muscle and therefore contains fewer muscle fibres. As a result, red cannot produce the same amount of power as white muscle (Rome 1994). Based these differences, as well as differences in red and white muscle metabolisms, unique roles have been proposed for red and white during swimming. According to Rome *et al.* (1993), red muscle is best suited to slow swimming which must be maintained for long periods of time (i.e. cruising).

In contrast, the high power production and large size of white muscle makes it suited for swimming at high speeds (i.e. burst swimming) (Jayne and Lauder 1994, Rome *et al.* 1993).

The proposed roles of red and white muscles during slow and fast swimming have been confirmed through measurements of red and white muscle activity using muscle electromyograms (EMGs) (Altringham and Johnston 1990, Bone 1978, Jayne and Lauder 1994, Rome *et al.* 1984, 1993). According to Jayne and Lauder (1994), red muscle activity reaches its maximum levels during continuous slow speed swimming. In contrast, very little white muscle activity occurs at slow swimming speeds. Instead, most of the white muscle activity in fish is recorded during periods of very fast swimming and is associated with some red muscle activity (Jayne and Lauder 1994)

The reason for the activity of the red muscle at high swimming speeds is unclear. According to Jayne and Lauder (1994), the activity of red muscle at high swimming speeds may be a consequence of muscle innervation patterns. Red muscle fibres are innervated by small motor neurons alone, while white muscle fibres are innervated by small and large motor neurons (Westerfield *et al.* 1986). When fish begin swimming, small motor neurons are stimulated and this causes red muscle to become active. Because white muscle is also stimulated by small motor neurons, some white muscle activity may also occur at low swimming speeds. As swimming speeds increase, larger motor neurons are activated in addition to small motor neurons, and white muscle activity increases (Henneman *et al.* 1965). To limit how much of their white muscle is used for slow speed swimming, fish muscle innervation patterns have evolved so that fewer small motor neurons innervate fast muscle fibres than slow muscle fibres (Henneman *et al.* 1965). This innervation pattern ensures that

predominantly aerobic fibres are involved in low swimming and that premature fatigue does not occur from increased levels of white muscle activity.

An additional role for the red muscle at high swimming speeds has been proposed by Hochachka and Somero (1984). According to Hochachka and Somero (1984), red muscle activity at high swimming speeds could play a role in prolonging white muscle activity. White muscle fatigue occurs as a result of muscle glycogen depletion and the accumulation of lactate and protons within the muscle. Red muscle is capable of oxidizing lactate (Hochachka and Somero 1984). Thus, during periods of fast swimming, some of the lactate produced by the white muscle could be transferred to the blood and delivered to the red muscle for oxidation. However, even with red muscle assisting with the clearance of lactate, the rapid hydrolysis of ATP and breakdown of glycogen by the white muscle limits its activity to periods of 20-30 seconds (Hochachka and Somero 1984).

Based on differences in red and white muscle metabolism, endurance and activity patterns during swimming, Beamish (1978) proposed that the swimming capabilities of fish could be classified under the categories of sustained swimming performance, prolonged swimming performance and burst swimming performance. Sustained swimming performance is applied to those speeds maintained for periods greater than 200 minutes without muscular fatigue. When swimming speeds exceed sustained performance levels, significantly more power is required to swim. Based on an examination of red and white muscle activity patterns it is now evident that this extra power comes from the white muscle. As a consequence, the length of time a fish spends swimming outside its range of sustained performance will depend on how active the white muscle is. Since the power required to swim increases faster than swimming speed, greater amounts of white muscle are required for

swimming and endurance decreases rapidly when sustained levels are exceeded (Beamish 1978). Beamish (1978) referred to swimming speeds maintained for a minimum of 20 seconds but no longer than 200 minutes as the prolonged swimming performance (Beamish 1978).

In some instances, the critical swimming speed (U_{crit}) is used to describe the swimming capabilities of fish. The concept of a critical swimming speed was first introduced by Brett (1964) to denote the maximum speed that a fish could maintain swimming for a prescribed period of time. It is obtained by swimming a fish over a range of water velocities that increase at specific intervals until the fish fatigues and then calculation the critical swimming speed using the formula:

$$U_{crit} = V + (t_i / t_{ii})u_i$$

Where: V is the highest velocity maintained for the prescribed period, t_i is the time elapsed at final velocity, t_{ii} is the time increment and u_i is the velocity increment, typically measured in body lengths (bl) (Brett 1964).

At the upper limit of their swimming range fish possess the ability to perform bouts of high speed (burst) swimming. Burst swimming is supported entirely by power supplied by the white muscle and is therefore limited to short periods of time. Beamish (1978) applied the term burst swimming performance to those speeds that fish maintain for periods less than 20 seconds. Collectively, the sustained, prolonged and burst swimming performances describe the overall swimming performance of fish.

Measurements of swimming performance are influenced by numerous environmental (or experimental) factors, including water temperature (Brett and Glass 1973, Beamish 1978, Randall and Brauner 1991), dissolved oxygen content (Beamish 1978, Randall and Brauner

1991) and salinity (Brauner *et al.* 1992, Glova and McInerney 1977, Randall and Brauner 1991). The influences of temperature and dissolved oxygen content may be particularly important in studies of swimming performance because of their influence on metabolic rate and the oxygen carrying capacity of fish blood (Randall and Brauner 1991). Fish are ectothermic and their metabolic rate (and therefore oxygen requirement) increases (or decreases) with increasing (or decreasing) temperature. Unfortunately, as water temperature increases its dissolved oxygen content decreases due the reduce saturation point of water for oxygen. Increased water temperature is also associated with a reduction in the oxygen affinity of fish blood (Satchell 1991). According to Satchell (1991), the decreased solubility of oxygen in fish blood at warm temperatures occurs from a reduction in blood pH. The decline in blood pH causes the oxygen-hemoglobin dissociation curve of fish blood to shift to the right (i.e. Bohr shift) and oxygen-carrying capacity declines (Randall and Brauner 1991). A decline in the oxygen-carrying capacity of fish blood may explain why studies have found that aerobic swimming performance decreases as temperature increases, even though the power produced by warm muscle is greater than that produced by cold muscles (Beamish 1978, Brett and Glass 1973, Randall and Brauner 1991, Rome *et al.* 1992).

In contrast to red muscle, white muscle does not require oxygen to generate ATP, and the relationship between elevations in temperature and blood oxygen level is probably unimportant for its activity (Beamish 1978, Hochachka 1994). Furthermore, the elevation in metabolic rate associated with an increase in temperature is probably unimportant to white muscle, which is only called upon for short periods of time. According to Beamish (1978), white muscle fatigue occurs so rapidly that any effect of temperature on its metabolic probably is probably unimportant and swimming involving the white muscle is largely

independent of temperature.

When water temperature decreases fish are probably not faced with oxygen limitations and are, therefore, unlikely to experience an oxygen-dependent decline in their aerobic (sustained) swimming performance. Nonetheless, declines in aerobic swimming are known to occur when fish are acclimated to cold water (Brett and Glass 1973, Beamish 1978, Rome *et al.* 1984). The most probable mechanism for a loss of aerobic swimming performance during cold acclimation concerns the influence of temperature on the contractile properties of fish muscle. According to Rome *et al.* (1984), the maximal speed of shortening and the amount of power produced by fish red muscle decreases 2 fold for every 10°C decline in temperature. Since the relationship between the power required for swimming and swimming speed does not change with temperature, fish must use a greater proportion of their aerobic muscle to swim when water temperatures are reduced. Because the numbers of red muscle fibres are limited, the increasing power demands for swimming require the recruitment of additional power from the white muscle. Thus, fish will experience some loss of aerobic swimming performance during periods of reduced temperature as a result of white muscle activity.

Declines in anaerobic (burst) swimming performance also occur when fish are acclimated to cold temperatures (Batty and Blaxter 1992). As noted previously, fish recruit more of their white muscle at cold temperatures to compensate for reduced red muscle power production. Since white muscle is also limited in size, the decline of anaerobic (burst) swimming performance probably causes the increased recruitment of white muscle at slower swimming speeds (Webb 1978).

The influence of salinity on the swimming performance of fish has not been well

studied (Beamish 1978). Moreover, studies of swimming performance relative to changes in salinity have produced conflicting results. For example, Nelson *et al.* (1996) found that the swimming performance of cod (*Gadus morhua*) was not affected by changes in salinity. However, decreases in the critical swimming speeds of juvenile coho salmon (*Oncorhynchus kisutch*) have been observed during transfer to seawater (Brauner *et al.* 1992). Atlantic salmon (*Salmo salar*) entering fresh water undergo a significant increase in the hemoglobin oxygen affinity of their blood resulting in a greater delivery of oxygen to their tissues (Maxime *et al.* 1990). Since sustained and prolonged swimming performance utilizes the aerobic muscle, the enhanced oxygen delivery to this muscle following acclimation to fresh water, suggests that these categories of swimming performance may be enhanced. Unfortunately, there have been few studies which have measured changes in the swimming performance of salmonids in response to metabolic adjustments to changes in salinity, and the influence of such adjustments on swimming performance remains unclear (Farmer and Beamish 1969, Madan Moan Rao 1971, Tang and Boutilier 1991).

In addition to environmental variables, biological factors such as body size, body proportions and nutritional condition can influence the swimming performance of fish (Beamish 1978). The influence of body size on swimming performance has been particularly well described and it is generally accepted that the power required to swim increases with fish size (mass and length) (Goolish 1991, Tang and Wardle 1992, Webb 1978). Small fish typically have a greater aerobic performance than larger fish during forced swim trials (Goolish 1991). This probably reflects the greater proportion of red muscle found in small fish compared to large fish (Goolish 1989, 1991). Furthermore, the bodies of smaller fish typically have lower resistance forces associated with them (Azuna 1994). As a result, the

power required by a small fish to swim at a given speed (relative to its body length) will not be as great as that of a larger fish for the same speed (Azuna 1994). Because red muscle makes up a greater proportion of the muscle mass of small fish, and small fish require less power to swim, their red muscle can support a greater range of swimming speeds than that of larger fish. For example, Tang and Wardle (1992) found that the sustained swimming speeds of Atlantic salmon smolts (fork length 15 cm) were approximately 1.6 times higher than those of large Atlantic salmon (fork length 45 cm) even though their red muscles produced one tenth as much power, and their tails generate significantly less thrust.

Body shape and proportions influence the swimming performance of fish through changes in the drag and pressure forces (resistance forces) acting on the body (Azuna 1994, Thomas and Donahoo 1977, Webb 1978). Since the power required to swim depends on the magnitude of the resistance forces acting on the body, small changes in body proportions can greatly increase the amount of power required to swim (Hawkins and Quinn 1996, Taylor and McPhail 1985). In some cases, the increased power required to compensate for the effects of body morphology can actually influence swimming performance. For example, Hawkins and Quinn (1996) found that steelhead trout (*Oncorhynchus mykiss*) had higher critical swimming speeds than cutthroat trout (*Oncorhynchus clarki clarki*). Associated with their higher critical swimming speeds, steelhead trout were found to have smaller heads and longer, deeper caudal regions than cutthroat trout. According to Azuna (1994), the smaller head and longer caudal region of steelhead trout would be better suited to swimming because they would result in laminar flow, and therefore produce smaller amounts of drag (Azuna 1994, Webb 1978).

The influences of biological factors on the swimming performance of fish have been examined, but only for a limited number of fish species (Beamish 1978, Kolok 1992). In contrast, more studies have examined the influence of starvation on fuel availability and the activities of enzymes involved in muscle metabolism (Jezierska *et al.* 1982, Mommsen *et al.* 1980, Lowery *et al.* 1987, Moon and Johnston 1980). Periods of starvation have been correlated with a decrease in intramuscular fatty acid levels (Jezierska *et al.* 1982), plasma fatty acid levels (Ballantyne *et al.* 1996) and muscle protein concentration (Mommsen *et al.* 1980). Starvation imposed on barred sandbass, (*Paralabrax nebulifer*) (Lowery *et al.* 1987) and plaice *(Pleuronectes platessa*) (Moon and Johnston 1980) has been shown to reduce white muscle glycolytic enzyme activity and lower total enzyme binding affinities. In barred sand bass, these changes were associated with a reduction in locomotory activity (Lowery *et al.* 1987).

The effects of environmental and biological factors on swimming performance may be particularly important for anadromous salmonids, which migrate between salt water and fresh water environments, and maintain activity over a seasonal basis despite not feeding. Furthermore, constraints on swimming performance may also be imposed by changes in sexual maturity which occur during this period (Brett 1995, McKeown 1984).

During their freshwater migrations, salmonids stop feeding and endogenous fuel sources are used to supply energy. The use of endogenous fuel sources results in significant losses of energy from the somatic and visceral tissues (Idler and Bitners 1958, Jonsson *et al.* 1997, Mommsen *et al.* 1980). Previous studies involving both Atlantic salmon (Jonsson *et al.* 1997) and Pacific salmons (reviewed by Brett 1995) have found that the greatest loss during migration occurs in body lipid. Lipid is an important fuel for red muscle (Zammit and

Newsholme 1979). Since lipid is also used for gonadal development, the period of starvation that accompanies the upstream migration of salmonids could influence their swimming performance by reducing the amount of lipid available for red muscle activity.

In an attempt to determine the influence of environmental and biological factors on the swimming performance of salmonids, most studies have measured changes in the swimming performance of fish with respect to a single variable (Beamish 1978, Brett 1982, Hawkins and Quinn 1996, Taylor and McPhail 1985, Williams and Brett 1987). However, due to the numerous environmental and biological factors experienced by salmonids in the wild, the influence of any one variable may be masked by the effect of another. For example, Williams and Brett (1987), despite correcting the critical swimming speeds of upstream migrating pink salmon (*Oncorhynchus gorbuscha*) for both temperature and body size, reported a significant reduction in the critical swimming speeds of male and female pink salmon during their upstream migration in the Thompson River, British Columbia. One factor that was not assessed, but could account for the decline in critical swimming speeds among migrating pink salmon, is a change in the biochemical and mechanical properties of red and white muscle.

Critical swimming speed is a category of prolonged swimming performance and therefore depends on both red and white muscles for power (Beamish 1978). Reductions in red and white muscle metabolism have been associated with changes in oxidative and glycolytic enzyme activities induced by starvation (Lowery *et al.* 1987). Furthermore, starvation results in a significant loss of protein from fish muscle and has been associated with a loss of muscle function (Ando *et al.* 1985, Mommsen *et al.* 1980). Thus, the decline

observed in the critical swimming speeds of pink salmon may have reflected the influence of starvation on red and white muscle metabolism.

To date there have been few studies which have examined the sustained, prolonged and burst swimming capabilities of anadromous salmonids (Brett 1964, Brett and Glass 1973, Taylor and McPhail 1985, Williams and Brett 1987). There are even fewer studies that have measured the swimming performance of salmonids in their natural environment (Ellis 1966, Quinn 1988). One reason for the bias towards laboratory studies is the ease at which fish can be obtained and tested, and experimental conditions controlled. In addition, until recently, few methods were available for assessing the swimming performance of fish in their natural environment.

One approach that has been used to measure the swimming speeds of fish outside of the laboratory has been to measure muscle activity and relate this to swimming speed. Muscle electromyograms (EMGs) have been used extensively to study the swimming performance of fish under laboratory conditions (Bone 1966, Rome *et al.* 1984, 1992, Jayne and Lauder 1994, Wardle *et al.* 1995). Laboratory equipment used in the measurement of muscle EMGs require that the fish be hard-wired to an amplifier and recording system. As a result, measurements of muscle activity have traditionally been limited to the laboratory studies. Recent advancements in physiological telemetry technologies now allow muscle EMGs to be recorded from fish in their natural environment. Applications of physiological telemetry are numerous (Kaseloo *et al.* 1992, Demers *et al.* 1996, McKinley and Power 1992, Økland *et al.* 1997, Rogers and Weatherley 1983, Weatherley *et al.* 1996). However, most of these studies have used physiological telemetry to measure the muscle activity of fish in the laboratory and then correlated these measurements with swimming speed and/or metabolic

rate in order to estimate the energy expenditures of fish in the wild (McKinley and Power 1992, Økland *et al* 1997, Weatherley *et al.* 1982).

Recently, muscle EMGs have been used to monitor the muscle activity patterns anadromous sockeye salmon in order to identify difficult stages during their upstream migration (Hinch *et al.* 1996). In this case, however, the criteria used to assess muscle activity patterns in upstream migrating sockeye salmon were based on the calibration with swimming speeds estimated from tail beat frequency (Hinch *et al* 1996). Furthermore, the correlation between muscle EMGs and swimming speeds used by Hinch *et al.* (1996) were obtained using captive sockeye salmon and then compared to wild fish. The swimming performance of captive fish can differ significantly from those of wild fish (Green 1964, Vincent 1960). In addition, when working with salmonids, many of the behavioural and physiological changes associated with their upstream migration may not occur, or at least to a lesser extent when these fish are held in captivity. These changes can include nutritional stress due to the cessation of feeding (Jonsson *et al.* 1997, Mommsen *et al.* 1980), changes in body proportions due to sexual maturation (Brett 1995) and behavioural changes associated with sexual maturation and preparations for spawning (Yamamoto 1969). Consequently, the data reported by Hinch *et al* (1996) may not accurately reflect the actual changes which occur in the muscle activity patterns and swimming speeds of sockeye salmon during their freshwater migration.

In order to report correct estimates of muscle activity, and correctly use these estimates to describe the changes which occur in the swimming performance of migrating salmon, muscle EMGs must be calibrated against the swimming speeds of salmon at various stages during their freshwater migration. In this way, normal changes that occur in the

behaviour and physiology of migrating salmon will be accounted for in the relationship between swimming speeds and muscle activity. Similar conditions would be true of any situation where the swimming performance of an individual is suspected to change and information concerning this change is being investigated by measuring muscle activity patterns (i.e. EMGs).

Studies concerning the swimming performance of anadromous salmonids have largely focused on species of Pacific salmon (reviewed by Webb 1995). Atlantic salmon also undertake extensive freshwater migrations; however there have been fewer studies of swimming performance for this species (Tang and Wardle 1992, Thorstad *et al.* 1997). Moreover, swimming performance studies involving Atlantic salmon have been limited to the laboratory and they offer little information about the swimming performance of migrating Atlantic salmon during their freshwater migrations. Furthermore, they do not allow inferences to be made concerning the changes which occur in the swimming performance of Atlantic salmon due to normal nutritional, behavioral and physiological changes associated with upstream migration.

Due to a paucity of information concerning the swimming performance of Atlantic salmon, the development of rivers used as spawning areas by this species represents a potential risk to the successful upstream migration of this species. Studies investigating the effects of hydroelectric developments on the Columbia River system have found that dams constructed over the past 20 years have caused an increase in the mean river temperature and have decreased mean river discharge (Quinn and Adams 1996). Moreover, these changes in are believed to have altered the arrival and upstream migration patterns of sockeye salmon in the Columbia River over the same period (Quinn and Adams 1996).

Increased water temperatures and altered river discharges are also believed to contribute directly to pre-spawning mortality. Specifically, these factors can increase the difficulty of upstream migration and require anadromous salmonids to expend more energy locomotion (Gilhousen 1990). Since anadromous salmon migrate with limited endogenous energy depots, any increase in energy expenditure has the potential to reduce reproductive success. and in extreme instances, influence survival.

There is already a large database for the swimming performance of Pacific salmon, however, without adequate information concerning the swimming performance of Atlantic salmon, the influences of human activities such as dam construction on this species are largely unknown. One approach that has been used to predict the influence of hydroelectric developments on Atlantic salmon has been to liken them to Pacific salmons and extrapolate current information from them. Although Atlantic salmon and Pacific salmons are believed to have shared a common ancestor (Tchernavin 1939) and continue to share many similarities in their migratory behaviors (i.e. both are anadromous), this comparison may be inappropriate because of the distinctly different migratory characteristics and life histories these salmons now possess. Pacific salmons, for example are semelparous and all individuals die following spawning (Higgs *et al.* 1995). In contrast, Atlantic salmon are iteroparous and some individuals survive spawning. According to Glebe and Leggett (1981), semelparous salmon have different energy requirements and may also have different swimming capabilities than iteroparous salmonids. Semelparous salmon invest large amounts of energy into reproduction and migration, and their migrations are typically undertaken as close to their maximal sustained swimming speed as possible. In contrast, iteroparous salmonids allocate less energy to reproduction and migrate close to their optimal speed (Glebe and

Leggett 1981). Since the swimming performance of anadromous salmon can differ greatly between iteroparous and semelparous forms, the extrapolation of swim speed data between these forms of salmon, for the purpose of fishway design, is clearly inappropriate.

It has been suggested that the lower allocation of energy to reproduction, and the slower migratory speeds may represent a strategy which allow iteroparous salmonids, such as the Atlantic salmon, to conserve energy for the purpose of over-wintering and to fuel a return migration to salt water (Glebe and Leggett 1981). Thus, an important stage in the life cycle of the Atlantic salmon is that which occurs after spawning is completed. Following spawning, spent adult Atlantic salmon (referred to as kelts) over-winter in fresh water before making a return migration back to the marine environment the following spring. Reproductive fitness for these individuals is determined by the number of times they spawn during their life; since the fitness of a single spawning event is considerably lower for an iteroparous salmon than for a semelparous salmon (Glebe and Leggett 1981).

Dams have the potential to decrease the reproductive fitness of iteroparous salmonids by increasing the energy expenditures associated with migration. Excessive energy lost during upstream migration can greatly influence the amount of energy available for over-wintering and the return migration of kelts to sea, and depletion of energy during these periods may lead to increased mortality among kelts. Since the number of surviving kelts is already small (typically less than 15% of the original upstream number of upstream migrants), any further losses can significantly influence the fitness of future spawning generations.

The primary goal of my research was to determine whether the swimming performance of anadromous Atlantic salmon changes during their freshwater spawning

migrations. Because of the concurrent changes which occur in environmental and biological factors during upstream migration, I wished to examine the influence of seasonal changes in temperature, and reproductive dependent changes in body proportions, and relate these to any changes observed in the swimming performance of upstream migrating Atlantic salmon. Atlantic salmon are unique from the Pacific salmons in their ability to survive spawning, and undertake a return migration back to the marine environment. Hydroelectric developments have the potential to reduce the survival of kelts because they often fail to incorporate structures which allow kelts to effectively bypass turbines and dams. Since may of the structures designed to pass kelts through or around dams require some form of swimming, it is important to understand how well this form of Atlantic salmon can swim. Since structures used to pass kelts are typically the same as those used to pass downstream migrating smolts, a comparison between the swimming capabilities of these groups is warranted. Thus, an additional aim of my research was to determine the swimming performance of kelts and compare this with that of downstream migrating smolts. I also compared the swimming performance of kelts with that of adults preparing for their upstream migration to determine the extent to which swimming performance changes during the upstream migration and over-wintering periods.

Anadromous salmonids depend heavily on lipid stored in their tissues during upstream migration, and some (e.g. Pacific salmons) can deplete their lipid reserves (Idler and Bitners 1958). The lipid requirements of migrating Atlantic salmon can differ greatly from Pacific salmons. For example, Jonsson *et al.* (1997) found that Atlantic salmon from the River Daamgard, Norway did not require significant amounts of protein to complete their freshwater migration. In contrast, sockeye salmon have been found to deplete most of their

lipid reserves prior to spawning (Idler and Bitners 1958). Plasma fatty acids are the most readily available form of lipid in fish. However, at present, the changes that occur in plasma non-esterified fatty acid levels during upstream migration have only been reported for anadromous sockeye salmon (Ballantyne *et al.* 1996). In Atlantic salmon, specific fatty acids have been found as necessary components of vitellogenin and in maturing ovaries (Weigand and Idler 1985). In contrast, fatty acids used as fuels by the red muscle are non-specific (Keissling and Keissling 1993). Since male and female Atlantic salmon may have different lipid requirements during upstream migration (Jonsson *et al.* 1991, 1997), but both require fatty acids as fuels for swimming, an investigation of plasma fatty acids levels in Atlantic salmon may provide important information about the importance of lipid during upstream migration. Plasma fatty acid levels may also provide additional information concerning the role of lipid following spawning before kelts leave fresh water, therefore plasma NEFA levels were also investigated in kelts.

Thesis Organization and General Format

This dissertation is divided into 6 chapters. Chapter 1 is a general introduction, including a review of pertinent literature relating to the swimming capabilities, migratory adaptations and basic physiology of salmonids. The main body consists of chapters 2 through 5, each of which is an independent study of anadromous Atlantic salmon during their migration in the Exploits River, Newfoundland. Chapter 6 is a general discussion of my research in relation to what is currently known, and how my work contributes to our understanding of the swimming performance and energy use of anadromous Atlantic salmon

during their freshwater migrations. Lastly, chapter 6 also discusses areas where further research is required.

My objectives for chapter 2: The swimming capabilities of adult and juvenile Atlantic salmon, (*Salmo salar* L.) during migratory stages of their life cycle were, (1) to investigate the swimming capabilities of anadromous Atlantic salmon during periods in their life cycle where migrations are undertaken. This includes the migrations undertaken by smolts and kelts, and the upstream migration of adults. The primary aim of chapter 3: *In situ* measurement of swimming performance in wild Atlantic salmon (*Salmo salar* L.) using radio transmitted electromyogram (EMG) signals, was to quantify changes that occur in the swimming capabilities and muscle activity patterns of Atlantic salmon during non-forced swimming at 12°C and 18°C in fresh water. I also wished to know whether radio transmitted EMG signals could be used to measure changes in the muscle activity indices of Atlantic salmon exposed to different environmental temperatures. Relationships between muscle activity and the swimming performance of migrating Atlantic salmon were further examined in chapter 4: Swimming capabilities and muscle activity patterns of wild Atlantic salmon (*Salmo salar* L.) at different stages of their freshwater spawning migration. The objectives of this study were to determine whether the swimming performance and muscle activity patterns of wild Atlantic salmon are influenced by either normal seasonal changes in temperature or, by sexual maturation, and whether these changes were sex dependent. In chapter 5, Plasma non-esterified fatty acid (NEFA) profiles of male and female Atlantic salmon (*Salmo salar* L.) during their freshwater spawning migration, the plasma non-esterified fatty acid levels of migrating Atlantic salmon were investigated and related to known reproductive events which occur in anadromous Atlantic salmon in order to provide new information about the role of

this fuel during upstream migration. Comparisons were made between the plasma NEFA levels of male and female salmon Atlantic salmon to establish whether plasma non-esterified fatty acid use differs between sexes.

Chapter 2

The swimming capabilities of adult and juvenile Atlantic salmon, (*Salmo salar* L). during migratory stages of their life cycle

Abstract

This study reports sustained, prolonged and burst swimming performances, as well as critical swimming speeds for seaward migrating Atlantic salmon smolts, upstream migrating adult Atlantic salmon and downstream migrating kelts collected from the Exploits River, Newfoundland, Canada. Atlantic salmon smolts possessed higher length dependent sustained swimming speeds (4.39±.0.09 bl sec^{-1}) than either upstream migrating adults (2.51±0.08 bl sec^{-1}) or kelts (0.97±0.04 bl sec^{-1}). Length dependent sustained swimming speeds of upstream migrating adults were significantly higher than those of kelts, even though their lengths were similar. The mean critical swimming speed of smolts was found to be 6.70±0.34 bl sec^{-1}. In contrast, the critical swimming speeds of upstream migrating adults and kelts were 3.67±0.08 bl sec^{-1} and 1.97±0.34 bl sec^{-1}, respectively. The burst swimming speed of smolts was found to be 10.24±0.08 bl sec^{-1}. The critical swimming speed of upstream migrating adults was 4.24±0.14 bl sec^{-1} and was significantly greater than that of kelts (2.67±0.10 bl sec^{-1}). The transition between sustained and burst swimming occurred over the range 0.50 to 2.80 bl sec^{-1} for kelts, 3.00 to 4.10 bl sec^{-1} for upstream migrating adults and between 5.00 and 8.20 bl sec^{-1} for smolts. The present study demonstrates differences in the sustained, prolonged and burst swimming performances between wild Atlantic salmon undertaking migrations at different stages of their life cycle.

Introduction

Anadromous Atlantic salmon (*Salmo salar*) are iteroparous salmonids which are born in fresh water but complete their growth in salt water. The movement from fresh water to salt water is initiated by a process referred to as smoltification (Thorpe 1984). During smoltification, Atlantic salmon parr undergo morphological, behavioral and physiological adaptations for life at sea. Smolts emerge from these adaptations and migrate to the ocean to complete their growth and return to freshwater as adults to spawn.

Iteroparous salmonids differ from semelparous salmonids in their ability to survive spawning. Thus, in addition to the downstream migrations of smolts and the upstream migrations of adults, a third migration is undertaken during the life cycle of Atlantic salmon when post-spawning adults (referred to as kelts) migrate downstream during their return journey to marine feeding areas.

The ability of Atlantic salmon to complete these periods of migration is important to their survival and are dependent on how well they swim. A frequently used method to describe the swimming capabilities of fish is to measure their sustained, prolonged and burst swimming performances (Beamish 1978). These can be obtained by swimming the fish at known speeds until fatigue. According to Beamish (1978) sustained swimming performance includes those speeds which fish can maintain swimming for a minimum of 200 minutes. Swimming speeds maintained for periods less than 200 minutes but more than 20 seconds are referred to as the prolonged swimming performance and burst swimming performance are those speeds maintained for periods of 20 seconds or less (Beamish 1978).

Although it has been possible to measure the swimming performance of Atlantic salmon, there have been only a few studies which have investigated their swimming performance (for example: Økland *et al.* 1997, Thorstad *et al.* 1997, Tang and Wardle 1992, Virtanen and Forsman 1977). Furthermore, most of these have been performed on hatchery raised individuals (Thorstad *et al.* 1997, Tang and Wardle 1992, Virtanen and Forsman 1977). In contrast, there have been numerous studies of swimming performance conducted on Pacific salmons, such as the sockeye salmon, and many of these have involved both hatchery raised and wild fish (see reviews by Brett 1995 and Webb 1995).

The reasons for the bias towards Pacific salmons are unclear. However, a need to provide information about the swimming performance of these species in order to minimize the potential impacts of development in rivers used by these species probably contributed (Quinn and Adams 1996). Specifically, the construction and operation of nearly 30 dams on tributaries of the Columbia river watershed coincided with numerous studies of anadromous sockeye salmon, pink salmon (*Oncorhynchus gorbuscha*) and coho salmon (Fraley *et al.* 1989). At present, similar developments are being considered for rivers used as spawning areas by Atlantic salmon. However, because of the limited information available on the swimming capabilities of this species, the potential threats posed by these developments are largely unknown.

One way in which data can be obtained for Atlantic salmon is to liken them to Pacific salmons and extrapolate information from them. However, this approach may not be appropriate because the historic divergence of these groups has resulted in different life history characteristics characterized by different migratory strategies (i.e. iteroparity vs. semelparity) (Riddell and Leggett 1981). For example, Atlantic salmon can survive

spawning and undertake a downstream migration as kelts. In contrast, Pacific salmons die following spawning. Glebe and Leggett (1981) suggest that iteroparous salmonids allocate less energy to locomotion and reproduction than semelparous salmonids in order to improve the odds of post-spawning survival. One way that this is achieved is by migrating at slower swimming speeds. Thus, in the adult form, Atlantic salmon can be expected to have different swimming capabilities than Pacific salmons. Furthermore, because Pacific salmon die following spawning, the swimming performance of post-spawning Pacific salmon has not been examined.

Because Atlantic salmon allocate less energy to reproduction, their reproductive fitness following a single spawning year will be lower than that of a Pacific salmon. To increase their reproductive fitness, Atlantic salmon must spawn several times during their life. Dams can greatly influence the survival of kelts by restricting their movement to marine feeding areas. Since these individuals have already depleted most of their energy, any barrier to migration can increase the chance of starvation among these individuals. At present, the downstream passage of kelts is facilitated using bypass structures designed to pass downstream migrating smolts. It is unclear whether these structures are effective because comparisons between the swimming performance of kelts and smolts have not been performed.

The most appropriate means of acquiring information about the swimming performance of Atlantic salmon is to measure it directly. This can only be done by conducting measurements of swimming performance from individuals obtained from wild population. The purpose of the present study was to measure the sustained, prolonged and burst swimming performances of Atlantic salmon during three stages of their life cycle when

migrations are undertaken in fresh water. These are the downstream migration of smolts, the upstream migration of adults and the downstream migration of kelts.

Materials and Methods

Animals

Atlantic salmon smolts, upstream migrating adults, and kelts were collected from fish traps located on the Exploits River, Newfoundland, Canada (49°N, 57°W). Collection of upstream migrating adults occurred between June 5th and June 30th, 1995 and kelts and smolts were collected between May 20th and June 30th, 1996. All Atlantic salmon were collected from a fish ladder situated at Bishop's falls, approximately 10 km from the mouth of the Exploits River. Prior to experimentation, Atlantic salmon were held for 24 hours under seasonal light:dark conditions. Smolts and kelts were held in 2 m wide by 4 m long by 1 m deep outdoor tanks, while adult Atlantic salmon collected during their upstream migration were held in tanks 5 m wide, 15 m long and 1 m deep. All tanks were supplied with river water at seasonal temperatures (11°C -14°C). In total, 30 upstream migrating adult salmon (15 male and 15 female), 30 kelts (15 male and 15 female) and 30 smolts were collected for swimming trials. Adult male Atlantic salmon were distinguished from female Atlantic salmon based on the presence of a hook like growth which develops on the lower jaw of sexually maturating males (Yamamoto 1969). The absence of secondary sex characteristics among smolts did not permit accurate sex determination, and individuals were therefore treated collectively. Lengths, weights and girths were measured at the termination of swimming trials. Condition factors (K) were calculated for each Atlantic salmon using the equation provided by Beamish (1978):

$$(K) = (WT / FL^3)*100 \qquad \text{equation 1}$$

where weight (WT) is in g and fork length (FL) is in cm.

Swimming chambers and experimental design

In the present study Atlantic salmon were tested using a Blazka-type swim speed chamber (Blazka 1960). Two swim chambers were used and chosen based on the fork length and girth (GT) of the Atlantic salmon. Smolts (FL=15-25cm, GT=12-16cm) were tested in a 35 litre swim chamber while upstream migrating adult Atlantic salmon and kelts (FL=48-62cm, GT=16-24cm) were tested in a 70 litre chamber. Ambient, untreated river water was supplied to the chamber via an external pump. Water velocity within the chamber was rheostatically controlled and the maximum water velocity could be reached within 5 seconds.

Swimming trials

In the present study swimming endurance and critical swimming speeds were determined for anadromous Atlantic salmon. These tests were conducted on all three forms of Atlantic salmon. Before any measurement of swimming performance or critical swimming speed, Atlantic salmon were allowed 3 hours to acclimate to the swimming chamber. The acclimation speed used was 0.5 bl sec^{-1}.

Swimming endurance was measured by swimming individual Atlantic salmon at a known velocity until they fatigued. The swimming speeds used in the present study were 0.5, 1.5, 3.0, 4.5 and 6 bl sec^{-1} for upstream migrating adults and kelts and 0.5, 4.0, 6.0, 8.0, 10.0 and 12.0 bl sec^{-1} for smolts. The sample sizes used at each velocity were as follows: 6 male and 6 female upstream migrating adults, 6 male and 6 female kelts and 5 smolts. Sustained, prolonged and burst swimming performances are components of swimming endurance (Beamish 1978). These stages were identified from the swimming endurance of

Atlantic salmon using the criteria of Beamish (1978). Sustained swimming performance was applied to swimming speeds which Atlantic salmon maintained for a minimum of 200 minutes, burst swimming applied to those swimming speeds maintained for less than 30 seconds, and prolonged swimming performance applied to those speeds maintained for less than 200 minutes and more than 30 seconds.

The critical swimming speed is a special category of prolonged swimming and is typically used to indicate the maximum swimming speed a fish can maintain for a prescribed period (Brett 1964). Critical swimming speeds were determined by swimming individual Atlantic salmon over a range of increasing speeds until they fatigued. However, before being swum, Atlantic salmon were allowed to acclimate to the swim chamber for 3 hours at a velocity of 0.5 bl sec^{-1}. Depending on the nature of the study, and fish being tested, different criteria can be used to determine critical swimming speeds. In the present study, Atlantic salmon were subjected to water velocities that were increased by 0.5 bl sec^{-1} at 10 minutes intervals, until fatigue. Critical swimming speeds (U_{crit}) were then calculated using the equation presented in Brett (1964):

$$U_{crit} = V + (t_i / t_{ii})u_i \qquad \text{equation 2}$$

Where: V is the highest velocity maintained for the prescribed period (m sec^{-1} or body lengths per second, bl sec^{-1}), t_i is the time elapsed at final velocity (min), t_{ii} is the time increment (min) and u_i is the velocity increment (m sec^{-1}). Critical swimming speeds were investigated using 5 male and 5 female upstream migrating adults, 5 male and 5 female kelts and 10 smolts. Because of the limited availability of upstream migrating adults and kelts, Atlantic salmon used in the measurement of prolonged swimming performance were also used to determine critical swimming speeds. However, these individuals were allowed 24 hours to

recover before additional testing.

Consistent measurements of swimming speeds depend on the consistent recognition of fish fatigue. Atlantic salmon were maintained in a swimming area (approximately 1.5-2 body lengths) by forward and rear non-electrified plastic grates. The criteria used to determine fatigue was the inability of the individual to continue swimming after touching the rear grate, despite three attempts to stimulate swimming by raising and lowering the water velocity.

Data analyses

Swimming speeds for each group were measured as water velocities (m sec^{-1}) and then translated to swimming velocities in body lengths per second. This was accomplished by dividing the water speed by the fork length of the fish. All values are represented as means ± their standard error. The sustained swimming performance, burst swimming performance and the critical swimming speed of male and female Atlantic salmon were compared using paired t-tests. These data were subsequently pooled to allow comparisons to be made with smolts which could not be sexed. Differences in sustained swimming performance, burst swimming performance and critical swimming speed between upstream migrating adults, kelts and smolts were investigated using ANOVAs. Differences in the fork lengths, girths, condition factors, and weights of upstream migrating adults, kelts and smolts were also investigated using ANOVAs. Linear regressions were used to examine the relationship between prolonged swimming speeds and time to fatigue. In all cases the accepted level of significance was $p<0.05$. Significant differences are denoted by plus signs (+), asterix (*) on graphs and by raised names in tables.

Results

Weight, fork length, girth and condition indices for Atlantic salmon are shown in Table 2.1. There were no differences in the fork lengths or girths of upstream migrating adult males and females. However, upstream migrating adult females weighed significantly more than males. Male and female kelts possessed similar fork lengths, weights, condition factors and girths. Upstream migrating adults did not differ from kelts in their fork lengths. They did, however, weigh 1.65 times as much, and possessed 1.2 times as much girth as kelts (Table 2.1). As a result, the condition factor of upstream migrating adults was significantly greater than that of kelts (Table 2.1). As expected, both upstream migrating adults and kelts weighed more, were longer and had significantly greater girths than smolts. Condition factors were similar for upstream migrating adults (0.84±0.04) and smolts (0.88±0.04).

There were no differences between the sustained, prolonged of burst swimming performances or critical swimming speeds of upstream migrating adults males and females or kelts (Table 2.2), and the data were subsequently pooled. Smolts possess the greatest sustained, burst and critical swimming speeds of any group relative to their fork length (Figure 2.1). The mean sustained swimming speed of smolts was found to be 4.39±0.09 bl sec^{-1} compared to only 2.51±0.08 bl sec^{-1} for upstream migrating adults and 0.97±0.04 bl sec^{-1} for kelts. Smolts also possessed the highest burst swimming speed of any groups. Smolts were capable of burst speeds in excess of 10.00 bl sec^{-1} while upstream migrating adults began burst swimming at 4.24±0.14 bl sec^{-1} and kelts at 2.67±0.10 bl sec^{-1}. The critical swimming speed of upstream migrating Atlantic salmon was found to be 3.67±0.08 bl sec^{-1} and was significantly lower than that of smolts (6.70±0.34 bl sec^{-1}). Kelts had the lowest

critical swimming speed of any group (1.97±0.34 bl sec^{-1}). The prolonged swimming speeds of upstream migrating Atlantic salmon, kelts and smolts are shown in Figure 2.2. The transition between sustained and burst swimming occurred over the range 0.50 to 2.80 bl sec^{-1} for kelts, 3.00 to 4.10 bl sec^{-1} for upstream migrating adults and between 5.00 and 8.20 bl sec^{-1} for smolts.

Table 2.1. Weights (WT), fork lengths (FL), condition factors (K) and girths (GT) for Atlantic salmon smolts, kelts and upstream migrating adults. Water temperatures ranged between 11°C and 14°C. Significant differences between males and females are indicated with an asterix (*). Groups which differ significantly are indicated by raised names.

	sex	*n*	upstream migrating adults	kelts	smolts
	M	15	1166.17±63.35*	847.42±35.10	NA
WT	F	15	1591.00±100.38	857.50±57.35	NA
(g)	mean	30	1409.07±84.65 kelts, smolts	852.80±31.44 smolts	49.69±5.55
	M	15	53.92±1.31	52.86±1.45	NA
FL	F	15	55.81±0.62	54.62±1.43	NA
(cm)	mean	30	55.00±0.68 smolts	53.80±1.01 smolts	17.71±0.76
	M	15	0.80±0.05	0.57±0.02	NA
K	F	15	0.91±0.04	0.53±0.03	NA
	mean	30	0.84±0.04 kelts	0.55±0.02 smolts	0.88±0.04
	M	15	26.17±0.48	21.86±0.83	NA
GT	F	15	25.88±0.90	20.86±0.80	NA
(cm)	mean	30	26.00±0.54 kelts, smolts	21.00±0.60 smolts	8.94±0.71

Table 2.2. Critical, sustained and burst swimming speeds of upstream migrating male and female Atlantic salmon and kelts. Water temperatures ranged between 11°C and 14°C during the experiment. There were no significant differences in the swimming speeds of males and females for either upstream migrating adults or kelts. Swimming speeds are in body lengths per second (bl sec^{-1}).

	group	*n*	pstream migrating adults	kelts
critical swimming	F	5	3.61±0.07	2.03±0.05
speed (bl sec^{-1})	M	5	3.73±0.09	1.94±0.04
	mean	10	3.67±0.08	1.97±0.34
sustained swimming	F	6	2.44±0.03	0.95±0.09
speed (bl sec^{-1})	M	6	2.57±0.15	1.00±0.03
	mean	12	2.51±0.08	0.97±0.04
burst swimming	F	6	4.06±0.24	2.51±0.22
speed (bl sec^{-1})	M	6	4.39±0.09	2.78±0.06
	mean	12	4.24±0.14	2.67±0.10

Figure 2.1. Sustained and burst swimming speeds and critical swimming speed of Atlantic salmon smolts (black), kelts (grey) and upstream migrating adults (open). Significant differences upstream migrating adults and kelts are indicated by an asterix (*). Differences between smolts and kelts are denoted by a plus sign (+).

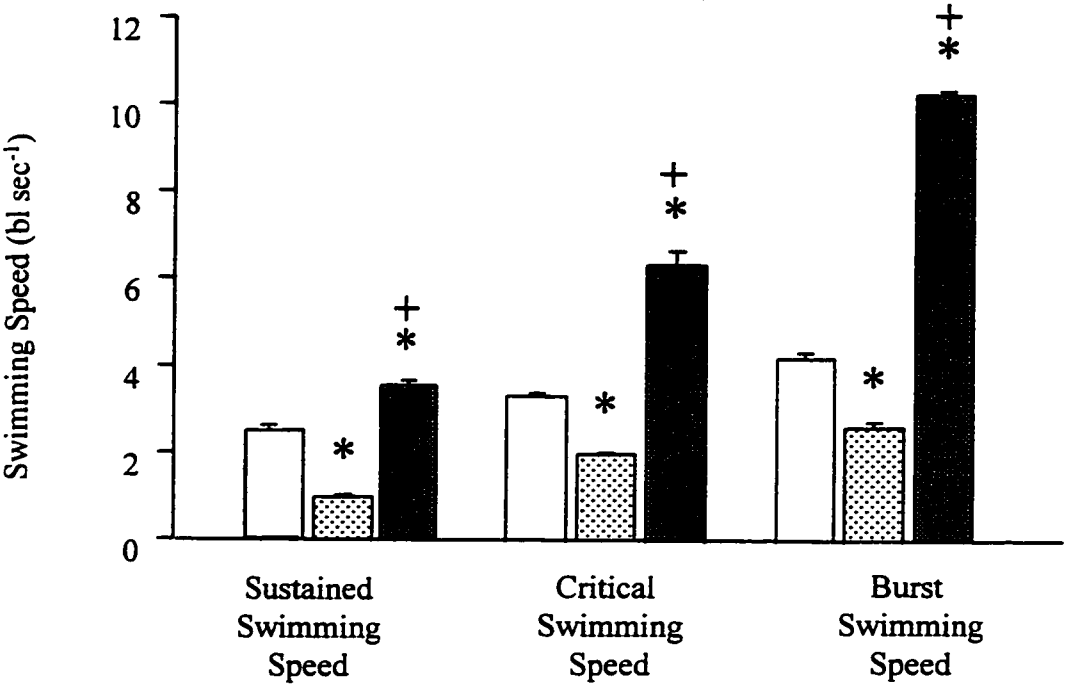

Swimming Speed (bl sec-1)
12
10
8
6
4
2
0
*
+
*
*
+
*
*
+
*
Sustained Swimming Speed
Critical Swimming Speed
Burst Swimming Speed

Figure 2.2. Prolonged swimming speeds of individual Atlantic salmon smolts (upside down triangle), kelts (square) and upstream migrating adults (triangle). Linear regressions are shown for each group. The relationship between swimming velocity (bl sec^{-1}) and time to fatigue (*log* min) for upstream migrating adults is described by the relationship:

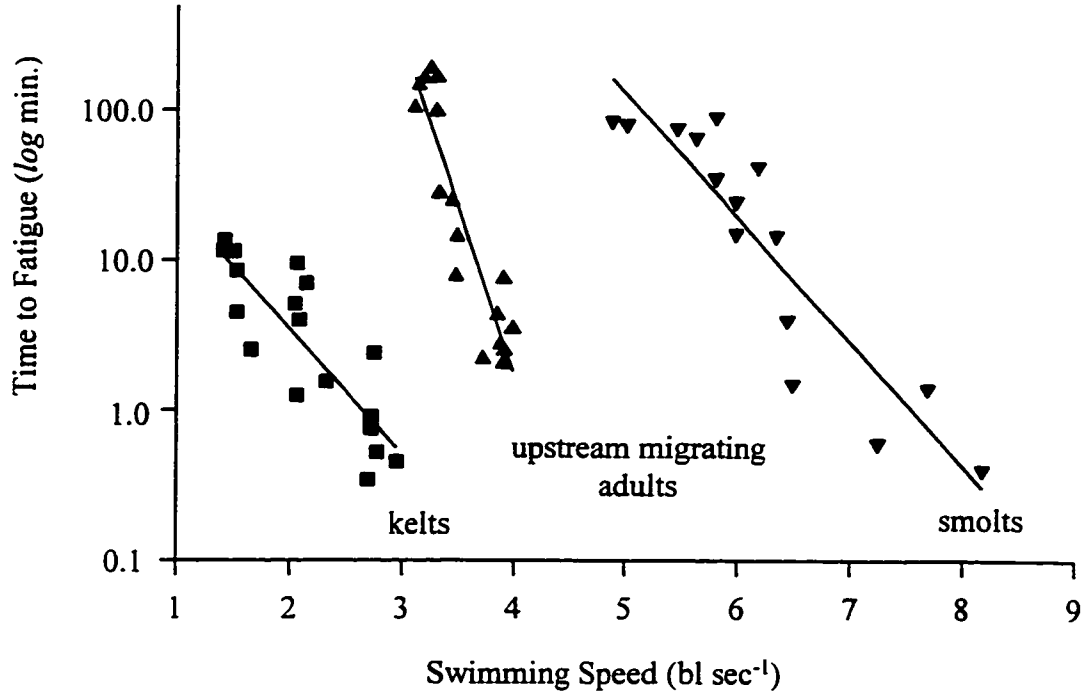
time (*log*) = 8.02-(1.91 * swimming velocity), r^2=0.72.

kelts by the relationship:

time (*log*) = 2.20-(0.83 * swimming velocity), r^2=0.86

and smolts by the relationship:

time (*log*) = 6.24-(0.83 * swimming velocity), r^2=0.83.


Time to Fatigue (log min.)
100.0
10.0
1.0
0.1
1
2
3
4
5
6
7
8
9
Swimming Speed (bl sec-1)
kelts
upstream migrating
adults
smolts

Discussion

Recently, Tang and Wardle (1992) reported that Atlantic salmon smolts require less power to swim at their sustained swimming speed than adults. As a result, the sustained swimming speed of smolts was nearly twice as fast as that of adults when expressed relative to their lengths (Tang and Wardle 1992). In the present study, Atlantic salmon smolts possessed higher sustained swimming speeds than upstream migrating adults. These findings are consistent with those of Tang and Wardle (1992). In addition, the present study found that smolts possessed significantly higher prolonged and burst swimming performances than upstream migrating adults. The differences observed in sustained, prolonged and burst swimming performances between smolts and upstream migrating adult Atlantic salmon are similar to those previously reported for migrating Pacific salmons. For example, Brett and Glass (1973) reported prolonged swimming speeds of 7.1 bl sec^{-1} for sockeye salmon smolts and 2.1 bl sec^{-1} for adults.

In a previous study of migrating pink salmon, Williams and Brett (1987) found that the critical swimming speed declined during upstream migration. It is not surprising, therefore, that Atlantic salmon kelts possessed slower swimming speeds than upstream migrating adults. What was surprising, however, was the magnitude of the reduction in swimming speeds. Relative to upstream migrating adult Atlantic salmon, the swimming speeds of kelts represent a reduction of 62% in sustained swimming speed, 47% in critical swimming speed, and 41% in burst swimming speed. The lower sustained, critical and burst swimming speeds of kelts may be an indication of the poor nutritional condition of these individuals. Compared to upstream migrating adults, kelts weighed 40% less. The large loss

of weight in kelts probably reflects a significant loss of lipid (Jonsson *et al.* 1997, Martin *et al.* 1993). Lipids, and in particular fatty acids, are important substrates for the aerobic muscle (Keissling and Keissling 1993, Moyes *et al.* 1989). Consequently, the low levels of plasma fatty acids found in kelts (Chapter 6), may explain the significant reductions in the sustained and prolonged swimming speeds of kelts.

Sustained and prolonged activity are dependent on red muscle activity, and therefore on aerobic metabolism. Therefore, a reduction in fatty acids, similar to that observed in plasma non-esterified fatty acids among migrating sockeye salmon (Ballantyne *et al.* 1996) can reduce the availability of these substrates to the red muscle, contributing to the decline in aerobic swimming. The loss of protein may be more important in explaining the reduction in burst swimming speeds.

Burst swimming is supported by the white muscle (Jayne and Lauder 1994). The white muscle is an anaerobic tissue, dependent on carbohydrate (i.e. glycogen) as a substrate for energy production (Hochachka 1994). As a result, white muscle metabolism would not be influenced by a decline in lipid as much as the metabolism of red muscle would be. Furthermore, the white muscle of salmonids is also used a source of protein during migration (Idler and Bitners 1960, Mommsen *et al.* 1980). Protein is the primary structural component of white muscle and its removal from the white muscle has been associated with the disorganization of this tissue (Ando *et al.* 1985). The lower burst swimming capabilities of kelts may therefore reflect the deterioration of their white.

Although the present study describes differences in the swimming performances of upstream migrating adults Atlantic salmon, kelts and smolts, it is important to note that swimming speeds in the present study are expressed relative to fork length. A more

appropriate description of swimming performance for management purposes may be to use water velocities of swimming speeds independent of length. For example, given that an average adult Atlantic salmon is 55 cm in fork length, a critical swimming speed of 3.67 bl sec^{-1} actually represents a swimming speed of 201.85 cm sec^{-1}. In contrast, an average smolt is 17.71 cm in fork length, and therefore a critical swimming speed of 6.7 bl sec-1 only results in an actual swimming speed of 118.67 cm sec^{-1}. Thus, although smolts have higher length dependent swimming capabilities, their actual swimming speeds are not greater than those of upstream migrating adult salmon. Furthermore, based on length dependent swimming speeds, smolts swim faster than kelts. When the swimming speeds of kelts are expressed independent of length, the sustained, critical burst swimming speeds are 52.19 cm sec^{-1}, 105.98 cm sec^{-1} and 143.65 cm sec^{-1}. These are very similar to the sustained, critical and burst swimming speeds of smolts; which are 77.70 cm sec^{-1}, 118.67 cm sec^{-1} and 177.71 cm sec^{-1}. Thus, for fishway management, water velocities used in smolt bypass systems may also be appropriate for the passage of kelts.

The present study provides new information concerning the swimming performance of Atlantic salmon kelts and upstream migrating adults. The information presented for smolts is not new, as previous studies have reported the swimming speeds of Atlantic salmon smolts (Thorpe 1984, Virtanen and Forsman 1977). Smolts were included in the present study because it was of interest to determine whether their swimming performance was similar to that of kelts since both of these groups use the same bypass structures to negotiate dams during their downstream migrations.

Based on measurements of sustained, prolonged and burst swimming speeds, this study demonstrates that wild Atlantic salmon smolts and upstream migrating adults possess

swimming speeds which are similar to those of Pacific salmons (Beamish 1978, Brett and Glass 1973). In addition, the present study reports significant differences in the sustained, prolonged (including critical swimming speed) and burst swimming speeds of Atlantic salmon kelts and upstream migrating adults. Although other studies have shown changes in the swimming performance of migrating Pacific salmons (e.g. Williams and Brett 1987), there have been no studies conducted on the changes that can occur in the swimming performance of Atlantic salmon during their spawning migrations. The results of this study suggest that adult Atlantic salmon may experience reductions in their swimming capabilities during upstream migration, although more work in this area is warranted.

Chapter 3

In situ measurement of swimming performance in wild Atlantic salmon (*Salmo salar* L.) using radio transmitted electromyogram signals

Abstract

Swimming performance and *in situ* measurements of muscle activity were obtained from adult Atlantic salmon swum at 12°C and 18°C. Critical swimming speeds were determined and correlated with integrated electromyogram (EMG*i*) signals obtained from the myotomal musculature using EMG radio transmitters. There were no differences in the swimming capabilities of radio tagged and untagged individuals at either temperature. Sustained swimming speeds were found to be 70% higher and critical swimming speeds 20% higher at 18°C than at 12°C. Temperature did not influence burst swimming speeds. Muscle activity indices obtained from Atlantic salmon during non-forced swimming at 18°C were significantly lower than activity indices obtained from Atlantic salmon at 12°C for similar water velocities. Ascent of an artificial fishway by Atlantic salmon at18°C required nearly twice as long as it did at 12°C. The faster ascent of the artificial fishway at 12°C was accompanied by higher muscle activity indices, suggesting that white muscle may be recruited at this temperature. These data demonstrate that Atlantic salmon may recruit white muscle fibres and incur an oxygen debt at colder temperatures as a strategy for increasing swimming speeds while ascending velocity obstructions such as fishways or rapids.

Introduction

Within the past century, many North American rivers have been developed for hydroelectric power generation. Rivers such as the Columbia and Snake Rivers of western North America have all been dammed for the purpose of power generation (Giorgi *et al.* 1988). Dams are permanent structures that can impede and potentially block the upstream migration of all anadromous species. In the Columbia River, for example, Quinn and Adams (1996) have demonstrated that the timing of the upstream migration of sockeye salmon has changed since the construction and operation dams. Furthermore, fluctuating water levels, particularly during peak generating periods, have been associated with delays in the upstream migration of sockeye salmon (Quinn and Adams 1996). Delays in upstream migration can increase the energy expenditures of anadromous salmon by increasing the amount of energy consumed during migration (Gilhousen 1990). Since anadromous salmon migrate with limited energy reserves, any reduction in energy content can potentially increase the chances of failed spawning and pre-spawning mortality (Gilhousen 1990).

Data obtained from studies of Columbia River sockeye salmon have raised concerns about the potential threats posed by developments on other rives such as the Thompson and Fraser rivers (Brett 1995, Williams and Brett 1987). To address concerns raised about the potential impact of hydroelectric developments on anadromous salmon populations, engineers now incorporate fishways, louvers and bypass systems into the design and construction of most dams. The main purpose of these structures is to provide anadromous salmon with *safe* passage through or around the dammed areas. Designs for these structures are numerous, however, common features to all designs are reduced water velocities

compared to those passing over or through the dams, and areas where salmon can rest or escape areas of high flow within the fishway (Katapodis 1990). However, to properly operate fish bypass structures, knowledge of the swimming capabilities of fish using them must be known. Unfortunately, the number of species for which swimming performance has been measured is small. As a result, fish passage structures are often designed and operated using the swimming performance of one species to estimate that of another.

To date, the swimming capabilities and muscle activity patterns are best studied for species of Pacific salmon (*Oncorhynchus* sp.) (see review by Brett 1995, Webb 1995). Presently, similar concerns are being expressed about the influence that similar developments might have on other anadromous salmonids such as the Atlantic salmon. Without information concerning the swimming capabilities of this species the effects of these developments on upstream and downstream migrations and survival are unknown.

One approach that has been used to predict the influence of hydroelectric developments on Atlantic salmon has been to liken them to Pacific salmons and extrapolate current information from them. This may be inappropriate because of the distinctly different migratory characteristics and life histories of these salmons. Pacific salmons, for example are semelparous and all individuals die following spawning (Higgs *et al.* 1995). In contrast, Atlantic salmon are iteroparous and some individuals survive spawning. According to Glebe and Leggett (1981), semelparous salmon have different energy requirements and swimming capabilities than iteroparous salmonids. Semelparous salmon invest large amounts of energy into reproduction and locomotion, and their migrations are undertaken as close to their maximal sustained swimming speed as possible to ensure that the spawning areas are reached with sufficient energy left to spawn. In contrast, iteroparous salmonids allocate less energy

to reproduction and migrate at speeds which are closer to their optimal swimming speed than their maximum swimming speed (Glebe and Leggett 1981). Since the swimming performance of anadromous used to evaluate fish way design must reflect the swimming performance at the time of migration, extrapolation of swim speed data between anadromous forms of salmon that are known to migrate at different speeds is clearly inappropriate.

To date, few studies have investigated the swimming performance of anadromous adult Atlantic salmon (Økland et al. 1997, Tang and Wardle 1991, Thorstad *et al.* 1997). Furthermore, most of these have been conducted in the laboratory using commercially available stocks or wild Atlantic salmon held in captivity (Tang and Wardle 1991, Thorstad *et al.* 1997). In the design of fishways, information about the swimming capabilities of freely swimming wild salmonids would be desired. However, acquiring direct assessments of the swimming capabilities of wild fish is extremely difficult because equipment cannot be easily taken into the field (Beamish 1978, Brett and Groves 1979, Fry 1947).

Within the past decade, physiological telemetry has been used to measure the muscle activity of fish during forced swimming trials in the laboratory and correlations between muscle activity and swimming speed have been used to estimate the swimming speeds of fish in their natural environment (Demers *et al.* 1996, Hinch *et al.* 1996, Kaseloo *et al.* 1992, McKinley and Power 1992, Weatherley *et al.* 1996). In addition, several other physiological telemetry techniques have also been used to estimate activity; these include the correlation between swimming speeds and heart rate (Armstrong *et al.* 1989, Lucas *et al.* 1993, Priede and Young 1977) or opercular rate (Rogers and Weatherley 1983). The use of muscle EMGs as indicators of muscle activity have been particularly successful for estimating the swimming speeds and energy expenditures of anadromous salmonids (Hinch *et al.* 1996,

Økland *et al.* 1997).

The objective of this study was to determine the swimming capabilities and muscle activity indices of anadromous Atlantic salmon under forced swimming conditions and to compare these to those of freely swimming Atlantic salmon during ascent of an artificial fishway. An additional aim was to determine whether measurements of muscle activity and swimming speeds implied from muscle activity indices change with temperature and thus whether they could be interpolated to environments which differ in temperature.

Materials and Methods

Animals and study site

Wild adult Atlantic salmon (55-60 cm) were collected from the Exploits River, Newfoundland, Canada (49°N, 57°W) between July 21st and 27th and September 27th and October 3rd. Ambient water temperatures at the time of collection averaged 18°C for July and 12°C for September. Atlantic salmon were transported to a hatchery facility situated on a tributary of the Exploits River, Noel Paul's brook. Individual Atlantic salmon were held in large rectangular outdoor pens (5 m wide, 15 m long and 1 m deep). These pens were positioned in a 100 m long raceway next to the river. Water flow into the raceway was regulated by a pair of valves positioned upstream and that controlled the flow of water in the raceway. Water velocity in the raceway was maintained at 40 cm sec^{-1}. All Atlantic salmon were allowed to recover from transportation for 3 days before being used in any experiment.

Surgical procedures

Individual Atlantic salmon were removed from a common enclosure and anaesthetised in an aerated and buffered solution of MS-222 (50-75 mg l^{-1}, pH 7.0). When a slow irregular operculum rate was observed, surgery was initiated. This stage of anaesthesia was generally reached within 4 minutes. Anaesthetised individuals were placed ventral side up onto a non-abrasive V-shaped surgical table and the gills were irrigated with fresh oxygenated river water.

To insert the transmitter, a 3 cm incision was made on the ventral surface at a position posterior to the pelvic girdle. The transmitter was gently inserted through the incision and pushed anteriorly into the body cavity above the pelvic girdle. Electrodes were positioned approximately 5 mm apart in the red muscle below the lateral line using 21 gauge rods. Once the electrodes were secure in the muscle, the rods were removed. The antenna was exited from the body via a 2 mm incision located anterior to the anal fin. The 3 cm incision was closed using three independent sutures (2/0 Ethicon silk) and, before the last suture was closed an injection of Liquamycin LP (1 ml kg^{-1} antibiotic) was provided intraperitoneally. Surgical time averaged 4-5 minutes.

Radio telemetry equipment

EMG transmitters used in the present study measured 50 mm in length and 13 mm in diameter and weighed less than 2% of the Atlantic salmon's body weight (Lotek Engineering Inc., Newmarket, Ontario). Muscle activity signals were detected via 18 carat gold tip sensors and transmitted along insulated stainless steel electrodes. A precision half wave rectifier and integrator processed the input EMG signals within the bandwidth 30-350 MHz. EMG signals were processed through an integrator, and a radio pulse corresponding to the pulse interval in milliseconds (ms) was transmitted when the sum of the integrated EMG (i.e.

EMG*i*) signals equalled the predetermined threshold value of 150 μV. Increasing muscle activity resulted in a corresponding decrease in the interval between successive radio pulses. Because of this inverse relationship, the term "increased muscle activity index" will be used in text to describe a decrease in the measured pulse interval (ms). Lifespan (i.e. battery life) of the transmitters was approximately two to three months. Transmitted signals were detected and recorded automatically using a W20 radio receiver/data-logger (Lotek Engineering Inc., Newmarket, Ontario) and downloaded to a laptop computer via an RS232 serial communication port. The receiver was programmed to record all EMG*i* signals from each tagged individual.

Swimming chambers and experimental design

Swimming performance trials and critical swimming speeds were measured using a Blazka-type swim speed chamber (Blazka *et al.* 1960). The diameter of the inner tube was 28 cm and the outer tube was 44 cm. The total volume of the chamber was 70 litres. A heat exchanger, which was attached to ensure a constant temperature within the chamber increased the total volume to 120 litres. Flow within the chamber was generated using an electric motor connected to an impeller and was rheostatically controlled. Calibration of motor speed and water velocity was performed at eight locations along the vertical mid-section of the swim chamber. The chamber could generate water velocities up to 2.4 m sec^{-1} within 5 seconds. Cross-sectional velocity profiles revealed that the edge effect was less than 4 cm at any speed. Ambient, untreated river water was pumped into the chamber. Maximum cross-sectional area of Atlantic salmon used in swim trials was measured to ensure that adjustments could be made for the influence of the fish's body on water flow within the

chamber, if necessary (Smit *et al.* 1971).

Swimming trials and measurement of muscle activity

In the present study swimming endurance and critical swimming speeds were determined for anadromous Atlantic salmon acclimated to 12°C and 18°C. Prior to any measurement of swimming performance or critical swimming speed, Atlantic salmon were allowed 3 hours to acclimate to the swimming chamber. The acclimation speed used was 0.5 bl sec^{-1}.

Swimming endurance was measured by swimming individual Atlantic salmon at a known velocity until they fatigued. The swimming speeds used in the present study were 0.8, 1.2, 1.6, 1.8, 2.0, 2.2 and 2.4 m sec^{-1}. Five Atlantic salmon were swum at each velocity. Sustained, prolonged and burst swimming performances are components of swimming endurance (Beamish 1978). These stages were identified from the swimming endurance of Atlantic salmon using the criteria of Beamish (1978). Sustained swimming performance should be applied to swimming speeds maintained for a minimum of 200 minutes, while burst swimming performance applied to those swimming speeds maintained for less than 30 seconds. Prolonged swimming performance occurs between sustained and burst swimming performances and are those speeds maintained for less than 200 minutes and more than 30 seconds. In the present study, however, a time of 120 minutes was chosen to define the sustained swimming performance. The reason for choosing 120 minutes instead of the standard 200 minutes pertains to the availability of Atlantic salmon. In the present study I was forced to collect Atlantic salmon from the river and hold them in outdoor pens during testing. Since this period of captivity could influence their swimming performance (Strange and Cech 1992), I chose to use a period of 120 minutes to minimize the length of the

experimental time, so that more Atlantic salmon could be tested in a shorter period of time. Because most of the swimming required for fishway ascent occurs in the prolonged and burst ranges. I was most interested in these swimming speeds.

The critical swimming speed is a special category of prolonged swimming and is typically used to indicate the maximum swimming speed a fish can maintain for a prescribed period (Brett 1964). Critical swimming speeds were determined by swimming individual Atlantic salmon over a range of increasing speeds until they fatigued. However, before being swum, Atlantic salmon were allowed to acclimate to the swim chamber for 3 hours at a velocity of 0.5 bl sec^{-1}. Depending on the nature of the study, and fish being tested, different criteria can be used to determine critical swimming speeds. In the present study, Atlantic salmon were subjected to water velocities that were increased by 20 cm sec^{-1} at 10 minutes intervals, until fatigue. Critical swimming speeds (U_{crit}) were then calculated using the equation presented in Brett (1964):

$$U_{crit} = V + (t_i / t_{ii})u_i \qquad \text{equation 2}$$

where: V is the highest velocity maintained for the prescribed period (m sec^{-1}), t_i is the time elapsed at final velocity (min), t_{ii} is the time increment (min) and u_i is the velocity increment (m sec^{-1}). Critical swimming speeds were investigated for 5 Atlantic salmon.

Consistent measurement of swimming speed depends on the consistent recognition of fish fatigue. Atlantic salmon were maintained in a swimming area (approximately 1.5-2 body lengths) by forward and rear non-electrified plastic grates. The criteria used to determine fatigue was the inability of the individual to continue swimming after touching the rear grate, despite three attempts to stimulate swimming by raising and lowering the water velocity. Critical swimming speeds were also determined for Atlantic salmon fitted with

transmitters (n=3) and without transmitters (n=3) so that any effect of the surgical procedure on the critical swimming speed of Atlantic salmon could be addressed.

Muscle activity was obtained from Atlantic salmon fitted with radio transmitters that were swum individually over the following range of water velocities: 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 and 2.2 m sec^{-1}. At each speed, Atlantic salmon were forced to swim for 5 minutes and muscle activity was measured during this period. Because muscle EMG*i* are recorded as a pulse interval signal, a large number of data is collected during the 5 min interval. To ensure that erroneous data associated with the increase in swimming speeds was not included only data recorded during the final 3 minutes were used. All raw data is shown in graphical format.

Muscle activities obtained from Atlantic salmon were plotted against their swimming speeds. Because muscle activity is based on an integrated EMG signal (EMG*i*), an activity index was used to describe muscle activity. The units for this index are milliseconds (ms). The muscle activity index used in the present study was based on that used in previous study involving measurements of muscle activity in lake sturgeon (McKinley and Power 1992).

Measurement of muscle activity in Atlantic salmon during ascent of an experimental fishway

Swimming speeds of wild Atlantic salmon were recorded during their ascent of an experimental fishway. The experimental fishway was constructed of plywood and plexiglass and consisted of a head pond and tail pond connected by a 20 m long sluice which measure 30 cm in width and 20 cm in height. The slope of the fishway was 1%, representing an offset vertical distance of approximately 44 cm. The water velocity within the fishway ranged from 1.85 m sec^{-1} at the upstream end to 2.6 m sec^{-1} at the downstream end (average 2.1 m sec^{-1}). Flow within the experimental fishway was not uniform and water velocities at the sides and

bottom were typically 0.1-0.2 m sec^{-1} less than in the centre of the channel. Atlantic salmon implanted with EMG transmitters ($n = 4$ at each temperature) were placed into the tail pond and were left to ascend the experimental fishway at will. Most of the fish attempted to ascend the flume in the evening and, therefore, continuous recordings were made during the evening hours. Data from evening recordings were analysed the following day.

Statistical analyses

All values are represented as means ± their standard error. Linear regression analyses were used to examine the relationship between swimming speeds and muscle activities. Unpaired *t*-tests were used to compare swimming endurance of fish at 12°C with that of fish at 18°C. In all cases $p < 0.05$ was the accepted level of significance.

Results

Relationship of muscle activity to swimming speed

Sustained and prolonged swimming speeds were influenced significantly by water temperature. Sustained swimming speeds were, on average, 1.0 m sec^{-1} slower at 12°C than at 18°C (Figure 3.1). Prolonged swimming speeds were also significantly lower at 12°C than at 18°C. In contrast, the maximum burst swimming speeds of Atlantic salmon were not different at 12°C and 18°C. Atlantic salmon were unable to maintain a swimming speed of 2.40 m sec^{-1} for periods greater than 10 sec at either 12°C or 18°C (Figure 3.1).

Critical swimming speeds of Atlantic salmon at 18°C and 12°C were found to be 2.16 ± 0.18 m sec^{-1} and 1.76 ± 0.06 m sec^{-1}, respectively, and were significantly different ($p <$

0.05). The mean critical swimming velocity of radio tagged Atlantic salmon was 2.16 ± 0.18 m sec^{-1} at 18°C and 1.76 ± 0.06 m sec^{-1} at 12°C. In contrast, the mean critical swimming speed of untagged Atlantic salmon was 2.10 ± 0.05 m sec^{-1} at 18°C and 1.80 ± 0.03 m sec^{-1} at 12°C. There were no statistical differences between the swimming capabilities of radio-tagged and untagged Atlantic salmon at either temperature.

Linear regressions indicated that muscle activity and swimming speeds were correlated at both 18 and 12°C (Figure 3.2). The relationships between muscle activity index and swimming speed for each temperature are described by the following equations:

at 18°C, muscle activity index = 2247.70-(332.75*swimming speed), $r^2 = 0.65$ equation 3

at 12°C, muscle activity index = 2599.30-(779.63*swimming speed), $r^2 = 0.88$ equation 4

where, muscle activity is in milliseconds and swimming speed is in m sec^{-1}.

The relationship between swimming speed and muscle activity index was significantly different at 18°C (slope 332.75) than at 12°C (slope 779.63). A greater slope indicates that more muscle is required to swim at a given speed. Thus, the greater slope measured for Atlantic salmon swum at 12°C indicates that these individuals required more of their muscle to swim than Atlantic salmon at 18°C.

Experimental fishway study

Atlantic salmon exhibited two distinct patterns of swimming during ascent of the flume. At 18°C, swimming was continuous and characterised by a constant increase in the muscle activity index throughout the ascent. In contrast, at 12°C, the ascent of Atlantic salmon was characterised by a rapid increase in the muscle activity index between 0 and 10-15 seconds at which point it remained elevated throughout the remainder of the ascent

(Figure 3.3).

At both 18 and 12°C, Atlantic salmon ascending the artificial fishway displayed muscle activity indices which exceeded the muscle activity index associated with their critical swimming speeds (horizontal line in Figure 3.3.). At 12°C, a greater proportion of the muscle activity index occurred above the muscle activity index corresponding to the critical swimming speed than at 18°C, suggesting that ascent at 12°C was more difficult (Figure 3.3). Mean and maximum muscle activity indices observed among Atlantic salmon ascending the fishway at 18°C were 1535±40 ms and 1280±26 ms, respectively. In contrast, the mean and maximum muscle activity indices of Atlantic salmon ascending the fishway at 12°C were 819±91 ms and 578±82 ms, respectively.

Overall, Atlantic salmon traversed the fishway significantly faster at 12°C than at 18°C, requiring 30±7 sec and 47±12 sec, respectively. Part of the reason for the quicker ascent at 12°C is that Atlantic salmon ascended the experimental fishway by swimming at significantly higher speeds than individuals ascending the fishway at 18°C. Based on a mean activity index of 1535 ms at 18°C and 819 ms at 12°C and using equations 3 and 4, Atlantic salmon ascended the fishway by swimming at a mean speed of 2.14 m sec^{-1} at 18°C and 2.28 m sec^{-1} at 12°C.

Figure 3.1. The swimming performance of wild Atlantic salmon measured at 18°C and 12°C. Five Atlantic salmon were swum at each speed. Dotted lines represent swimming endurance (i.e. time to fatigue) greater than 120 minutes for all individuals at those swimming speeds.

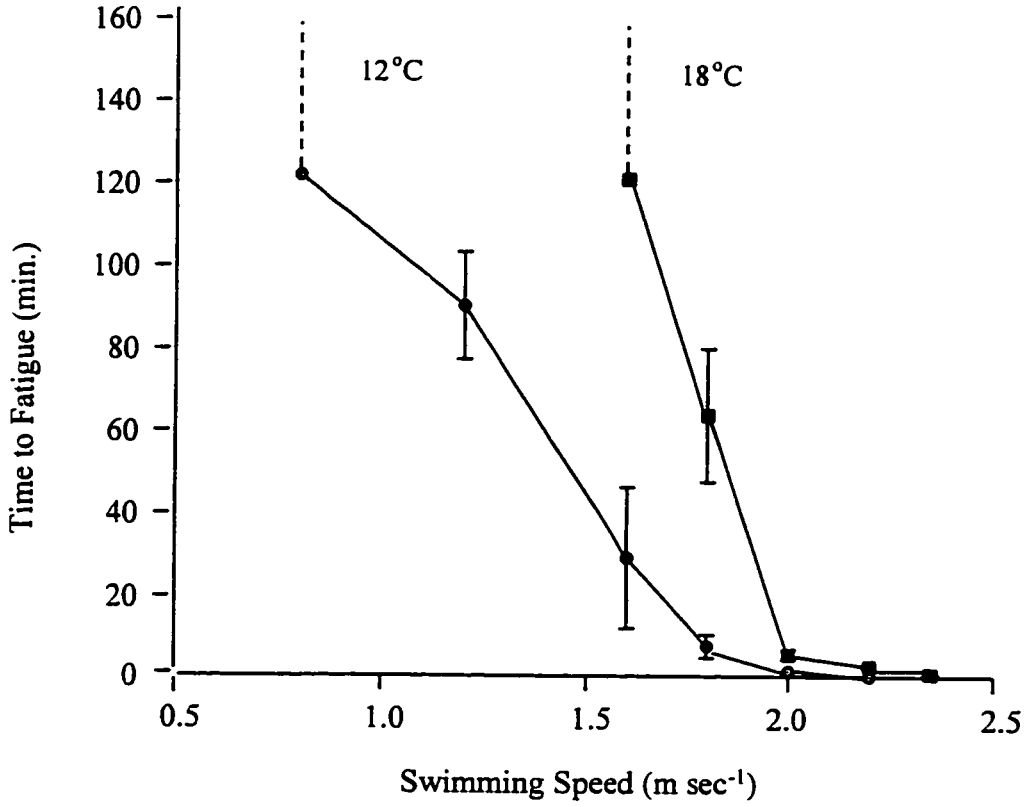

160
140
120
100
80
60
40
20
0
12°C
18°C
Time to Fatigue (min.)
0.5
1.0
1.5
2.0
2.5
Swimming Speed (m sec-1)

Figure 3.2. Calibration of muscle activity index with swimming performance in wild Atlantic salmon at 18°C and 12°C. Muscle activity index was measured using radio transmitted electromyogram signals obtained from the axial musculature of adult Atlantic salmon under forced swimming conditions using a Blazka-type swim chamber. Linear regressions are shown.

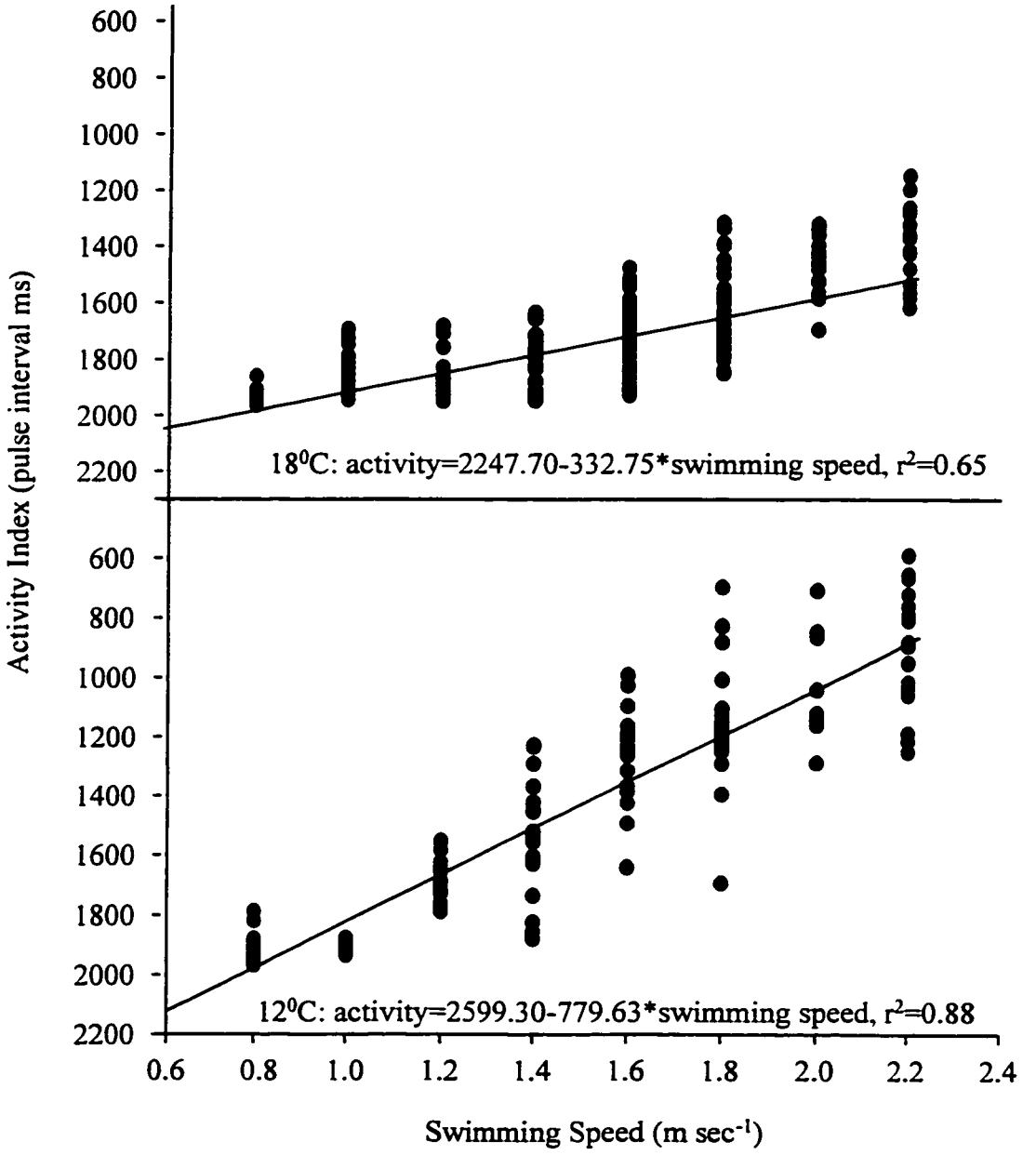
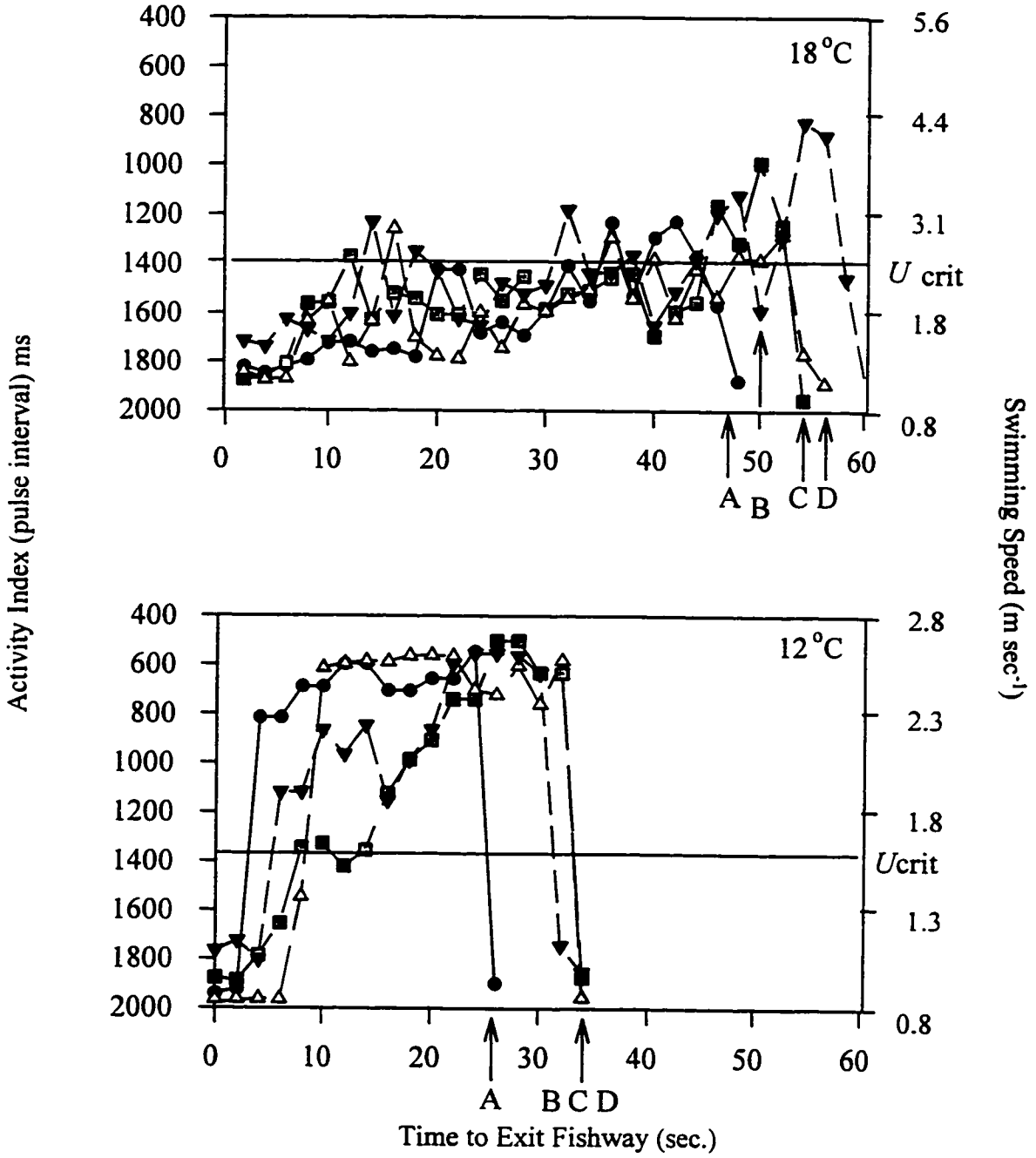

Activity Index (pulse interval ms)
600
800
1000
1200
1400
1600
1800
2000
2200
18°C: activity=2247.70-332.75*swimming speed, r²=0.65
12°C: activity=2599.30-779.63*swimming speed, r²=0.88
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
Swimming Speed (m sec⁻¹)

Figure 3.3. Muscle activity indices recorded of wild Atlantic salmon ($n = 4$) during ascent of a 20 m long experimental fishway. Data were recorded in late summer (i.e. 18°C) and early fall (i.e. 12°C). Each symbol represents actual mean measurements of the muscle activity index for an individual salmon. Critical swimming speeds, calculated using swimming speeds corresponding to the observed muscle activity indices according to equations 3 and 4, are shown on the Y axis. A, B, C, D represent the exit times of each Atlantic salmon from the flume.


400
600
800
1000
1200
1400
1600
1800
2000
18°C
5.6
4.4
3.1
U crit
1.8
0.8
0
10
20
30
40
50
60
A
B
C
D
12°C
2.8
2.3
1.8
Ucrit
1.3
0.8
Activity Index (pulse interval) ms
Swimming Speed (m sec-1)
Time to Exit Fishway (sec.)

Discussion

Barriers, either mechanical or hydraulic, routinely impede the natural migratory progress of anadromous salmon. The presence of dams can greatly influence the energy expenditures of migrating salmon and subsequently influences spawning success. How much influence these barriers have on the energy expenditure of migrating salmon during fishway passage, ultimately depends on how well fish using them can swim, and whether swimming performance changes on a seasonal basis.

Seasonal changes in the swimming performance of Atlantic salmon could occur from the effects of seasonal declines in temperature on muscle function. Atlantic salmon possess spatially distinct regions of oxidative red muscle and glycolytic white muscle (Love 1980). Examinations of these muscles have revealed that they contain different types of muscle fibres. Red muscle is composed of slow twitch fibres that generate small amounts of power but have great endurance. In contrast, white muscle contains fast twitch muscle fibres that generate large amounts of power but fatigue rapidly (Rome 1994). Since less power is required to swim at slower speeds the power production of red muscle is well suited for swimming at low speeds, and the higher power output of white muscle is well suited for swimming at high speeds. The lower sustained swimming velocities of Atlantic salmon at 12°C, suggest that red muscle function may be compromised at low temperatures. Previous laboratory studies have demonstrated reduced shortening velocities and decreased power output of the red muscle in fish acclimated to cold temperatures (Rome *et al.* 1984). White muscle activity, however, is less dependent on temperature (Rome et al. 1984), and may explain why sustained and prolonged swimming performances differ, and burst swimming

performances are similar between Atlantic salmon acclimated to 12°C and 18°C.

When fish are acclimated to cold temperatures, the reduced power output of the red muscle must be compensated for if fish are to continue swimming at a given speed, or over a range of speeds. Laboratory studies have shown that fish recruit power from their white muscle to offset the effects of reduced temperature on red muscle power production (Jayne and Lauder 1994, Rome *et al.* 1990, 1992). In the present study, the significant increase in muscle activity observed in Atlantic salmon swum at 12°C compared to 18°C may reflect an increased recruitment of white muscle. However, since the EMG transmitter used could not discriminate between red and white muscle activity, it must be assumed that the increase in muscle activity reflects the recruitment of white muscle. Certainly, there is sufficient evidence to suggest that this is the case (Altringham 1994, Jayne and Lauder 1994, Rome *et al.* 1984, 1990, 1992). However, further investigation of the EMG signal derived from radio telemetry is required before definite conclusions about red and white muscle activities can be made from measurements of muscle activity obtained from radio transmitted EMGs.

Previous studies have shown that a significant oxygen debt is acquired during burst activity (Wood *et al.* 1983). Beamish (1978) reported that the energetic costs of repaying this oxygen debt could be greater than the aerobic scope of the fish. Furthermore, exhaustive exercise may contribute to increased post-exercise mortality among fish (Wood *et al.* 1983). At 18°C, the water velocity within the fishway (i.e. 2.10 m sec^{-1}) was below the critical swimming speed of Atlantic salmon determined under laboratory conditions. Activity indices of Atlantic salmon during ascent of the flume at 18°C were consistent with the activity based estimates of swimming speeds obtained in the swimming chamber under

laboratory conditions, and indicate that ascent of the flume at 18°C involved swimming within the aerobic capacity of Atlantic salmon. In contrast, the muscle activity measured from Atlantic salmon ascending the fishway at 12°C was greater than at 18°C. The greater muscle activity of Atlantic salmon ascending the fishway at 12°C indicates that more of their muscle was active at this temperature than at 18°C. As noted previously, the transmitter used in the present study cannot differentiate between red and white muscle activities. However, previous studies have shown that red muscle is predominant at slow swimming speeds, while white muscle is predominant at high swimming speeds (Jayne and Lauder 1994, Rome *et al.* 1984). Furthermore, these studies have also shown that white muscle is recruited at lower swimming speeds when fish are acclimated to cold temperatures. Thus, the increased muscle activity of Atlantic salmon ascending the flume at 12°C most likely indicates that these salmon have recruited their white muscle during fishway ascent and therefore exceeded their aerobic scope.

This study demonstrated that radio transmitted muscle activity can be correlated with swimming performance for anadromous Atlantic salmon. Using this procedure, the swimming capabilities of Atlantic salmon were investigated during the ascent of an experimental fishway. Although Atlantic salmon were found to be less effective swimmers at 12°C, they were able to ascend the flume through the use of burst swimming activity and the acceptance of an oxygen debt. Collectively, these results suggest the swimming capabilities and muscle activity indices of Atlantic salmon are dependent on temperature, such that lower temperatures reduce swimming capabilities but increase muscle activity necessary to swim.

Chapter 4

Swimming capabilities and muscle activity patterns of wild Atlantic salmon (*Salmo salar* L.) at different stages of their freshwater spawning migration.

Abstract

The present study examined the swimming performance and muscle activity patterns of male and female Atlantic salmon during their upstream migration in the Exploits River, Newfoundland, Canada during the study period May 26th to October 17th 1996. The influences of seasonal changes in temperature and body morphology on the swimming performance of Atlantic salmon were also investigated. Morphological measurements of adult Atlantic salmon indicated that stomach (including the pyloric caeca) weight declined by approximately 88% in both male and female Atlantic salmon while liver weight declined by approximately 59% in females and 26% in males. Just prior to spawning, ovaries weighed 218.68±23.77 g and were significantly heavier than testes (55.38±2.26 g). Significant increases in girth and cross-sectional area were associated with ovary maturation. Sustained, critical and burst swimming speeds were correlated with temperature and cross-sectional area for females, but only with temperature for males. Sex dependent differences in muscle activity indices were apparent at the critical swimming speed (2 bl sec^{-1}) and were significantly higher for females. Muscle activity indices were correlated with both temperature (r^2=0.64) and cross-sectional area (r^2=0.74) for females, but only to temperature for males (r^2=0.63). The results of the present study indicate that environmental temperature is an important determinant of swimming performance in anadromous Atlantic salmon. Changes in the body morphology of females appear to place additional demands on their locomotory muscle, and may be responsible for the significantly lower sustained and critical swimming capabilities of female Atlantic salmon.

Introduction

The Atlantic salmon is an important migratory fish species throughout North America and Europe. Considerable progress has been made towards understanding the energy requirements (Jonsson *et al.* 1991, 1997), migratory patterns (McCleave *et al.* 1978, Power and McCleave 1980) and reproductive behavior (Heggberget 1988, Bagliniere *et al.* 1990) of this species. However, there is a paucity of information concerning the swimming capabilities and efficiencies of Atlantic salmon during their spawning migration.

Atlantic salmon migrate with limited energy reserves (Jonsson et al. 1997). Consequently, any changes in the difficulty of their migration could have consequences on the amount of energy available spawning. According to Glebe and Leggett, iteroparous salmonids such as the Atlantic salmon minimize energy losses by migrating at speeds close to their optimal sustained levels. However, several factors can influence the swimming capabilities of migrating Atlantic salmon, including changes in water temperature (Quinn *et al.* 1996), body size/morphology (Taylor and McPhail 1985, Goolish 1991, Hawkins and Quinn 1996) and reproductive status (Thorstad *et al.* 1997).

During their migrations salmonids experience seasonal fluctuations in temperature as well as changes in their body morphology due to the effects of starvation and morphogenic changes associated with sexual development. Previously, Williams and Brett (1987) observed reductions in the critical swimming speeds of male and female pink salmon over the course of their spawning migration in the Thompson River, British Columbia. However, their results, were standardized to a temperature of 15°C and corrected for differences in body size, making it unclear what factors may have caused the reduction in critical swimming

speeds. Previous studies have suggested that salmonids undergo a metabolic reorganization during their reproductive migrations as a result of changes in energy availability (Mommsen *et al.* 1980, Guderley *et al.* 1986). Reductions in the swimming performance of pink salmon may, therefore, indicate limitations imposed on muscle activity by fuel depletion during the final stages of migrations. Unfortunately, it is difficult to assess changes that influence fuel availability and the swimming capabilities of fish in the field. Under laboratory conditions, muscle EMGs have been used to examine the influence of temperature and swimming speed on muscle activity patterns (Rome *et al.* 1990, 1992, Jayne and Lauder 1994). Therefore, measurements of muscle EMGs may be useful in understanding how changes in muscle activity relate with changes in the swimming performance of wild fish.

Numerous studies have used radio transmitter electromyogram (EMG) signals to examine the swimming performance and energy expenditures of fish in their natural environment (McKinley and Power 1992, Demers *et al.* 1996, Hinch *et al* 1996, Weatherley *et al.* 1996). As a result of these studies, methods for surgical implant of EMG transmitters and treatment of data obtained through the measurements of muscle EMGs have been established. The origin of physiological signals (i.e. red muscle or white muscle), however, is less clear. While most studies place the electrodes of the transmitter in the red muscle, the signals received, particularly when the fish are startled or handled, suggest that white muscle activity is also recorded. In the laboratory, white muscle activity is also measured when electrodes are placed in the red muscle and care is not taken to ensure discrete electrode placement (Wardle and Videler 1994). Because the electrodes of the radio transmitter are larger than those used in the laboratory, there is a good chance that they may also record white muscle activity. This may be particularly true at high swimming speeds since the

nature of the signal originating from the white muscle greatly exceeds that of the red muscle (Rome *et al.* 1992). More baseline work needs is required in order to fully understand the nature of the EMG signal recorded by physiological radio telemeters (Lucas *et al.* 1993, Økland *et al.* 1997).

A number of laboratory based studies have demonstrated that the intensity and duration of EMGs obtained from the red and white muscles of fish are dependent on swimming speed (Jayne and Lauder 1994, Wardle *et al.* 1995) and temperature (Rome *et al.* 1990, 1992, Wardle *et al.* 1995). Furthermore, these studies have also found swimming speeds can be maintained, and are independent of external factors, when the number of active muscle fibres are increased (Rome *et al.* 1984,1990, Jayne and Lauder 1994). For example, carp have been shown to recruit their white muscle to maintain a constant swimming speed when temperature is decreased (Rome *et al.* 1984). By recruiting white muscle carp are capable of attaining similar swimming speeds at different temperatures although a decline in aerobic swimming (i.e. swimming supported by the red muscle) has clearly occurred. Thus, a failure to measure a change in swimming performance does not mean that external factors have not influenced the ability of a fish to swim.

Although previous studies have described the swimming capabilities of Atlantic salmon (Tang and Wardle 1992, Økland *et al.* 1997), there have been no studies describing the sustained, critical and burst swimming capabilities of Atlantic salmon during their freshwater migrations. Furthermore, studies of swimming performance have not measured changes in muscle activity patterns or the relationships between muscle activity and swimming speed. The purpose of the present study, therefore, was to examine the influence of body morphology and temperature on the swimming performance and muscle activity

indices of male and female Atlantic salmon during their annual freshwater spawning migration.

Materials and methods

Animals and study site

Wild Atlantic salmon (1.0-2.6 kg) were collected from the Exploits River, Newfoundland, Canada during their spawning migration (May 26th to October 17th, 1996). Collections of Atlantic salmon were based on their peak migration at one estuary site and e four sampling locations approximately 5 km, 20 km and 100 km from the river mouth. These sampling locations are shown in Figure 4.1. Collection of Atlantic salmon from the estuary involved capturing Atlantic salmon is a tended gill net. The net was monitored constantly and fish were typically removed from the net within 2-3 minutes. These salmon were immediately placed in fresh aerated river water. River collections involved dip netting Atlantic salmon from fish traps located at each of these sites and placing them in an aerated tank until transfer to the Noel Paul's Brook research facility. At the research facility, Atlantic salmon were held in outdoor tanks 5 m wide, 15 m long and 1 m deep supplied with river water at ambient temperature. All tanks were supplied with river water at seasonal temperatures (11°C -14°C). Atlantic salmon used as spawning individuals were collected from site 4 approximately 2 weeks prior to spawning, and taken to the Noel Paul's Brook hatchery facility (site 5) where they were held in an artificial spawning channel until fully mature. Determination of sexual maturity involved monitoring for indications of redd

making activity by females and was confirmed later confirmed for both sexes by measuring plasma hormone concentrations and gonadal weight.

Due to the current legislation regarding the management of Atlantic salmon in the Exploits River, only a limited number of individuals could be obtained, and sacrificed, from each site. For this reason, the numbers of Atlantic salmon used for morphological analyses and measurements of muscle activity are less than the numbers used for assessment of swimming performance.

Blood sampling

Individual Atlantic salmon were removed from a holding tank using a dip-net and immediately placed in a solution of MS-222 (tricaine methanosulfonate) buffered to pH 7.0 using sodium bicarbonate ($NaCO_3$). Once anaesthetized, Atlantic salmon were removed and placed on a sterile surgery table. A 5 ml aliquot of blood was drawn from the caudal vasculature using a 10 ml heparinized syringe and distributed among 1.5 ml Eppendorf tubes. Samples were centrifuged for 4 min at 7000 *g* to isolate the plasma fractions. Plasma was then transferred into 1.5 ml Eppendorf tubes and immediately placed in liquid nitrogen until frozen. Plasma samples were stored at -80°C until analyses were conducted. In total, 4 male and 4 female Atlantic salmon were sampled from each site.

Hormone analyses:

Plasma samples were analyzed for testosterone and 17β-estradiol content using the radioimmunoassay (RIA) method of McMaster *et al.* (1992). The antiserum used was anti-11 ketotestosterone (ICN Biomedicals, St. Laurent PQ.). This antiserum reacted 100% with testosterone and less than 0.1% with 17β-estradiol. In summary, 1 ml plasma samples were

mixed with 5 ml of ether to release steroids bound to plasma proteins. The ether was then evaporated at 50°C and the remaining plasma reconstituted in 1 ml phosphogel (5.75 g Na_2HPO_4, 1.28 g NaH_2PO_4 hydrous, 1 g gelatin, 0.1 g thimersol dissolved in 800 ml distilled water and pH adjusted to 7.6). Two hundred microlitres of sample or standard (1000 ng ml^{-1} testosterone or 17β estradiol) were pipetted into 12x75 mm borosilicate glass tubes and spiked with 200 µl of radiolabeled steroid (2,4,6,7-^{3}H-17β-estradiol or 1,2,6,7-^{3}H-testosterone). After an overnight incubation, 200 µl of dextran-coated carbon solution (0.5 g activated carbon, 0.05 g dextran and 100 ml phosphogel) was added and the mixture was incubated for 10 min. Following incubation, samples were centrifuged at 3000 RPM for 12 min at 4°C. The supernatant containing steroids was decanted into scintillation vials and 5 ml of scintillation fluid (Scintiverse, Fisher Scientific, Toronto, ON.) was added. Radioactivity was measured on a scintillation spectrophotometer (Sequola-Turner model 450).

Morphological measurements

Measurements of fork length, girth, total weight, gonad weight, liver and stomach weight were obtained from male and female Atlantic salmon. Included in the stomach weight are the weight of the pyloric caeca and anterior intestine. Individual Atlantic salmon were cross sectioned horizontally at the position of the first dorsal fin ray and measurements of body depth and width were obtained. Cross-sectional area (XS) was calculated from these measurements as: cross-sectional area = width X depth X (π/4). In total, 8 male and 8 female Atlantic salmon were sampled from each site. The exceptions were Atlantic salmon collected at site 4 and taken to site 5 to mature. Only 7 males and 7 females from these representative spawning Atlantic salmon were sampled.

Surgical procedures

Individual Atlantic salmon were removed from a common enclosure and anaesthetised in an aerated and buffered solution of MS-222 (50-75 mg l^{-1}, pH 7.0). When a slow irregular operculum movement was observed, surgery was initiated. This stage of anaesthesia was generally reached within 4 minutes. Anaesthetised individuals were placed ventral side up onto a non-abrasive V-shaped surgical table and the gills were irrigated with fresh oxygenated river water.

To insert the transmitter, a 3 cm incision was made on the ventral surface at a position posterior to the pelvic girdle. The transmitter was gently inserted through the incision and pushed anteriorly into the body cavity above the pelvic girdle. Electrodes were positioned approximately 5 mm apart in the red muscle below the lateral line using 21 gauge rods. Once the electrodes were secure in the muscle, the rods were removed. The antenna was exited from the body through a 2 mm incision located anterior to the anal fin. The 3 cm incision was closed using three independent sutures (2/0 Ethicon silk) and, before the last suture was closed an injection of Liquamycin LP (1 ml kg^{-1} antibiotic) was provided intraperitoneally. Surgical time averaged 4-5 minutes.

Radio telemetry equipment

Transmitters measured 50 mm in length and 13 mm in diameter and were less than 2% of the experimental animals' body weight (Lotek Engineering Inc., Newmarket, Ontario). Muscle activity signals were detected through 18 carat gold tip sensors and transmitted along insulated stainless steel electrodes. A precision half wave rectifier and integrator processed the input EMG signals within the bandwidth 30-350 MHz. EMG signals were processed through an integrator and a radio pulse corresponding to the pulse interval in milliseconds

(ms) was transmitted when the integrated EMG (i.e. EMG*i*) equalled the predetermined threshold value of 150 μV. Increasing muscle activity resulted in a corresponding decrease in the interval between successive radio pulses. Because of this inverse relationship, the term "increased muscle activity index" will be used in the text to describe a decrease in the measured pulse interval (ms). Transmitters were designed to broadcast within an operating band of 148 to 150 MHz. Transmitted signals were detected and recorded automatically using a W20 radio receiver/data-logger (Lotek Engineering Inc., Newmarket, Ontario) and downloaded to a laptop computer via an RS232 serial communication port. The receiver was programmed to record all EMG*i* signals from each tagged individual.

Swimming chambers and experimental design

Swimming performance trials were conducted (at least one week after surgery) using a Blazka-type swim speed chamber (Blazka *et al.* 1960). This type of swim speed chamber is characterised by a tube within a tube design that permits water to be circulated within a smaller volume compared to Brett-type tunnel swim speed chambers (Brett 1964). The diameter of the inner tube was 28 cm and the outer tube was 44 cm. The total volume of the chamber was 70 litres. A heat exchanger was attached to the swim speed chamber to ensure a constant temperature during measurements of swimming speeds and determination of muscle activity indices. Water flow within the chamber was generated using an electric motor connected to an impeller and was rheostatically controlled. Calibration of motor speed and water velocity was performed at eight locations along the vertical mid-section of the swim chamber. The chamber could generate water velocities up to 2.4 m sec^{-1} within 5 seconds. Cross-sectional velocity profiles revealed that the edge effect was less than 4 cm at any

speed. Ambient, untreated river water was pumped into the chamber. Maximum cross-sectional area of Atlantic salmon used in swim trials was measured to ensure that adjustments could be made for the influence of the fish's body on water flow within the chamber, if necessary (Smit *et al.* 1971).

Measurement of swimming performance and critical swimming speed

In the present study swimming endurance and critical swimming speeds were determined for anadromous Atlantic salmon collected at various stages during their upstream migration. Prior to any measurement of swimming performance or critical swimming speed, Atlantic salmon were allowed 3 hours to acclimate to the swimming chamber. The acclimation speed used was 0.5 bl sec^{-1}.

Swimming endurance was measured by swimming individual Atlantic salmon at a known velocity until they fatigued. The swimming speeds used in the present study were 0.5, 1.5, 3.0, 4.5 and 6 bl sec^{-1}. For each velocity, 5 male and 5 female Atlantic salmon from each site were tested. Different individuals were used for each velocity. Sustained, prolonged and burst swimming performances are components of swimming endurance (Beamish 1978). These stages were identified from the swimming endurance of Atlantic salmon using the criteria of Beamish (1978). Sustained swimming performance was applied to swimming speeds which Atlantic salmon maintained for a minimum of 200 minutes, burst swimming applied to those swimming speeds maintained for less than 30 seconds, and prolonged swimming performance applied to those speeds maintained for less than 200 minutes but more than 30 seconds.

The critical swimming speed is a special category of prolonged swimming and is typically used to indicate the maximum swimming speed a fish can maintain for a prescribed

period (Brett 1964). Critical swimming speeds were determined by swimming individual Atlantic salmon over a range of increasing speeds until they fatigued. However, before being swum, Atlantic salmon were allowed to acclimate to the swim chamber for 3 hours at a velocity of 0.5 bl sec^{-1}. Depending on the nature of the study, and fish being tested, different criteria can be used to determine critical swimming speeds. In the present study, Atlantic salmon were subjected to water velocities that were increased by 0.5 bl sec^{-1} at 10 minutes intervals, until fatigue. Critical swimming speeds (U_{crit}) were then calculated using the equation presented in Brett (1964):

$$U_{crit} = V + (t_i / t_{ii})u_i \qquad \text{equation 2}$$

where: V is the highest velocity maintained for the prescribed period (m sec^{-1}), t_i is the time elapsed at final velocity (min), t_{ii} is the time increment (min) and u_i is the velocity increment (m sec^{-1}). Critical swimming speeds were investigated using 5 male and 5 female upstream migrating adults from each site.

Consistent measurements of swimming speeds depend on the consistent recognition of fish fatigue. Atlantic salmon were maintained in an exact swimming area (approximately 1.5-2 bl) by forward and rear grates. The criteria used to determine fatigue was the inability of the individual to continue swimming after touching the rear grate, despite three attempts to stimulate swimming by raising and lowering the water velocity.

Muscle activity was measured for Atlantic salmon swimming over the same range of speeds as those used to measure the critical swimming speed. Sample sizes are as follows: sites 2 through 4, n=4 males and n=4 females, sites 1 and 5, n=3 males and n=3 females. Muscle activity data were related to the swimming speeds for Atlantic salmon from each of the sites, and relationships between a muscle activity index and swimming speed were

established from these associations. In addition, muscle activity was also measured for Atlantic salmon at rest, and swum at 1.5 and 3.0 bl sec^{-1}.

Statistical analyses

Comparisons between sexes and among sites were accomplished using two-way ANOVA. Significant differences were identified using LSMEANS (Steele and Torrie 1980). The accepted level of significance in all cases was $p<0.05$. Linear regressions were used to develop relationships between muscle activity indices and swimming speeds. Means are shown ± their standard error. Linear regressions were also used to investigate relationships between changes in temperature and cross-sectional area with swimming performance and muscle activity indices. Multiple linear regressions were used to investigate the influence of cross-sectional area, temperature and water velocity on the swimming performance and muscle activity indices of male and female Atlantic salmon.

Results

Figure 4.1 shows the sampling sites and times of Atlantic salmon collection from the Exploits river. Temperature increased during the initial 10 weeks of sampling and then declined gradually until spawning (Figure 4.1). The highest water temperature was observed during July and was 22.2°C; the lowest temperature observed was 8.8°C and was in October.

Female Atlantic salmon were significantly heavier than males at each site, as well as at the time of spawning. The mean weight of males decreased significantly between site 1 and site 4 and then increased between sites 4 and 5 so that the weight of spawning males (site

5) did not differ from males at the start of their upstream migration (site 1)(Table 4.1). A similar trend in weight was observed among females, however, the final increase in weight was significantly greater in females than males and spawning females (site 5) weighed significantly more than females sampled at the outset of their upstream migration (site 1)(Table 4.1). There were no differences in mean length of male and female Atlantic salmon. Furthermore, length did not change significantly during upstream migration (Table 4.1). Significant decreases were observed in stomach and liver weight between site 1 and site 5 (Table 4.1). For female Atlantic salmon, a reduction of approximately 45% was observed in stomach weight and 58% in liver weight. By comparison, males lost 53% of their total stomach weight and only 23% of their liver weight. Ovaries weighed significantly more than testes throughout upstream migration. The mean weight of ovaries prior to spawning was almost 4 times that of testes (ovaries: 218.68±23.77g vs. testes: 55.38±6.91g). There were no significant differences in the girth or cross-sectional areas of males at any point during their upstream migration (Table 4.1). In contrast, the mean girth and cross-sectional area of females increased significantly between during upstream migration (Table 4.1).

Within 2 weeks of their collection from site 4, adult Atlantic salmon began courtship behavior (pairing of males and females) and within 3 weeks females began nest building activities. These conditions were used to identify the onset of spawning. The concentration of plasma testosterone in these males and was 1.24±0.17 pg μl^{-1} and was significantly higher than plasma concentrations from males at any other time. The concentration of 17β-estradiol in spawning females was found to be 0.67 pg μl^{-1} (Figure 4.2). The maximum concentration of plasma 17β-estradiol was found in female Atlantic salmon collected at site 4,

approximately 4 weeks prior to the initiation of spawning behaviors by Atlantic salmon collected at site 4 and allowed to complete maturation at the Noel Paul's Brook hatchery facility. Atlantic salmon collected at site 4 and taken to site 5 to finish maturation will hereafter be referred to as spawning Atlantic salmon (male and female).

There were no differences in sustained and burst swimming speeds of male and female Atlantic salmon between sites 1 and 4 (Figure 4.3). However, by the onset of spawning, the sustained swimming speeds of females were significantly lower than males. The critical swimming speeds female Atlantic salmon were also significantly lower than those of spawning males. Burst swimming speeds of spawning males and females were not significantly different (Figure 4.3). Both males and females were found to have significantly lower sustained, prolonged and burst swimming speeds at the onset of spawning than at any site sampled prior to spawning.

The relationship between muscle activity index and swimming speed changed over the course of upstream migration, and was sex dependent (Figure 4.4). Between sites 1and 4, male and female Atlantic salmon possessed similar muscle activity indices for a given speed (Figure 4.4). However, between site 4 and the onset of spawning the slope of the relationship between the muscle activity index and swimming speed for both male and female Atlantic salmon increased significantly (females site 4: -5.74±0.39 male site 4: -4.28±0.27, compare, spawning females -13.73±1.05 spawning males -9.62±0.51). The increase in slope was significantly greater for females than males (Figure 4.4).

Muscle activity indices from resting Atlantic salmon did not differ between sampling locations, and were independent of sex (Figure 4.5). However, significantly higher muscle

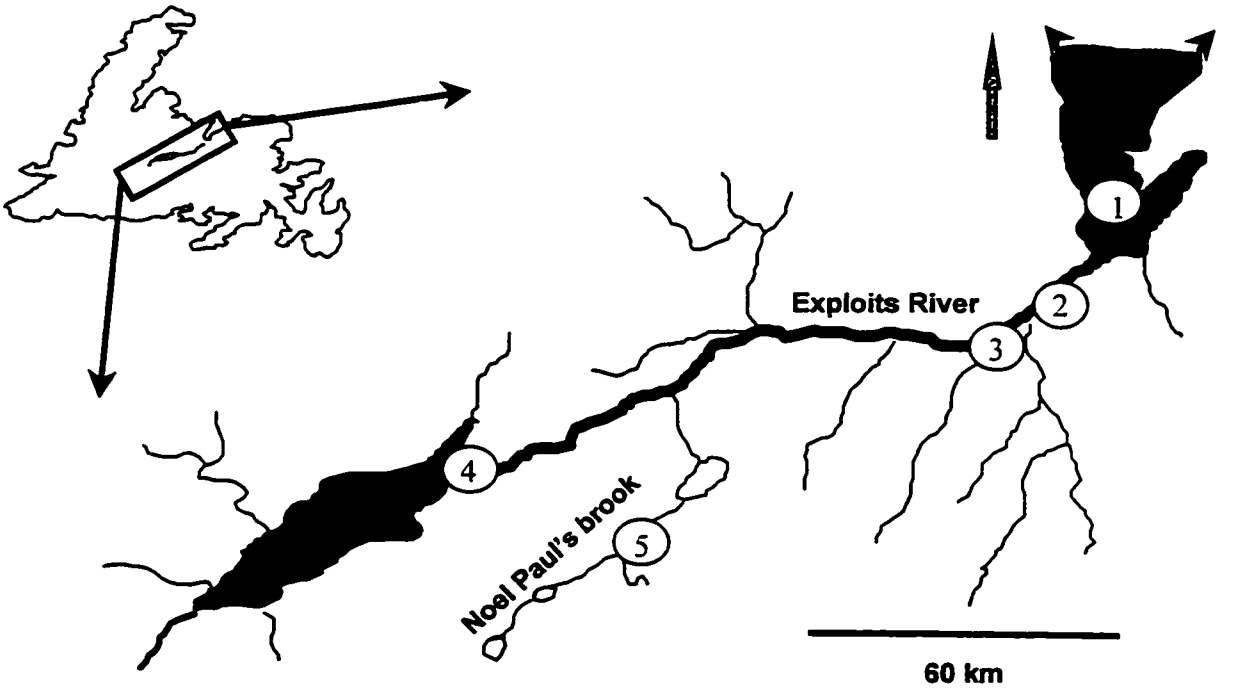
activity indices were observed in spawning male and spawning female Atlantic salmon swum at 1.5 bl sec^{-1} compared to those sampled from sites 1 through 4 (Figure 4.5). The muscle activity indices of spawning Atlantic salmon were also independent of sex. Swimming at 3.0 bl sec^{-1} resulted in the most noticeable change in the muscle activity index between sites as well as between sexes (Figure 4.5). Muscle activity indices of female Atlantic salmon sampled at site 5 and swimming at 3.0 bl sec^{-1} were significantly greater than those of males sampled at site 5 and females sampled at site 4 (Figure 4.5).

Changes observed in the sustained, critical and burst swimming speeds of male and female Atlantic salmon were correlated with the changes which occurred in water temperature over the migratory period (Table 4.2). Critical swimming speeds were influenced to a greater extent by temperature than either sustained or burst swimming speeds. The sustained, critical and burst swimming speeds of female Atlantic salmon were also correlated with changes observed in cross-sectional areas during the upstream migration (Table 4.2). The sustained swimming speeds of females was more dependent on cross-sectional area than either critical or burst swimming speeds (sustained r^2=0.58, critical r^2=0.39, burst r^2=0.40). The swimming capabilities of males were almost independent of changes in cross-sectional over the upstream migration (sustained r^2=0.06, critical r^2=0.01, burst r^2=0.05).

Changes in the muscle activity indices of male and female Atlantic salmon swum at 1.5 bl sec^{-1} and 3.0 bl sec^{-1} were correlated with changes in temperature over the migratory period (Table 4.2). The influence of temperature on muscle activity was greatest for Atlantic salmon tested at 3.0 bl sec^{-1} (Table 4.2). Cross sectional was correlated with changes in the muscle activity patterns of females swimming at 1.5 and 3.0 bl sec^{-1}, but not males (Table

4.2). Collectively, changes in the swimming capabilities and muscle activities of females are influenced to a greater extent by temperature and cross-sectional area than male Atlantic salmon.

Figure 4.1. Sampling sites along the migratory route of Atlantic salmon in the Exploits River, Newfoundland, Canada. Mean water temperature and sampling dates are shown. Map is to scale.



site	location	sampling date	temperture (°C)
Site 1	Bay of Exploits	May 26 -27th	14.6±0.3
Site 2	Bishop's Falls	June 18 - 24th	19.3±0.4
Site 3	Grand Falls	July 16 - 20th	22.2±1.1
Site 4	Red Indian Dam	September 11-14th	14.0±0.3
Site 5	Noel Paul's Brook	October 12-17th	8.8±0.4

Table 4.1. Fork lengths (FL), weights (WT), girths(GT), and cross sectional areas (XS) of male and female Atlantic salmon sampled at various sites during their spawning migration in the Exploits River, Newfoundland, Canada. Weights of the gonad, liver and stomach (including caeca) are listed as well. Sample sizes are indicated in the table. Significant differences between sexes are indicated by an asterix (*) and between sites by raised numbers. In all cases the accepted level of significance was $p<0.05$.

	Sex	site1 (n=8M,8F)	site 2 (n=8M,8F)	site 3 (n=8M,8F)	site4 (n=8M,8F)	site5 (n=8M,8F)
FL	M	55.32±0.65	55.72±1.11	54.80±0.94	53.98±1.26	55.81±0.81
(cm)	F	55.55±0.61	54.78±0.89	56.10±0.54	58.24±2.11	59.58±1.57
WT	M	1.54±0.06 *	1.47±0.08 *	1.37±0.06 * [1,2]	1.33±0.05 * [1,2]	1.49±0.05 * [3,4]
(kg)	F	1.60±0.07	1.57±0.07	1.47±0.04 [1,2]	1.76±0.22 [1,2,3]	1.74±0.22 [1,2,3]
GT	M	26.53±1.05	24.97±0.66	24.68±0.70	26.94±0.66	25.95±0.51
(cm)	F	28.36±0.57	27.75±0.31	25.19±1.06	27.80±1.94	32.75±1.18 [2,3,4]
XS	M	53.90±1.72	53.40±1.65	49.54±1.44	57.27±2.58	54.41±2.26 *
(cm^2)	F	65.81±1.43	61.82±0.86	54.99±2.90	63.49±4.95	75.89±4.78 [1,2,3,4]
WT_{gonad}	M	3.13±0.27 *	5.69±0.741 *	10.62±1.63 *	39.04±4.78 * [1,2,3]	55.38±6.91 * [1,2,3]
(g)	F	16.04±2.85	30.93±2.03	31.59±3.81	194.20±19.54	218.68±23.77 [1,2,3]
WT_{liver}	M	22.36±1.42	13.28±0.53 *	15.35±0.91 *	12.44±0.60	17.05±3.85 *
(g)	F	26.51±1.93	24.33±0.69	21.97±2.34	12.21±1.40 [1,2,3]	10.82±0.98 [1,2,3]
$WT_{stomach}$	M	73.06±9.08	30.46±0.84 * [1]	27.53±0.74 [1]	9.91±1.34 [1,2,3]	8.48±4.17 [1,2,3]
(g)	F	72.16±6.88	44.17±3.27 [1]	30.82±3.03 [1]	15.17±4.08 [1,2,3]	8.55±0.55 [1,2,3]

note: M represents male Atlantic salmon and F, female Atlantic salmon.

Figure 4.2. Changes in the plasma concentration of testosterone (males) and 17-β estradiol (females) during the spawning migration of Atlantic salmon during their in the Exploits River, Newfoundland, Canada. Sampling periods with different letters are significantly different. Vertical bars indicate the onset of spawning.

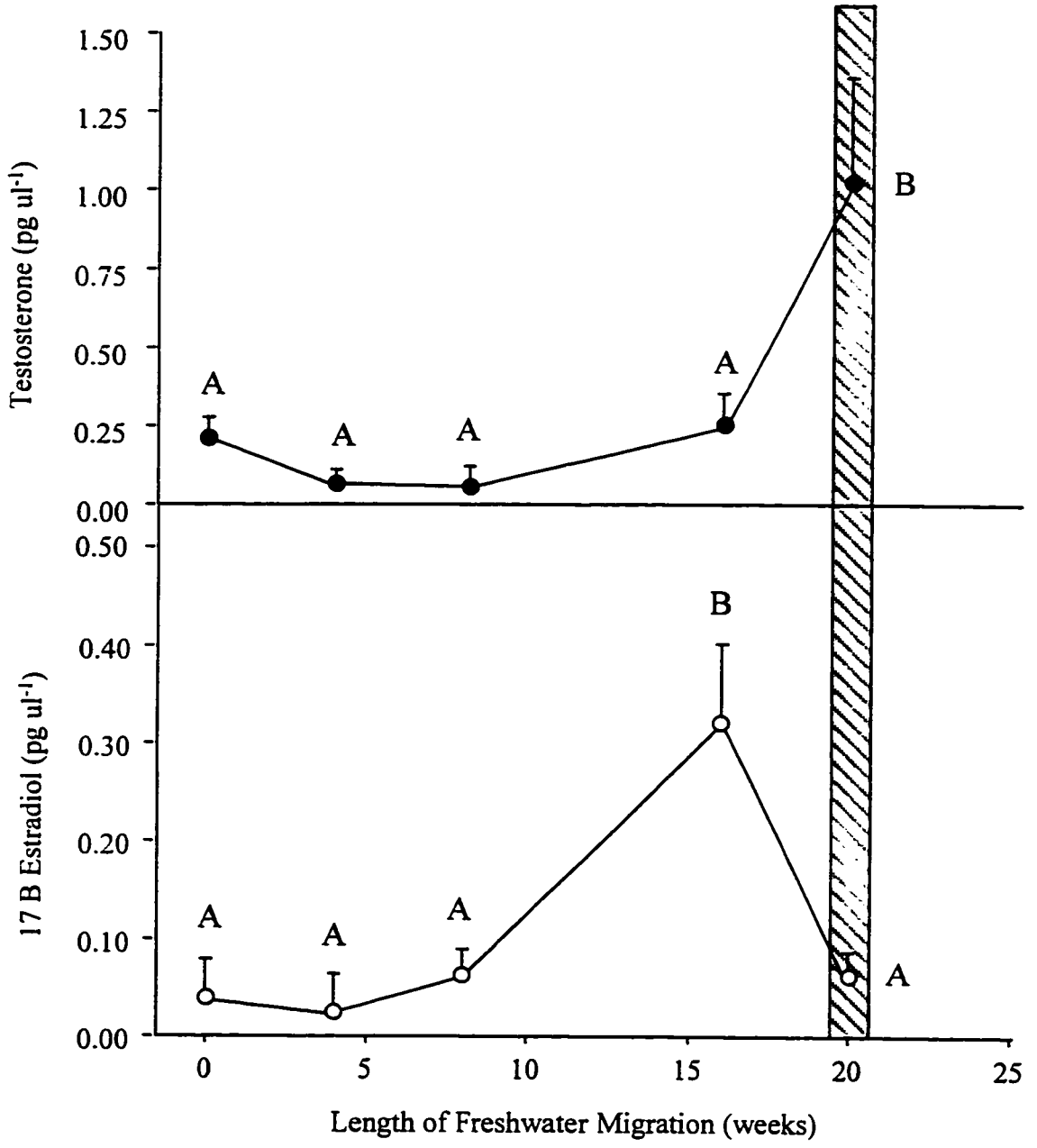

Testosterone (pg ul-1)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
A
A
A
A
B
17 B Estradiol (pg ul-1)
0.50
0.40
0.30
0.20
0.10
0.00
A
A
A
B
A
0
5
10
15
20
25
Length of Freshwater Migration (weeks)

Figure 4.3. The sustained (i), critical (ii) and burst (iii) swimming speeds of male (solid circle) and female (white circle) Atlantic salmon during their spawning migration. Sampling periods with similar letters are not significantly different. Vertical bars indicate the onset of spawning. Significant differences between sexes are indicated by an asterix (*).

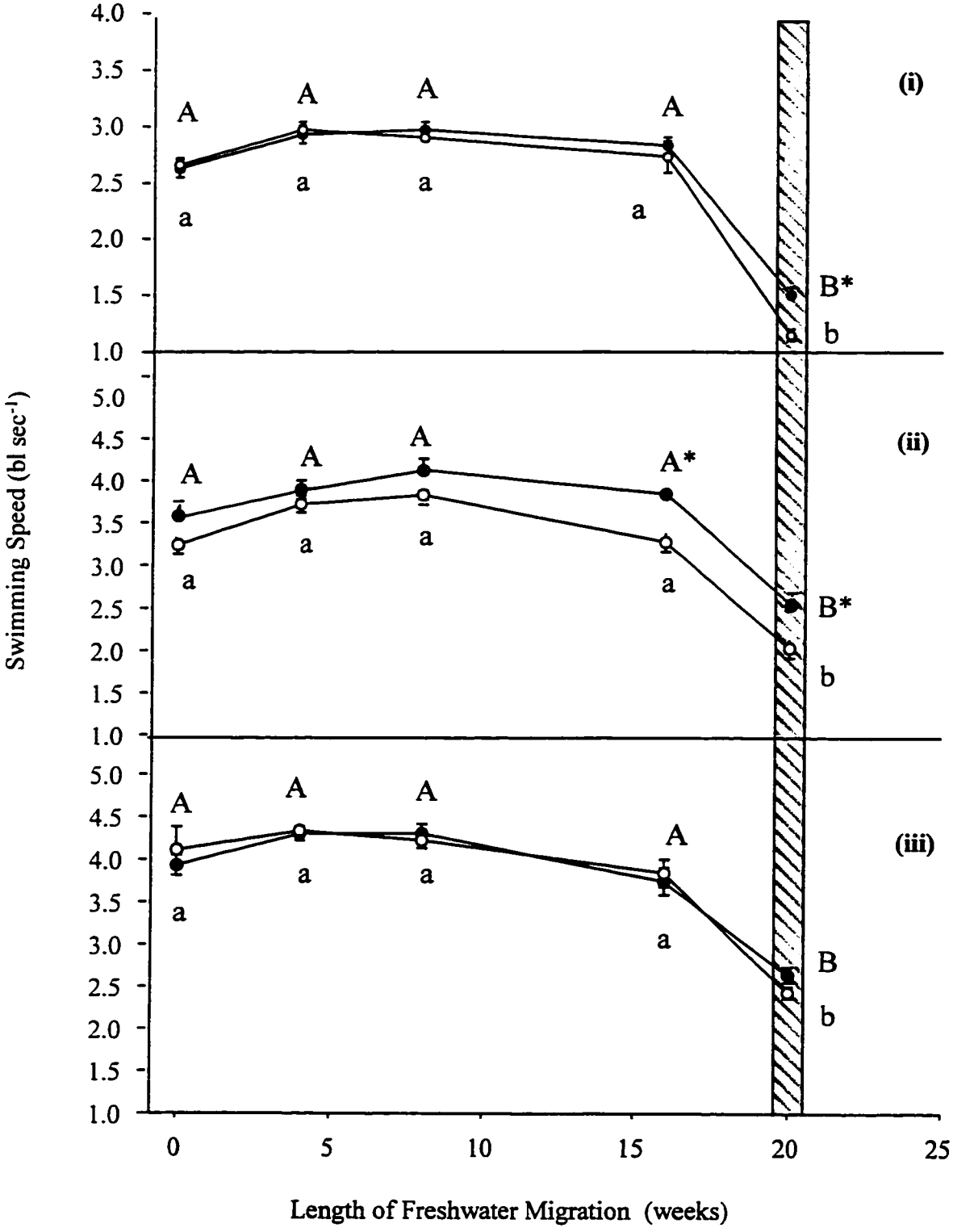

Swimming Speed (bl sec-1)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
(i)
A
A
A
A
a
a
a
a
B*
b
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
(ii)
A
A
A
A*
a
a
a
a
B*
b
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
(iii)
A
A
A
A
a
a
a
a
B
b
0
5
10
15
20
25
Length of Freshwater Migration (weeks)

Table 4.2. Multiple squared regression coefficients and significance levels from comparisons of girth and temperature with (i) sustained, critical, burst swimming speeds and (ii) muscle activity indices measured during swimming at 1.5 and 3.0 bl sec^{-1} from wild Atlantic salmon collected during their spawning migration. In total, 18 Atlantic salmon were used for each comparison. (i.e. sites 2, 3and 4, n=4, sites 1 and 5, n=3).

	female		male	
	temperature	cross-sectional area	temperature	cross-sectional area
sustained	r^2=0.59, p<0.001	r^2=0.58, p<0.015	r^2=0.41, p<0.001	r^2=0.06, p=0.075
critical	r^2=0.67, p=0.001	r^2=0.39, p<0.001	r^2=0.54, p<0.001	r^2=0.01, p=0.057
burst	r^2=0.28, p<0.001	r^2=0.40, p<0.001	r^2=0.20, p<0.001	r^2=0.05, p<0.001
activity(1.5bl)	r^2=0.62, p<0.001	r^2=0.71, p<0.001	r^2=0.49, p=0.002	r^2=0.02, p=0.563
activity(3.0bl)	r^2=0.64, p<0.001	r^2=0.74, p<0.001	r^2=0.63, p<0.001	r^2=0.14, p=0.020

Figure 4.4. Relationships between muscle activity indices and swimming speeds for male (solid) and female (white) Atlantic salmon at various stages of their spawning migration. Site 1 represents sampling just after entry to fresh water and site 5 represents sampling during the spawning period. Sample sizes are as follows sites 2 through 4, n=4 males and n=4 females, sites 1 and 5, n=3 males and n=3 females.

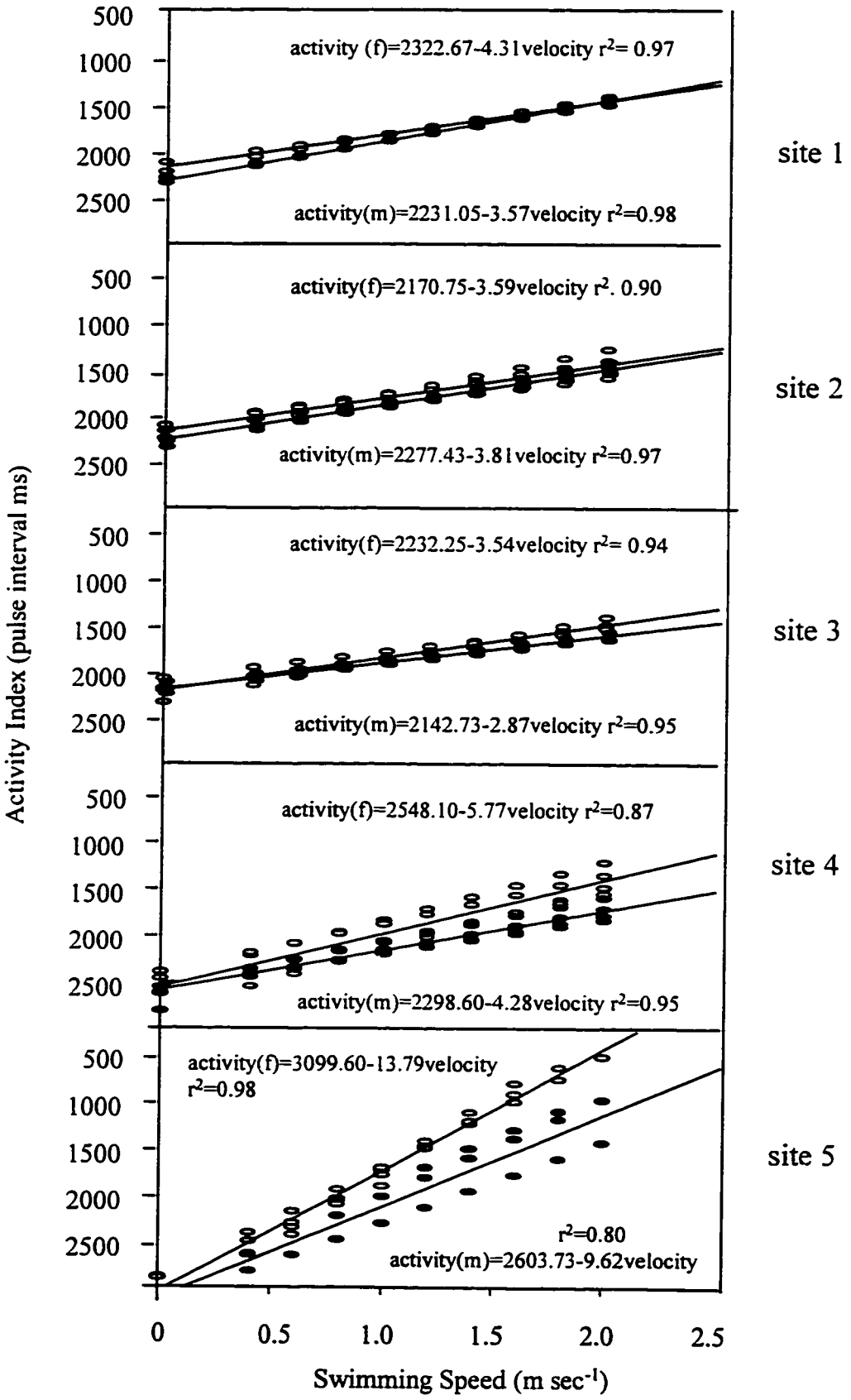
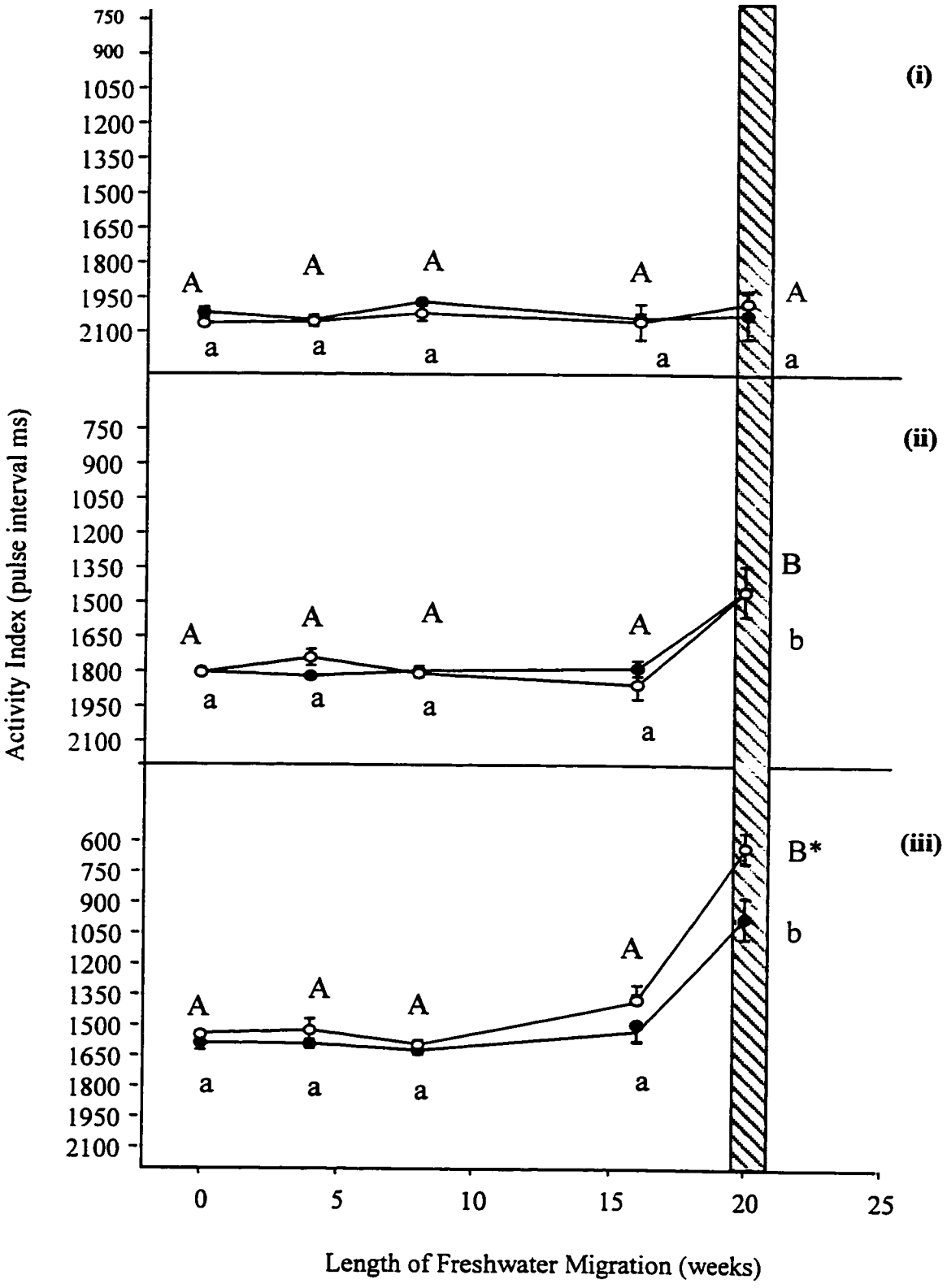

activity (f)=2322.67-4.31velocity r²= 0.97
activity(m)=2231.05-3.57velocity r²=0.98
site 1
activity(f)=2170.75-3.59velocity r². 0.90
activity(m)=2277.43-3.81velocity r²=0.97
site 2
activity(f)=2232.25-3.54velocity r²= 0.94
activity(m)=2142.73-2.87velocity r²=0.95
site 3
activity(f)=2548.10-5.77velocity r²=0.87
activity(m)=2298.60-4.28velocity r²=0.95
site 4
activity(f)=3099.60-13.79velocity
r²=0.98
r²=0.80
activity(m)=2603.73-9.62velocity
site 5
Activity Index (pulse interval ms)
500
1000
1500
2000
2500
0
0.5
1.0
1.5
2.0
2.5
Swimming Speed (m sec⁻¹)

Figure 4.5. The muscle activity indices of male (solid circle) and female (white circle) Atlantic salmon at rest (i) and swum at 1.5 (ii) and 3.0 (iii) bl sec^{-1}. Hatched bar indicates the pre-spawning period. Vertical bars indicate the onset of spawning. Sampling periods with similar letters are not significantly different. Significant differences between sexes are indicated by an asterix (*).


Activity Index (pulse interval ms)
750
900
1050
1200
1350
1500
1650
1800
1950
2100
(i)
(ii)
(iii)
A
a
B
b
B*
0
5
10
15
20
25
Length of Freshwater Migration (weeks)

Discussion

The sustained, prolonged and burst swimming performance of Atlantic salmon do not change during their upstream migration, prior to spawning. At the time of spawning, however, all three categories of swimming performance declined significantly. Furthermore, the loss of swimming performance appears greater for females than males. There is also a decline in critical swimming speed, which like the decline in sustained swimming speed is greater among spawning females than spawning males. The reasons for the decline in swimming performance at the time of spawning may be related to several factors, including the depletion of energy (lipid in particular), behavioral and morphological changes associated with reproductive maturity and the decrease in water temperature which occurred just prior to spawning.

Lipid and proteins obtained from their somatic and visceral tissues supply most of the energy for swimming and gonadal development (Jonsson *et al.* 1997). The stomach and liver may be important sources of energy in upstream migrating Atlantic salmon in the Exploits River. The stomach lost significantly more weight than the liver during migration, suggesting that energy from lipid stored around the stomach may be used before that of other sources such as the liver. The greater loss of weight from the stomach is consistent with the findings of Jonsson *et al.* (1997), who reported significant reduction in lipid from stomach tissue in upstream migrating Atlantic salmon from the River Daamgard, Norway.

The loss of weight from the liver in the latter stages of migration could be related to sexual maturation. The liver is an important source of lipoproteins, and during the final stages of ovarian maturation the liver supplies large amounts of vitellogenin for egg

production. The observation that the significant increase in ovary weight occurred during the same period as the largest decline in liver weight are consistent with the role of the liver in the supply of vitellogenin to maturing ovaries (Weigand and Idler 1985). Vitellogenin is not required by maturing males and this is reflected in the smaller loss of weight from the livers of upstream migrating male Atlantic salmon. The largest increase in plasma 17β-estradiol concentration occurred during the same period as the largest increase in ovary weight. The increase in 17β-estradiol is consistent with its role in ovarian maturation (Olin and Von Der Decken 1987). The highest concentration of testosterone was observed in spawning male Atlantic salmon. The finding that testosterone peaked prior to 17β-estradiol is intriguing. Since, males undergo less gonadal development during migration than females, circulating levels of testosterone will remain lower than that of 17β-estradiol, which is required for ovarian maturation. The increase in plasma testosterone concentration at the time of spawning is consistent with the role of this steroid in the control of reproductive behavior in males (Kindler *et al.* 1989). Since males are preparing to spawn and will have to compete for females. increased testosterone at this time suggests males are, in fact, be preparing to spawn (Kindler *et al.* 1989).

According to Brett (1995), anadromous salmonids experience changes in body morphology during their spawning migrations. Most of these changes occur during the freshwater phase, and are associated with acquisition of secondary sex characteristics and (Yamamato 1969) and increases in gonad size during sexual maturation. Morphogenic changes occur to a greater extent in males than females, and are thought to function as stimuli in mate selection and male dominance displays (Yamamoto 1969). Changes in gonad size

occur in both males and females. However, females may experience greater changes in body shape than males because the increase in ovary weight is greater than that of the testes (Brett 1995). Furthermore, since the sexual maturation of both males and females occurs over the course of the upstream migration, changes in body morphology associated with gonad development probably occur gradually over the same period.

The larger cross sectional areas of spawning female Atlantic salmon compared to male Atlantic salmon may have contributed to the greater reductions observed in the sustained, critical and burst swimming speeds and the increase in muscle activity indices of females. Regression analyses confirmed that the sustained, critical and burst swimming speeds of females were dependent on cross sectional area. One possibility for the relationship between cross sectional area and swimming speed is the effect of cross sectional area on power required for swimming. According to Azuna (1994), as cross sectional area increases so does the power required to swim. To obtain the additional power required to swim, female Atlantic salmon may have to recruit their white muscle (Beamish 1978, Jayne and Lauder 1994, Rome *et al.* 1993). The increased muscle activity and reductions in sustained swimming speeds of females support this conclusion (Beamish 1978).

Recruitment of white muscle is also known to occur when the power required to swim is increased by the effects of temperature on muscle mechanics (Rome *et al.* 1993). It is not surprising, therefore, that the sustained, critical and burst swimming speeds and muscle activity indices of upstream migrating male and female Atlantic salmon were correlated with temperature. At the outset of migration, the initial increase in temperature probably favors upstream migration, by increasing muscle power output and increasing swimming speeds (Rome *et al.* 1984). However, the decline in temperature prior to spawning would impair

swimming performance. Given that a decrease in temperature occurred prior to spawning, and was greater than the increase which occurred at the outset of migration, it is not surprising that a decrease in swimming performance was observed prior to spawning and an increase at the start of the upstream migration was not.

In addition to the effects of temperature and cross sectional area, reductions observed in sustained, critical and burst swimming speeds and increase in muscle activity indices may reflect the influence of a metabolic readjustment due to the cessation and the mobilization of visceral and somatic energy sources. During their upstream migration, Atlantic salmon depend on lipids for most of their energy (Jonsson *et al.* 1997). In the present study, Atlantic salmon experienced significant reductions in their plasma non-esterified fatty acid levels, suggesting that fatty acids are an important source of lipid during migration. Furthermore, females experienced larger declines in their plasma fatty acid levels, suggesting that females have higher lipid requirements than males (R.K. Booth, unpublished data, chapter 5). Fatty acids are important substrates for ovarian development in female Atlantic salmon (Weigand and Idler 1985). At least some of the change in female plasma fatty acid levels may reflect the use of lipid during gonadal maturation. However, since the greatest period of ovarian development occurs just prior to spawning, the declines in plasma fatty acids at the outset of migration suggest additional uses of plasma fatty acids. Fatty acids are important substrates of the red muscle (Keissling and Keissling 1993). Intramuscular fatty acids may provide the immediate source of fatty acids during muscle activity, however, after these are depleted, fatty acids must be obtained from extramuscular sources. This mobilization may explain, in part, the initial rise in plasma fatty acid levels at the outset of migration, and the similar decline of plasma fatty acids in both male and female Atlantic salmon during the initial

portion of their upstream migration. In the final stages of their upstream migration, reductions in sustained and critical swimming speeds could reflect constraints placed on red muscle activity by reduced plasma fatty acid levels. Since white muscle does not use lipid as fuel, it is unlikely that reduced lipid levels would influence burst swimming speeds. The reduced burst swimming speeds of Atlantic salmon at the time of spawning may be related to a greater use of white muscle to offset the effects of fatty acid depletion on red muscle activity.

At burst swimming speeds the muscle activity indices obtained from upstream migrating female Atlantic salmon were significantly higher than those of upstream migrating males. As noted previously, white muscle may be recruited to supply additional power to offset the effects of temperature and increased cross sectional area on red muscle power output. There is also evidence to suggest that as power requirements increase, more white muscle is recruited (Jayne and Lauder 1994, Rome *et al.* 1993). Since females experienced greater increases in their cross sectional area, swimming at burst speeds may require females to recruit additional power from their white muscle. This may explain why burst swimming speeds of female Atlantic salmon were more dependent on cross sectional area than those of males. The burst swimming speeds of both upstream migrating male and female Atlantic salmon were mildly influenced by temperature.

The highest muscle activity indices were observed in spawning females at their burst capabilities. These results imply that spawning females may depend on white muscle activity to a greater extent than males, and consequently, may exceed their aerobic capacity more frequently than males. Priede (1983) suggested those fish which exceed their aerobic scope increase their potential for mortality. Thus, the higher muscle activity indices of females may

indicate that female Atlantic salmon are at a greater risk of dying as a result of upstream migration than males. This is in contrast to Jonsson *et al.* (1991) who suggested mortality to be higher in migratory male Atlantic salmon because of the large depletion of their lipid reserves during spawning.

An interesting finding of this study was the change observed in the relationship between muscle activity index and swimming speed during upstream migration. The findings suggest that when radio transmitted EMG*i* are used to measure the swimming performance of upstream migrating salmonids, muscle activity indices must be based on relationships with swimming speeds obtained at periods during migration. Failure to correlate muscle activity indices with swimming speeds at periods during migration could lead to errors when estimating the swimming performance of upstream migrating salmonids. Furthermore, correlations should also be sex specific to reflect differences in morphology which could be related to the degree of sexual maturity.

Over the course of their freshwater spawning migrations, Atlantic salmon will experience changes in water temperature. They will also undergo rapid sexual maturation. Under such conditions, any factors, including normal spawning behavior, which increase swimming activity will impose stress on Atlantic salmon which may already be performing close to, or beyond their aerobic limits (Brett 1995, Gilhousen 1980). During their upstream migration, male and female Atlantic salmon were found to experience decreases in their sustained, critical and burst swimming speeds and increases in their muscle activity indices. However, these declines were not apparent until the onset of spawning, at which time these salmon will have already reached their spawning areas and will not be required to migrate any further. Thus, the decline observed in the swimming performance of Atlantic salmon

appears to be well suited to their anadromous life cycle. That burst swimming does not differ between males and females may attest to the importance of this form of swimming in courtship and nest building activities (Brett 1995). Furthermore, since burst swimming is not dependent on lipid as a substrate, it is unlikely to be influence by the decline in lipid over the migratory period, whereas, sustained swimming is. Additionally, this study found that the relationship between muscle activity index and swimming speed of anadromous Atlantic salmon changes during upstream migration. The change is characterized by a consistent increase in muscle activity as migration proceeds. These results suggest that Atlantic salmon can maintain their swimming abilities through greater muscular effort. Since this increased muscular effort comes at the price of increased energy expenditures, Atlantic salmon may be required to tap into additional energy stores, such as protein, to fuel upstream migration.

With respect to the management of Atlantic salmon, this study demonstrates that care should be taken when operating fish passage structures which require Atlantic salmon to swim at or beyond their sustained swimming performance because this parameter changes during migration and between sexes. My results also show that the swimming capabilities of Atlantic salmon are greater at the outset of migration are greater than when they are preparing to spawn. Consequently, when Atlantic salmon are required to swim at specific velocities during the final stages of their freshwater migration, they swim at these speeds with an additional energetic cost. Therefore, when Atlantic salmon are forced to maintain specific swimming speeds more energy may be used for swimming prior to spawning and this could limit the availability of energy for reproduction. I suggest that swimming performance data used in the operation of fish bypass structures for anadromous Atlantic salmon use swimming speeds of migrating salmonids obtained at various stages of their migration, or at least from

Atlantic salmon subjected to environmental and physiological stresses similar to those experienced during their upstream migration.

Chapter 5

Plasma non-esterified fatty acid profiles of male and female Atlantic salmon (*Salmo salar* L.) during their freshwater spawning migration.

Abstract

In the present study plasma non-esterified fatty acid (NEFA) profiles of migrating Atlantic salmon were examined to determine their role(s) during freshwater migration, preparation for spawning and the post spawning period. Changes in gonadal weight were also investigated to determine reproductive status. Sex specific patterns of gonadal development were identified in Atlantic salmon. Gonadal maturation was most rapid in males with testicular growth commencing after the second month of migration, whereas maximum ovarian growth did not occur until the fourth month. Significant increases in plasma NEFA content occurred in both males and females during the first month of upstream migration. Female plasma NEFA levels were significantly higher than those of males and remained higher throughout the first 4 months of their migration. However, during the month before spawning total NEFA levels declined significantly in females and at spawning female NEFA levels were 27% lower than those of males. The rapid decline in the plasma NEFA content of females coincided with the largest increase in their gonadosomatic index. Plasma levels of 16:0 (palmitic), 16:1 (palmitoleic), 18:1n9 (oleic) and 20:5n3 (timnodonic) varied significantly between males and females throughout the migratory period. Plasma NEFA levels declined in both males and females following spawning. There were no sex specific differences in any fatty acids of kelts. Collectively, these results show that fatty acids are important substrates during the migration and sexual development of Atlantic salmon. Additionally, these data provide information regarding the role of fatty acids in Atlantic salmon kelts during their seaward migration.

Introduction

During their spawning migrations, salmonids stop feeding and depend entirely on their somatic and visceral tissues to supply the energy for locomotion and gonadal development (Love 1980). Previous studies of migrating sockeye salmon (Idler and Clemens 1959, Idler and Bitners 1960), lake whitefish *Coregonus clupeaformis* (Bernatchez and Dodson 1985, Lambert and Dodson 1990), cisco *Coregonus artedii* (Bernatchez and Dodson 1985, Lambert and Dodson 1990) and Atlantic salmon, (Jonsson *et al.* 1997) have demonstrated that fat and proteins are the most readily available sources of energy in fish tissues. Moreover, fats appear to be used in preference to protein. There is, however, considerable variability in the lipid requirements of migrating fish. These differences may be related to differences in their life histories and the length of their migrations. For example, members of the genus *Oncorhynchus.* which includes most Pacific salmonids, undertake freshwater migrations that can exceed 500 km in length. The extreme energy requirements of these migrations result in a significant depletion of body lipids throughout the spawning period (Idler and Bitners 1958, Idler and Clemens 1959, Patton *et al.* 1970). Muscle proteins are often mobilized by Pacific salmons in the latter stages of migration when fat depots have become depleted. The enormous demands placed on the limited body reserves and the physiological stress associated with migration and reproduction ultimately lead to death soon after spawning. In contrast, Atlantic salmon and anadromous coregonids are iteroparous and death does not always occur after spawning. In these species, lipids are used throughout the

migratory period with little or no decline in muscle protein content (Jonsson *et al.* 1991, 1997).

Tissue and plasma non-esterified fatty acid levels have been investigated in an attempt to determine the physiological requirements of fish for lipid (Plisetskaya 1980, Henderson and Tocher 1987, Ballantyne *et al.* 1996). Plasma non-esterified fatty acids may be particularly well suited for studying the lipid requirements of anadromous fish because they vary during different phases of migration and reflect changes in nutritional status (Jezierska *et al.* 1982, Black and Skinner 1986), state of sexual development (Weigand and Idler 1985, Ballantyne *et al.* 1996) and activity level (Henderson and Tocher 1987, Weber *et al.* 1996). Environmental factors including temperature, salinity and pH are also known to influence the proportions of fatty acids in fish (Hazel and Williams 1992). Specifically, polyunsaturated fatty acids appear to be most responsive to environmental factors because of their structural roles in biological membranes (Bell *et al.* 1986). Consequently, the drop in salinity and increase in water temperatures experienced by salmon as they migrate into fresh water will influence tissue and plasma fatty acid compositions.

To my knowledge, migration-dependent changes in plasma NEFA levels have only been reported for sockeye salmon (Patton *et al.* 1970, French *et al.* 1983, Ballantyne *et al.* 1996). Clearly, the variability that exists in the lipid requirements of anadromous fish infers that fatty acid requirements may also differ between migratory species. In salmonids such as the Atlantic salmon, which are capable of surviving spawning, it is thought that feeding may not be resumed until the return to the marine environment. Consequently, the additional energetic demands of over-wintering in fresh water and the return migration to salt water are placed on the already limited body reserves of spent Atlantic salmon. At present there are no

studies of plasma NEFA profiles in migrating Atlantic salmon during their upstream migration or downstream freshwater migrations. The purpose of this study, therefore, was to determine the roles of plasma non-esterified fatty acids in male and female Atlantic salmon during their spawning migration. Plasma NEFA levels in post spawning Atlantic salmon were also examined to determine whether fatty acids provide an important source of energy during the return migration to salt water.

Materials and Methods

Animals and study site

Wild Atlantic salmon (1.0-2.6 kg) were collected from one salt water site in the Bay of Exploits and 3 fresh water sites on the Exploits River, Newfoundland, Canada between May 10th and October 14th, 1996 (Figure 5.1). Individuals were collected from the Bay of Exploits using a small mesh gill net. The net was monitored constantly and entangled fish were typically removed within 4 minutes and immediately placed in fresh aerated seawater. Fish collections from each of the three Exploits River sites occurred during the peak migrations of salmon at each site (Figure 5.1). Collection of fish from each site involved dip-netting individuals from traps located at fish ladders located 5km, 20km and 100km from the river mouth. Spawning individuals were collected from site 3 and taken to the Noel Paul's brook hatchery facility where they were held in an artificial spawning channel until fish were preparing to spawn which was signified by the initiation of redd making activity by female salmon.

Due to the current legislation regarding the management of Atlantic salmon in the Exploits River only 6 individuals (3 males and 3 females) could be obtained from each site for comparison of sex specific differences in plasma NEFAs.

Blood sampling and gonadal somatic indices

Individual Atlantic salmon were removed from the tank using a dip-net and immediately placed in a solution of MS-222 (tricaine methanosulfonate) buffered to pH 7.0 using sodium bicarbonate ($NaCO_3$). Anaesthetized salmon were removed and placed on a sterile surgery table and a 5 ml aliquot of blood was immediately drawn from the caudal vasculature using a 10 ml heparinized syringe. Whole blood was immediately distributed among 1.5 ml Eppendorf tubes and centrifuged for 4 min at 7000 X *g* to isolate the plasma fractions. Fresh plasma was transferred into three 1.5 ml Eppendorf tubes and immediately frozen in liquid nitrogen. Total body and gonad weights were determined for each fish to calculate gonadosomatic index (GSI).

Analysis of plasma non-esterified free fatty acids

Plasma non-esterified fatty acids were methylated using the method of Singer *et al.* 1990. Briefly, 150 µl of plasma and 15 µl of a known standard (heptadecanoic acid, 17:0) was placed in 5 ml of 6:1 v/v acetyl chloride/methanol solution and incubated for 45 min. at 27°C. The reaction was halted with 3 ml of potassium carbonate. Following this, 150 µl of hexane was added and the solution was vortexed for 30 sec and then spun at 2000 X g for 10 min. Following centrifugation, 300µl of hexane was added to the tube and the mixture was allowed to sit for 1 min. Using a Hamilton syringe, 300 µl of hexane was drawn off and stored in a 0.5 ml vials sealed under N_2 gas. Prior to analysis the hexane was blown off under

N_2 gas and the methyl esters were re-suspended in 25 µl of carbon disulphide (CS_2) and stored in 0.5 ml dram vials. One microlitre of CS_2 containing methyl esters was injected into a gas chromatograph (Hewlett-Packard HP5890A) fitted with a flame ionization detector and an automatic injector (Hewlett-Packard 7673A). Fatty acid methyl esters were resolved on a fused silica column (DB-225 Megabore, Chromatographic Specialties Inc.) using helium as the carrier gas. The Initial oven temperature was 150°C and this was programmed to increase to 210°C upon injection of the sample, where it remained for 30 min. while the sample was analyzed. Retention times of fatty acid methyl esters were compared with those of known standards and the final concentrations were determined by comparison with the internal standard. All chemicals were obtained from Sigma Chemicals, St. Louis, Mo.

Statistical analyses

An analysis of variance was used to determine differences between sites and sexes. Significance was determined between sites and sexes using LSMEANS (Steele and Torrie 1980). Means are shown ± the standard deviation. The accepted level of significance in all cases was $p<0.05$.

Results

Changes in the mean GSI for male and female Atlantic salmon are shown in Figure 5.2. Both males and females experienced gonadal growth throughout the migratory period. The development of male testes occurred earlier into migration than the development of female ovaries and is reflected in the increase in male GSI after 2 months while female GSI

did not increase until nearly 4 months. At the time of spawning, females possessed gonads which were substantially larger in size than males as reflected by the significantly higher GSI of females

Total plasma NEFAs increased significantly in both male and female Atlantic salmon upon entering fresh water (Figure 5.3). Total plasma NEFAs were significantly higher in females before entering fresh water and remained significantly higher than male levels until the fourth month of their spawning migration. By the fifth month, when Atlantic salmon were preparing to spawn, female NEFA levels were 25% lower than those of males. In the period following spawning, total plasma NEFA contents declined significantly but were not different between the sexes during the post spawning period. It is noteworthy, however, that post-spawning plasma NEFA levels in females represented only 9.8% of their maximum pre-spawning level of 5476.60 nmol ml^{-1} whereas the levels in males represented 15% of their peak pre-spawning level of 4617.20 nmol ml^{-1}.

Monounsaturated (MUFA), polyunsaturated (PUFA) and saturated (SFA) NEFAs followed a trend similar to that of the total NEFA profiles (Figure 5.4). Over most of the pre-spawning period females maintained higher levels of MUFAs and PUFAs and SFAs than levels in males. However, by the fifth month, when Atlantic salmon were preparing to spawn, the plasma concentrations of MUFAs, PUFAs and SFAs were lower among females than males. The post-spawning concentrations of MUFAs, PUFAs and SFAs were significantly lower than pre-spawning in both sexes. The levels of MUFAs, PUFAs and SFAs declined significantly in males during the post-spawning period. In contrast, only MUFAs and PUFAs declined significantly in females. SFAs also declined, but the plasma

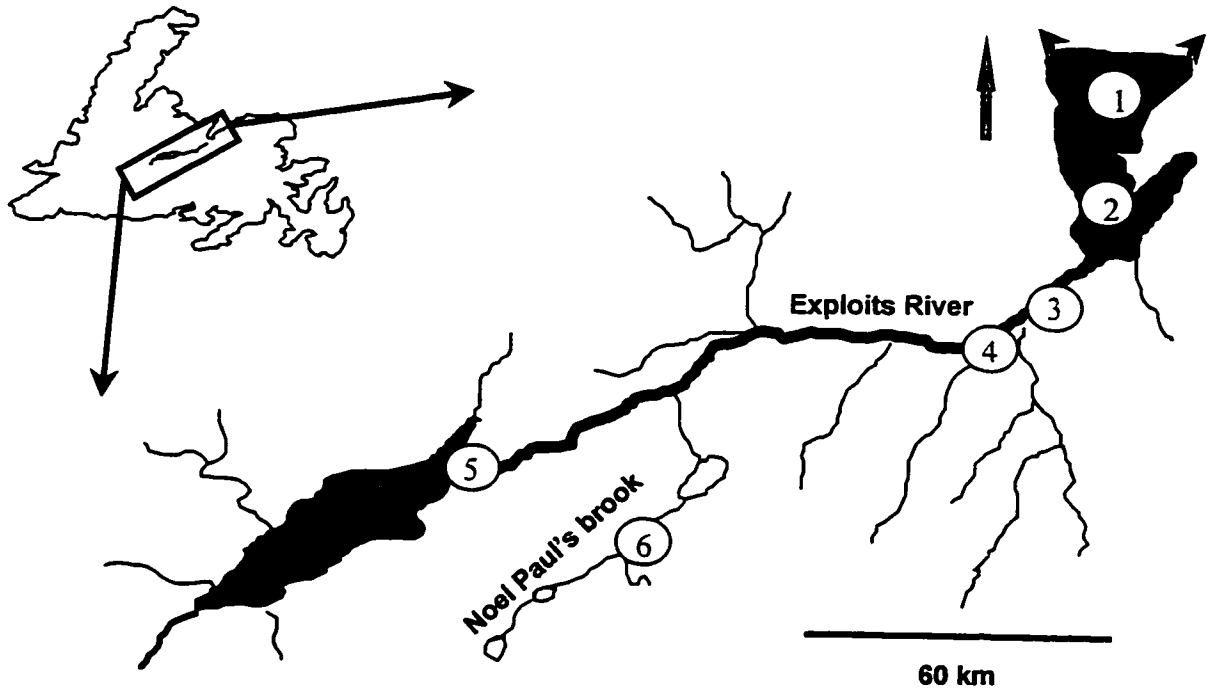
levels in female kelts were not significantly different from those of spawning females (Figure 5.4)

Throughout the migratory period, 14:0 (myristic), 16:0 (palmitic), 16:1 (palmitoleic), 18:1n9 (oleic), 22:6n3 (cervonic) and 20:5n3 (timnodonic) represented more than 78.0±3.5% of the total plasma NEFAs in male and female Atlantic salmon. The seasonal profiles for these fatty acids are shown in Figure 5.5. Prior to entering fresh water these NEFAs were found in similar concentrations in both males and females, with the exception of 18:1n9 (oleic acid) which was significantly lower in males. During the first 2 months of migration, plasma concentrations of 16:0 and 18:1n9 increased significantly in males but not females. In contrast, plasma 16:1 levels declined in males but increased significantly among females (34.0% increase). Myristic acid was found in identical concentration in both males and females and declined steadily throughout migration (Figure 5.5). Male and female Atlantic salmon also exhibited similar seasonal profiles of 22:6n3. The most striking difference between males and females was in the plasma levels of the NEFA 20:5n3 (Figure 5.5). Over the first 4 months females possessed significantly higher plasma concentrations of these NEFAs than males. Between four months and the onset of spawning levels of this NEFA did not differ between the sexes. Males and female kelts possessed significantly lower levels of the NEFAs 16:0, 16:1, 18:1n9, 20:5n3 and 22:6n3 than spawning males and females.

Eicosanoid precursors found in the plasma of Atlantic salmon include 20:3n6 and 20:4n6 (Figure 5.6). The plasma concentrations of these NEFAs were similar in males and females prior to entering fresh water but increased significantly in females upon entering fresh water. After 2 months of freshwater migration, plasma concentrations of 20:3n6 and

20:4n6 were 43.0% and 53.0% higher in females than in males. Plasma concentrations of 20:3n6 declined in male Atlantic salmon during the first 2 months in fresh water but then increased so that plasma levels were not different at spawning than levels prior to entering fresh water (Figure 5.6). Females maintained significantly higher levels of 20:4n6 in their plasma compared to males until the onset of spawning, at which time plasma concentrations of 20:4n6 were no longer different. After 12 months in fresh water, plasma concentrations of 20:3n6 and 20:4n6 were similar between males and females. The levels of these NEFA in male kelts were also similar to those of Atlantic salmon soon after entry to fresh water (site 2). In contrast, the levels of these NEFAs in female kelts were significantly lower than females soon after entry to fresh water.

Figure 5.1. Schematic diagram showing the Bay of Exploits and the Exploits River, Newfoundland Canada. Sampling sites are shown and sampling times are presented in the legend. Note that representative spawning Atlantic salmon were obtained from site 5 and transferred to a spawning area at site 6 for sampling.



Sampling Dates		
Site 1	Bay of Exploits (salt water)	May 27-30th
Site 2	Mouth of Exploits River (esturine)	June 12-14th
Site 3	Bishop's Falls (fresh water)	July 5-9th
Site 4	Grand Falls (fresh water)	August 2-4th
Site 5	Red Indian Dam (fresh water)	September 11-12th
Site 6	Noel Paul's Brook (fresh water)	October 12-14th
note:	kelts were sampled from site 2 from May 10-20th	

Figure 5.2. Mean gonadosomatic indices for male (open circle) and female (solid circle) Atlantic salmon at various stages of maturation during their upstream spawning migration. Saltwater and freshwater phases of migration are indicated by the shaded and white areas respectively. The onset of spawning is indicated by the vertical dotted line. Values are means of 3 males and 3 females. Standard errors are shown. Sites with the same letter markers are not significantly different. Differences between sexes at each site are denoted with an asterix.

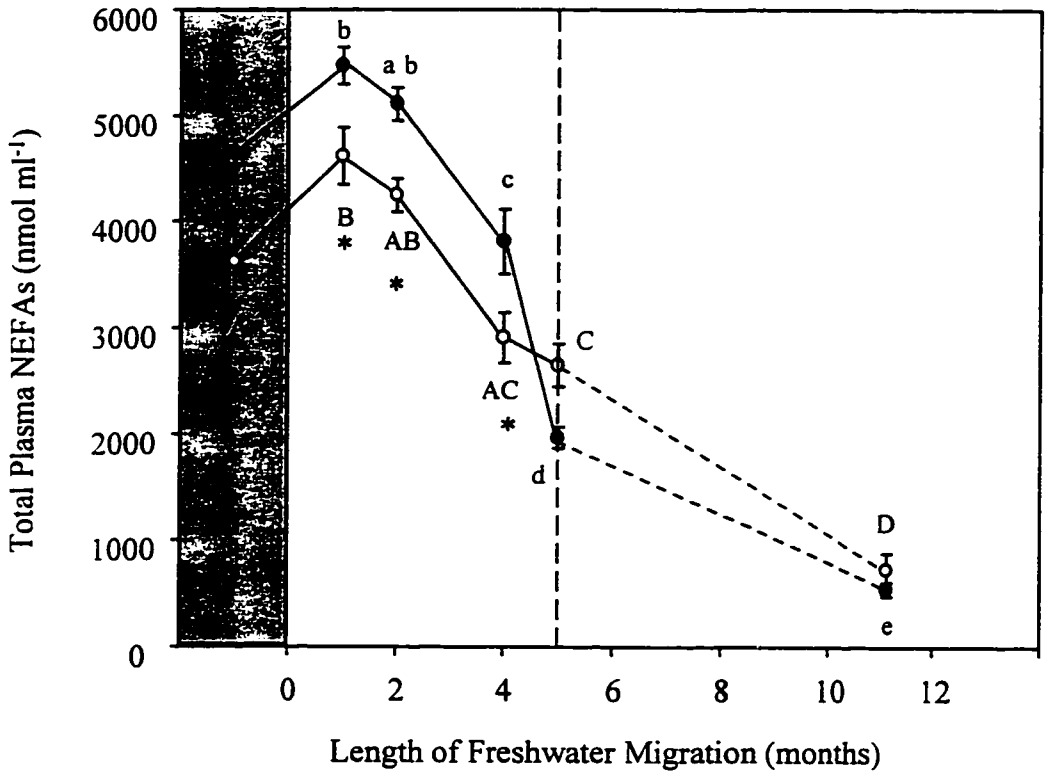

6000
5000
4000
3000
2000
1000
0
Total Plasma NEFAs (nmol ml-1)
0
2
4
6
8
10
12
Length of Freshwater Migration (months)
b
a b
c
d
e
B
AB
AC
C
D
*

Figure 5.3. Total non-esterified fatty acid concentrations in the plasma of male (open circle)and female (solid circle) Atlantic salmon sampled at various stages of their freshwater migration and during the post-spawning period. Salt water and fresh water phases of migration are indicated by the shaded and white areas, respectively. The onset of spawning is indicated by the vertical dotted line. Standard errors are shown. Sites with the same letter markers are not significantly different. Differences between sexes at each site are denoted with an asterix.

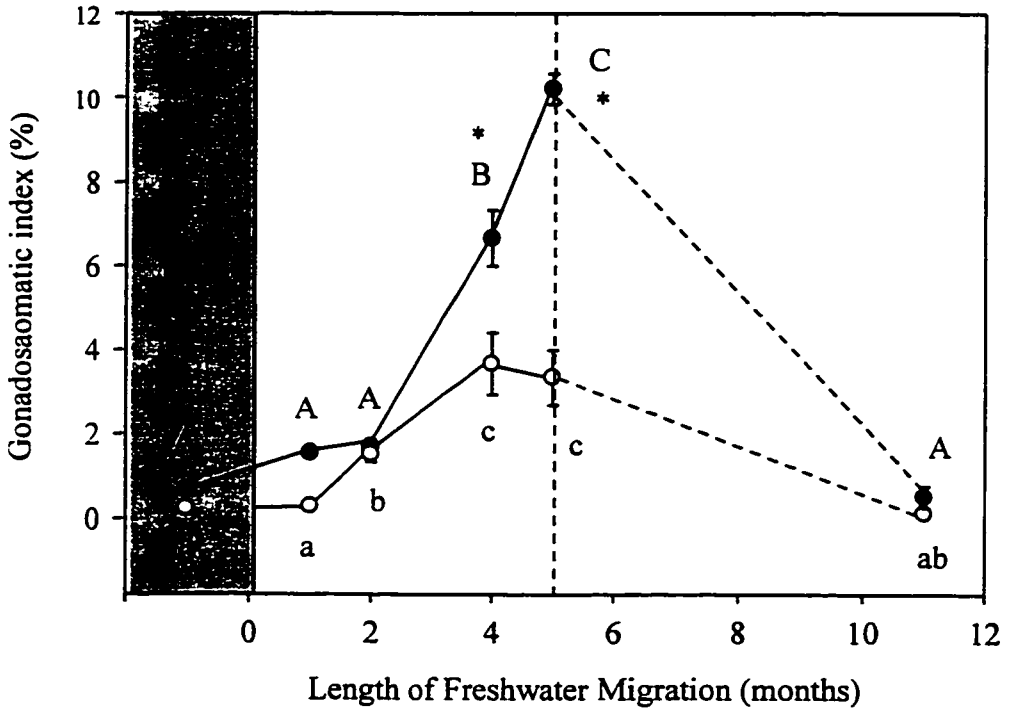

Gonadosaomatic index (%)
12
10
8
6
4
2
0
A
A
B
C
a
b
c
c
A
ab
0
2
4
6
8
10
12
Length of Freshwater Migration (months)

Figure 5.4. Plasma concentrations of the 3 classes of non-esterified fatty acids in the plasma of male (open circle) and female (solid circle) Atlantic salmon: A) monunsaturated B) polyunsaturated and C) saturated fatty acids. Samples are shown for males and females before and after spawning. Saltwater and freshwater phases of migration are indicated by the shaded and white areas respectively. The onset of spawning is indicated by the vertical dotted line. Standard errors are shown. Sites with the same letter markers are not significantly different. Differences between sexes at each site are denoted with an asterix.

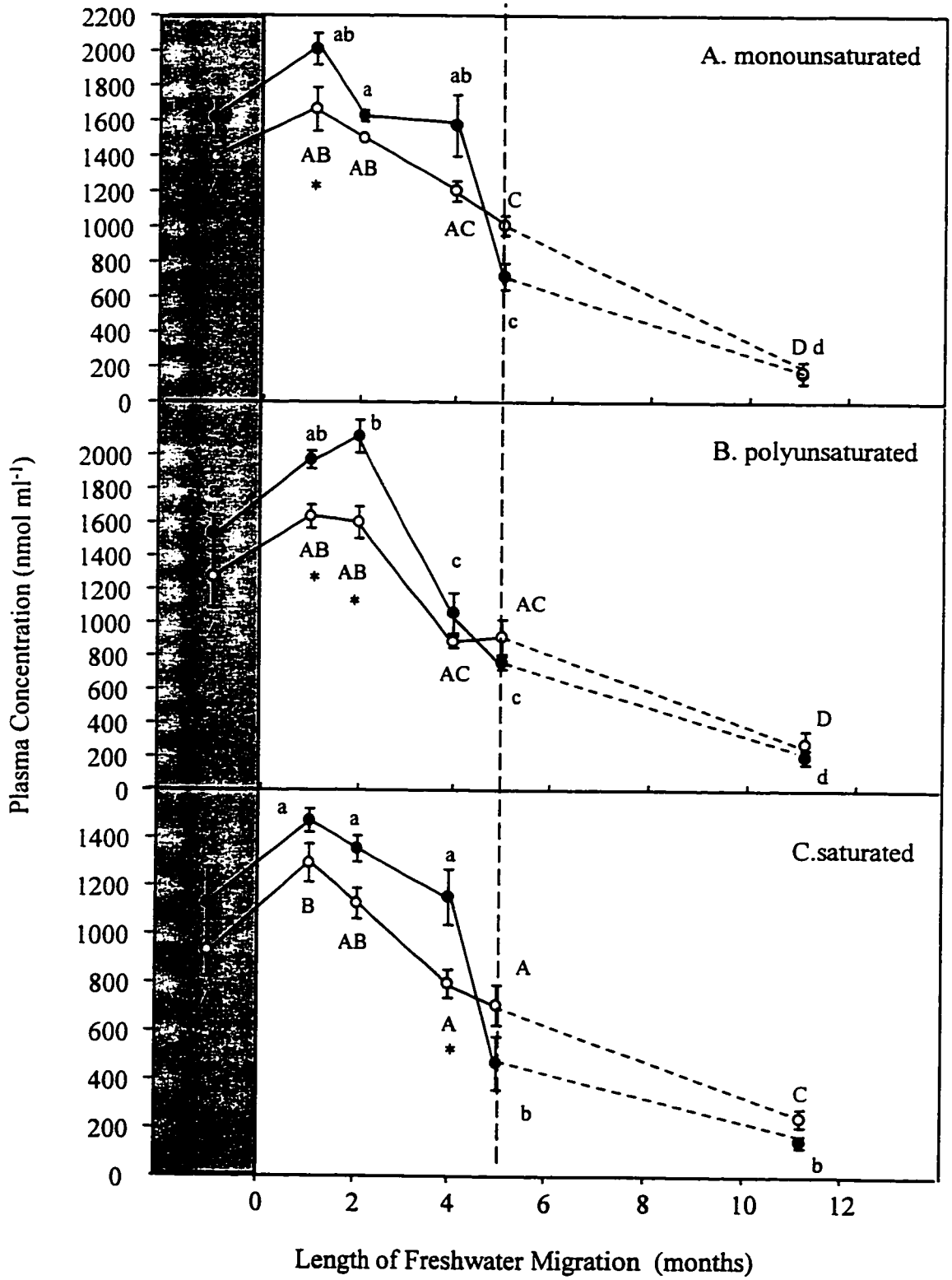

A. monounsaturated
B. polyunsaturated
C.saturated
Plasma Concentration (nmol ml-1)
Length of Freshwater Migration (months)
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
2
4
6
8
10
12
ab
a
ab
AB
AB
C
AC
c
D d
ab
b
c
AB
AB
AC
AC
c
D
d
a
a
a
B
AB
A
A
b
C
b

Figure 5.5. Changes in the dominant plasma non-esterified fatty acids of male (open circle) and female (solid circle) Atlantic salmon during their upstream spawning and downstream post-spawning migrations. Saltwater and freshwater phases of migration are indicated by the shaded and white areas respectively. The onset of spawning is indicated by the vertical dotted line. Standard errors are shown. Sites with the same letter markers are not significantly different. Differences between sexes at each site are denoted with an asterix.

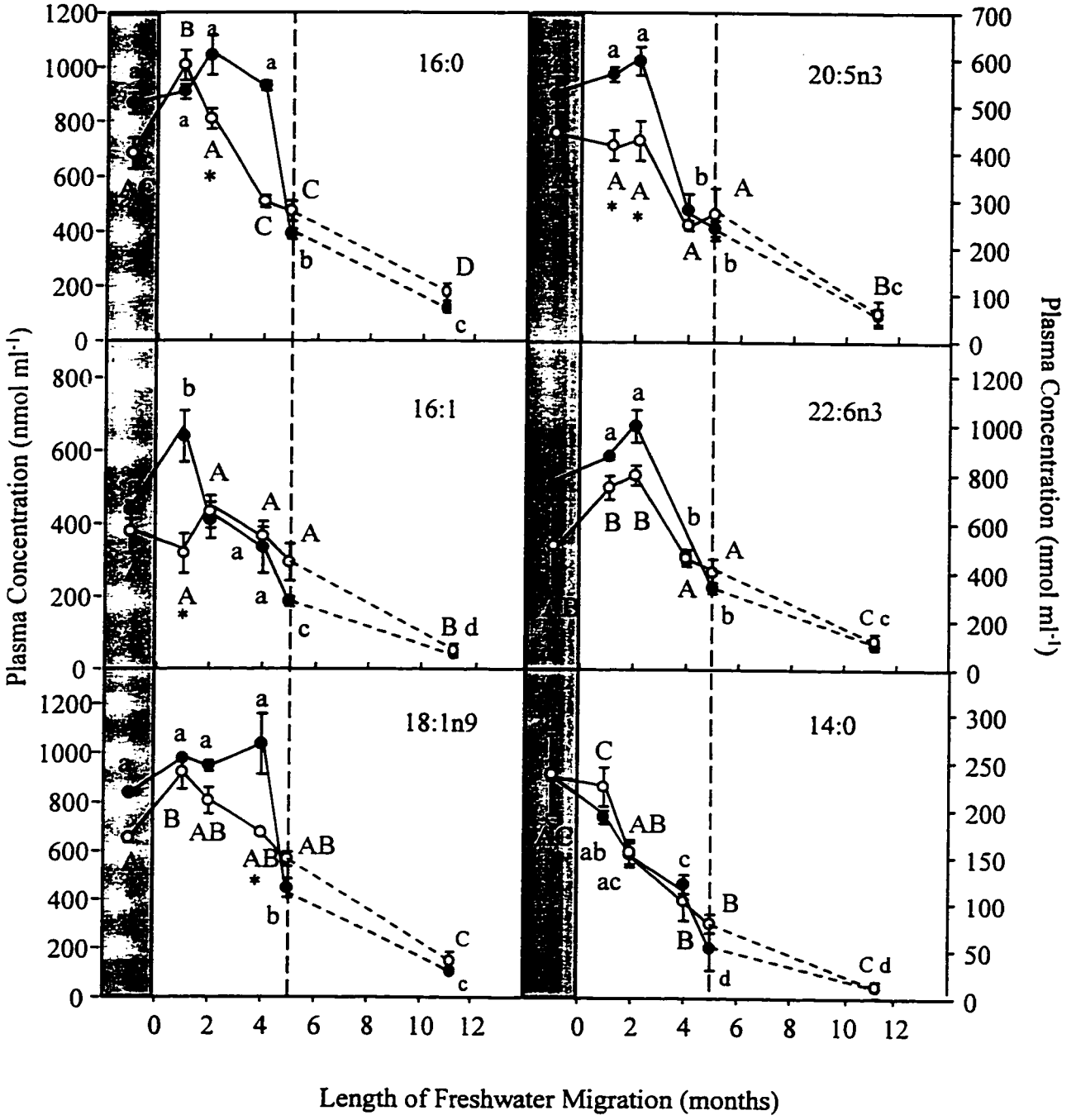
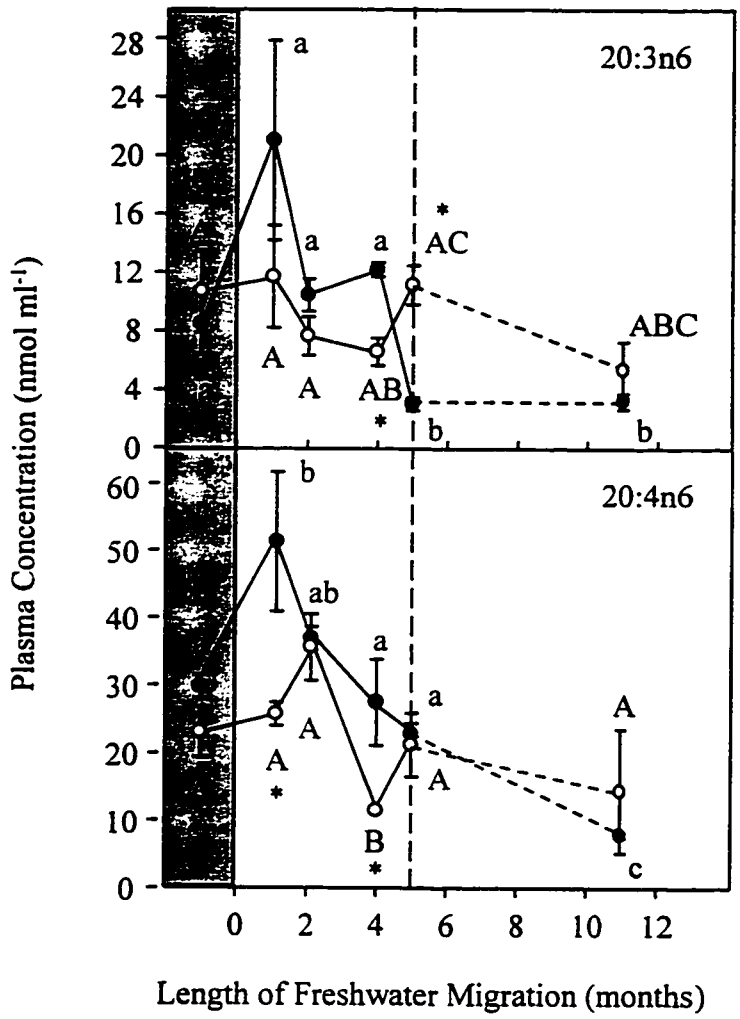

16:0
20:5n3
16:1
22:6n3
18:1n9
14:0
Plasma Concentration (nmol ml-1)
Plasma Concentration (nmol ml-1)
Length of Freshwater Migration (months)

Figure 5.6. Changes in the plasma concentration of 20:3n6 and 20:4n6 fatty acids in the plasma of sexually maturing male (open circle) and female (solid circle) Atlantic salmon during their spawning and post spawning migrations. Saltwater and freshwater phases of migration are indicated by the shaded and white areas respectively. The onset of spawning is indicated by the vertical dotted line. Standard errors are shown. Sites with the same letter markers are not significantly. Differences between sexes at each site are denoted with an asterix.


20:3n6
20:4n6
Plasma Concentration (nmol ml-1)
Length of Freshwater Migration (months)
0
2
4
6
8
10
12
0
4
8
12
16
20
24
28
0
10
20
30
40
50
60
a
a
a
AC
ABC
A
A
AB
b
b
b
ab
a
a
A
A
A
A
B
c
*

Discussion

The freshwater spawning migrations undertaken by anadromous Atlantic salmon are associated with periods of nutritional stress, characterized by significant mobilization of stored lipid (Jonsson et al. 1991, 1997). Plasma NEFAs are one of the most metabolically active forms of lipid in the blood of salmonids and are involved in the transfer of lipid from storage sites to the sites of utilization. The initial increase in plasma NEFAs in Atlantic salmon suggests that entrance into fresh water is associated with the mobilization of lipid.

Several factors may be responsible for the increase in plasma NEFA levels of Atlantic salmon in fresh water. Changes in salinity are known to induce osmoregulatory stress and increase the resting metabolism and oxygen requirements of salmonids (Hochachka and Somero 1984, Maxime *et al.* 1990). The increase I observed in plasma NEFAs during the transition from the marine to fresh water environment could reflect the mobilization of lipid to meet the energy requirements of osmoregulation. In addition, the increase in water temperature as Atlantic salmon migrate into fresh water may also be a factor. Specifically, the large increase in PUFAs could account for most of the increase in total plasma NEFA. PUFAs are important components in the phospholipids of cell membranes (Bell *et al.* 1986). In cold acclimated fish, PUFA concentrations increase in the membranes to conserve their fluidity, while in warm water, membrane concentrations of PUFAs decline (Bell *et al.* 1986). The increase in total plasma NEFAs and PUFAs specifically, may reflect the mobilization of NEFAs from the membranes so that they can be used as fuel during upstream migration.

It is unlikely, that changes in specific plasma NEFA during freshwater adaptation, alone, could explain the significant increase in plasma NEFAs. An additional factor that may

explain the increase in plasma NEFA levels during this period is a change in the activity level of salmon as they migrate into fresh water. Water velocities are substantially higher in rivers than in the oceans and the increased activity necessary for upstream migration would therefore require additional energy that could be provided from plasma NEFAs. This energy could be derives from PUFAs, and the mobilization of these NEFA from cell membranes could be linked to the increased energy requirements of Atlantic salmon during their upstream migration.

Declining plasma NEFA levels throughout the freshwater migration suggest that fatty acids provide an important source of lipid during the pre-spawning period. Previous studies have also shown a diminution in plasma NEFAs among anadromous salmonids during their spawning migration (French *et al.* 1983, Ballantyne *et al.* 1996). However, compared to the total plasma NEFAs reported for sockeye salmon (Ballantyne *et al.* 1996), plasma NEFA levels were nearly 1.5 times higher for male and female Atlantic salmon at the outset of upstream migration and 1.9 times higher in males and 1.7 times higher in females at spawning. The reason for the higher plasma NEFA levels in Atlantic salmon compared to sockeye salmon is unclear. However, fish in general exhibit considerable differences in plasma NEFA levels (Ballantyne *et al.* 1993) and the basis for these differences are unknown.

At least one reason for the difference in plasma NEFAs between Atlantic salmon from the present study and those reported previously for Pacific salmon (Ballantyne *et al.* 1996) may be the storage patterns of lipid within the tissues. Previous studies have demonstrated that when lipids are stored in tissues other than the muscle, large amounts of lipid must be mobilized from these depots and transported to the muscle as plasma NEFAs (Plisetskaya 1980). It is possible, therefore, that the higher circulating NEFA levels in

Atlantic salmon compared to sockeye salmon may reflect the transfer of lipid from storage sites, such as the liver, to the muscle.

Male and female sockeye salmon partition similar amounts of energy into locomotion during their spawning migration (Idler and Clemens 1959, Ballantyne *et al.* 1996). Weber *et al.* (1996) have shown that while intramuscular fatty acids are the preferred substrates for ATP production by mitochondria in the aerobic red muscles of some mammals, plasma NEFAs may be more important as energy sources in other animals such as fish. However, the relative importance of plasma NEFAs versus endogenous lipids as energy sources for muscular work has not yet been examined. Considering red muscle is capable of oxidizing a variety of fatty acids, utilization of NEFAs to support locomotion should result in a proportional decrease in all plasma NEFAs (Ballantyne *et al.* 1996). In this study the decline in total plasma NEFA levels of males mirrored that of females during the initial 4 months of migration. Similar trends were also seen among monoeonic, polyenic and saturated fatty acid levels. In particular, the decline in two fatty acids 16:0 and 14:0 indicate that saturated fatty acids may be an important source of energy for locomotory muscle since the declines in males and females were similar throughout the migratory period. On the basis of declines in plasma NEFA levels in both sexes and the similar declines of specific fatty acid levels in male and female Atlantic salmon, at least some of the energy required for sustained swimming appears to be derived from the oxidation of plasma NEFAs by the red muscle.

Over the 7 month period following spawning plasma NEFA levels continued to decline in wild Atlantic salmon. The presence of plasma NEFA in spent fish suggests that fatty acids continue to be an important source of energy during the downstream post-spawning migration. Previous studies of post-spawning cisco and lake whitefish have

demonstrated that protein is used during the over-wintering period (Lambert and Dodson 1990). Protein concentrations were not measured in the present study, however, Jonsson *et al.* (1997) found that protein content was similar in both male and female Atlantic salmon before spawning and remained stable during the spawning period. The small declines in protein reported by Jonsson *et al.* (1997), and my observation that fatty acid levels continue to decline in spent Atlantic salmon suggests that fatty acid oxidation may reduce the need to catabolize protein and act to minimize the dependence on a single energy source. This would be important since Atlantic salmon have not resumed feeding at this point but still require energy for their return migrations to the marine environment. Previous studies involving Pacific salmonids have demonstrated that the consumption of muscle protein during migration results in the severe disorganization of the swimming musculature (Mommsen *et al.* 1980, Ando *et al.* 1985). Decreased consumption of muscle proteins by Atlantic salmon may help to maintain the functional integrity of the swimming musculature during their downstream migration.

Besides the costs of locomotion, significant amounts of energy are also directed into gonadal growth and sexual maturation. Wild Atlantic salmon can partition as much as 52% of their available energy into spawning (Jonsson *et al.* 1991). A considerable amount of this energy appears to have been devoted to gonadal development rather than locomotion since the total distance migrated by Atlantic salmon in their study was only 1 kilometre. More recently, Jonsson *et al.* (1997) determined that as much as 60-70% of available energy is devoted to migration and spawning in wild Atlantic salmon. Increases in the GSI of male and female Atlantic salmon in the present study suggest that gonadal development occurs throughout the migratory period but at different rates for males than females. Based on

trends in GSIs, it appears that the testes matured earlier than ovaries. In fact, female GSIs increased most significantly in the month just prior to spawning. The rapid increase in the GSI of female Atlantic salmon after 4 and 5 months in fresh water probably reflects the final stages of ovarian development prior to spawning. Overall, the trend observed in gonadal development among Atlantic salmon in the present study is similar to trends reported previously for Atlantic salmon (Jonsson *et al.* 1991) and sockeye salmon (Ballantyne *et al.* 1996).

Previous studies have demonstrated that spawning female salmonids possess more lipid and specifically, they have greater amounts of plasma NEFAs than males (Henderson and Tocher 1987, Ballantyne *et al.* 1996). It has been suggested that the higher lipid levels in females may represent a necessary condition required to meet the energy and substrate requirements for yolk production and to complete the development of maturing eggs. A previous study by Jonsson *et al.* (1991) revealed that the ovaries from spawning Atlantic salmon contained about 4 times as much fat as the testes. Total plasma NEFA levels in Atlantic salmon at the outset of their migration were approximately 20% higher in females than males, however, at the time of spawning the plasma NEFA levels of females had been reduced by 72% while males had decreased by only 40%. Moreover, the largest decline in plasma NEFAs among females coincided with the largest increase in GSI, indicating that NEFAs are probably being used for maturation of the ovaries.

The types and amounts of fatty acids used in gonadal development differ considerably between males and females. Ballantyne *et al.* (1996) were able to demonstrate differences in the proportions of the fatty acids 16:0, 18:1 and 20:5n3 among anadromous male and female sockeye salmon. Weigand and Idler (1985) found that the monoenes 16:1 and 18:1

comprised the major fatty acids in the neutral lipid of ovaries in landlocked Atlantic salmon and thus the plasma levels of these fatty acids would be expected to be greater in maturing females than males. The significant increase in the levels of 16:0 among females but not males in the present study is consistent with the role of this fatty acid in ovarian but not testicular development (Weigand and Idler 1985). The decline of 16:0 in females prior to spawning coincides with the greatest increase in gonad size which compares with the role of 16:0 in ovarian development previously described by Weigand and Idler (1985) for landlocked Atlantic salmon. The significant decline in plasma levels of 18:1n9 prior to spawning suggests that this fatty acid may be important in the final stages of ovarian growth. Weigand and Idler (1985) also found that 18:1n9 is an important fatty acid in the ovarian neutral lipids of landlocked Atlantic salmon and similar increases in plasma 18:1n9 levels have been described for female sockeye salmon during their spawning migration. Plasma levels of 18:1n9 did not increase in male sockeye and remained significantly lower than in females throughout migration. In contrast, plasma levels of 18:1n9 in male Atlantic salmon increased significantly at the outset of migration and then declined over the remainder of their upstream migration. Male Atlantic salmon did not experience sharp declines in 18:1n9 levels at any time which would indicate its additional mobilization for gonadal development and suggest that this fatty acid may be recruited for other purposes such as locomotion because of its constant availability in the plasma.

In addition to their role in gonadal development, fatty acids are also involved in the production of hormones and have been shown to have steroidogenic effects in fish (Bell *et al.* 1986, White *et al.* 1986, Henderson and Tocher 1987, Wade *et al.* 1994). Specifically, arachidonic acid (20:4n6) appears to be an important precursor for the production of

eicosanoids such as prostaglandins (PG) (Stacey and Goetz 1982, Bell *et al.* 1986, Henderson and Tocher 1987) which are involved in the stimulation of steroid production in fish (Wade *et al.* 1994, Mercure and Van Der Kraak 1995). The decline in plasma levels of arachidonic acid among male Atlantic salmon suggest that this fatty acid is important throughout the migratory period. Prostaglandins have been identified in the testes of numerous fish and the decline in arachidonic acid among males suggests that this fatty acid may be used for PG synthesis in males. The early development of testes among male Atlantic salmon would require PG to be readily available soon after entering fresh water and this is where the greatest decline in plasma 20:4n6 levels was seen. In female Atlantic salmon the greatest decline in plasma 20:4n6 levels occurred between 3 and 4 months into their migration. Gonadal maturation and the onset of ovulation are dependent on PG synthesis and the decline in arachidonic acid at this time is consistent with the final stages of ovarian development and preparation for spawning among females.

During their freshwater migrations anadromous Atlantic salmon use plasma NEFAs to support locomotion as well as gonadal development. Females have greater amounts of plasma NEFA at the outset of their migrations and devote most of this to ovarian growth. Different fatty acids appear to be required by females compared to males and it is assumed that these are involved in ovarian development and hormone production. The continued decline of plasma NEFAs in spent salmon suggests that fatty acids continue to be important energy sources during the over-wintering period and during the return migration of Atlantic salmon to saltwater. Given the limited number of studies describing NEFA profiles in anadromous salmonids, including spent individuals more work is clearly warranted in this area.

Chapter 6

General Discussion

In the preceding sections, I have described several new findings concerning the changes that occur in the swimming performance and lipid (i.e. plasma NEFA) use of anadromous Atlantic salmon during their upstream migration. Key findings include the maintenance of swimming capabilities throughout the active period of upstream migration, a reduction in swimming performance during the onset of spawning, and a greater reduction in the swimming capabilities of females compared to males. In addition, swimming capabilities of Atlantic salmon kelts were compared to those of upstream migrating adults and were found to be significantly lower. Plasma NEFA levels declined significantly in both males and females, however, greater reductions in plasma NEFA occurred during the upstream migration of females. Plasma NEFA levels continued to decline following spawning and were lowest in kelts.

Unlike previous studies that measured the swimming capabilities of migratory salmonids under laboratory conditions, using commercially available stocks, this study reports the swimming capabilities of wild Atlantic salmon obtained during their upstream migration. Because I was primarily interested in changes which occur in swimming performance and lipid use during migration, I could not use hatchery reared Atlantic salmon for several reasons. First, hatchery reared fish are typically obtained from the same stock and therefore possess few differences in their age, size, weight and environmental history. In addition, hatchery reared fish have been found to differ significantly in their physiological and behavior compared to wild fish. More importantly, hatchery fish have been shown to

differ from wild fish in their swimming abilities (Green 1964, Vincent 1960). For these reasons, the swimming capabilities of hatchery reared Atlantic salmon may not accurately represent those of wild Atlantic salmon. Furthermore, it would be difficult to replicate the environmental, behavioral and physiological conditions associated with the freshwater migrations and sexual maturation of wild Atlantic salmon.

In order to conduct my studies of Atlantic salmon I was first challenged with the problem of collecting wild individuals from the Exploits River. The Exploits River provides many opportunities for collecting Atlantic salmon because it is accessible by road along most of its length. Moreover, it has fish ladders at three points along its length to facilitate the collection of Atlantic salmon. Unfortunately, collection sites were not suitable for establishing a field station for measuring swimming performance and performing surgical procedures such as the implantation of EMG transmitters. Therefore, I chose to use an existing, but unused, government owned hatchery facility located on a tributary of the Exploits River, Noel Paul's Brook. The use of this facility allowed me to set up swim chambers and hold fish in river water under natural regimes of temperature and photoperiod. However, Atlantic salmon had to be transported from their sites of collection to the field station. Previous studies have suggested that transport and confinement may be associated with a significant amount of stress that can affect the swimming performance of fish (McDonald *et al.* 1993, Strange and Cech 1992). Prior to using any fish, I allowed them to recover for at least 72 hours, which according to Wood *et al.* (1983) is sufficient time for recovery from stress. In addition, stress normally associated with transplanting salmon from natural environments to hatchery environments was probably minimized in the present

studies as a result of holding fish in river water and at ambient temperatures and under a natural photoperiod.

An additional problem that I encountered with my experimental setup concerned the influence of isolating Atlantic salmon prior to measuring their swimming performance. My first attempts at holding Atlantic salmon involved placing 4 to 6 individuals in tanks measuring 1 m wide by 1.5 m long by 1.5 m deep. Several problems were associated with this holding system. First, I noticed that the swimming speeds measured of these Atlantic salmon differed greatly between individuals. Furthermore, the swimming speeds of these salmon were generally lower than Atlantic salmon that I collected but did not hold before testing.

Previous studies have demonstrated that the swimming capabilities of fish are influenced by periods of confinement (Strange and Cech 1992, Tang and Bosclair 1993). Therefore, in order to minimize the effects of confinement stress on the swimming capabilities of Atlantic salmon used in my studies, I chose to increase the size of the pens to 8 m wide, 15 m long and 0.8 m deep. In addition, I chose to collect Atlantic salmon on a weekly basis in an attempt to minimise the effects of confinement stress on swimming capabilities and fish health. Atlantic salmon used for experimentation following the implementation of these modifications exhibited greater consistency in their swimming capabilities than individuals used previously. Moreover, the incidence of fungal infections among adult Atlantic salmon declined significantly. On this basis, this procedure was used to hold all of the adult Atlantic salmon used in this study, with the exception of those collected as spawning individuals, which, as noted in chapter 4, were held in a spawning channel where they were allowed to complete their maturation before being tested.

Problems associated with establishing a useful protocol for collecting, transporting and measuring the swimming performance of Atlantic salmon had to be solved before valid data could be obtained. The observed differences in sustained, critical and burst swimming capabilities between smolts and upstream migrating adults were expected. The higher length dependent sustained, prolonged and burst swimming performances of smolts relative to adults is consistent with previous studies of conducted on juvenile and adult sockeye salmon (Brett 1964). It is important to note, however, that when swimming speeds are expressed independent of length, upstream migrating adults can swim against significantly faster water currents than smolts. Thus, interpretation of swim speed data must be done with caution. For management purposes it is suggested that swimming speeds be measured as water velocities (cm sec^{-1} or an equivalent) because in the wild fish will be swimming against water flows, and therefore their swimming speeds in relation to their body lengths may be inappropriate.

The lower swimming capabilities of kelts compared to upstream migrating adults was also expected. However, the difference in swimming capabilities was larger than anticipated since previous studies have suggested that the physiological disturbance in kelts following burst exercise does not differ greatly from that of upstream migrating adults (Brobbel *et al.* 1996). The reason for the reduced swimming capabilities of kelts is unclear since this is the only study I am aware of that has measured the swimming capabilities of kelts. However, as described in chapter 5, kelts possess very low plasma NEFAs levels. Since aerobic swimming depends on fatty acids as a source of energy (Kiessling and Kiessling 1993, Zammit and Newsholme 1979), the reduction observed in sustained swimming capabilities may reflect substrate limitations. With respect to prolonged and burst swimming, these

activities are known to involve white muscle activity (Wardle *et al.* 1995). According to Mommsen *et al.* (1980), white muscle also provides an important source of amino acids during migration. Consequently, the lower prolonged and burst swimming capabilities of kelts may reflect white muscle degradation due to metabolism of muscle protein over the migratory period. Unfortunately, there is limited information regarding the influence of protein catabolism on muscle function and further studies in this area are clearly warranted.

Another reason for the reduced swimming capabilities of kelts may concern the behavioral changes these salmon experience during their downstream migration. Previous studies have shown that the orientation of Atlantic salmon changes from highly rheotactic to indifferent during smoltification (Thorpe 1984). Associated with this change in orientation is a loss of swimming performance (Glova and McInerney 1977, Thorpe 1984). Physiological and behavioural changes observed in smolts are believed to be adaptations for their passive displacement downstream. Since kelts also undertake downstream migrations, similar changes may account for some of the loss of swimming performance observed in these individuals. Given the lack of information about the swimming capabilities and physiology of kelts, more work in these areas is clearly warranted.

An important question raised by the observed differences between upstream migrating adults and downstream migrating kelts is whether the observed loss of swimming abilities occurs gradually over the migratory period, rapidly over the pre-spawning period or during the long period of overwintering which follows spawning. Changes observed in the sustained, critical and prolonged swimming capabilities of male and female Atlantic salmon indicate that the loss of swimming ability among adults occurs just prior to spawning and is associated with the most pronounced increase in gonadal development and the lowest

recorded temperature. One limitation of my studies involved the sampling of Atlantic salmon. As noted in chapter 4, adult Atlantic salmon were collected from site 4 and held in an artificial spawning channel during their final maturation. Since confinement stress has been associated with a loss of swimming performance, this factor cannot be ruled out as a cause of the reduction of swimming performance in Atlantic salmon. However, given that the spawning area was quite large and that Atlantic salmon are not migrating large distance during this period, I feel that the effects of captivity on the swimming performance of Atlantic salmon would be minimal. Furthermore, similar reductions in swimming performance before the onset of spawning have been reported for wild pink salmon, and support the conclusion that the effect of captivity on the swimming performance of Atlantic salmon is probably minimal. Although it would have been preferable to sample naturally spawning individuals, this was not possible on the Exploits River because of current management legislation.

Measurements of *in situ* muscle activity obtained from Atlantic salmon at different stages of their migration indicate that both males and females recruit additional muscle fibres in order to compensate for the effects of temperature and cross-sectional area on swimming capabilities. Furthermore, muscle activity index patterns suggest that prolonged swimming by spawning females involved increased white muscle activity. Measurements of muscle activity using existing physiological techniques do not allow for the separation of red and white muscle activity. One assumption, proposed in chapter 3, is that increased muscle activity at high swimming velocities and colder temperatures may indicate the recruitment of white muscle fibres. Given the existing technology for measuring red and white muscle activity under laboratory conditions, future studies employing radio transmitted

electromyograms would benefit from calibrations between radio telemetered EMG*i* signals and red and white muscle EMGs obtained using more sophisticated myographical laboratory equipment. Furthermore, because factors such as temperature and body characteristics can change during the upstream migration of salmonids, future studies should also consider calibrating muscle activity or muscle EMG*i* to swimming speeds under the specific conditions of migration or laboratory equivalents. These calibrations would greatly reduce the possibility of error when applying these calibrations to freely swimming salmonids in their natural environments.

An important assumption which underlies any study of upstream migration is whether Atlantic salmon from different fresh water sites have been allowed similar amounts of time to acclimatize to river conditions whether they possess similar energetic resources. In the preceding studies, it was assumed that all Atlantic salmon captured from a site had been in the river a similar length of time (i.e. weeks, months). This assumption was based on data reported by O'Connell *et al.* (1983) which showed that adult Exploits River Atlantic salmon typically migrate in groups which peak at a given site within a period of about 1 week. At Grand Falls for example, adult Atlantic salmon counts peaked during the week of July 23rd-30th in 1977, August 4th-11th in 1979 and July 25th-August 1st in 1981. Although the study of O'Connell *et al.* (1983) supports these assumptions, they can only be verified through extensive tagging of Atlantic salmon as they enter the river. Knowledge of tag numbers, dates of entry, weight and time of capture would greatly increase the accuracy of statements concerning residency time. However, such efforts are extremely time consuming and would require tagging thousands of Atlantic salmon to ensure that the few required would be available. As a result, it was assumed that Atlantic salmon taken from each site had been in

the river for a similar period of time.

During their freshwater migrations anadromous Atlantic salmon use plasma NEFAs to support locomotion as well as gonadal development. Females have greater amounts of plasma NEFA at the outset of their migrations and most of this is likely directed into egg development (ovarian maturation). Differences in the concentration of specific NEFAs between males and females most likely reflects the different fatty acid requirements of egg production versus sperm production. Plasma NEFAs continued to decline and were lowest in kelts, suggesting that fatty acids continue to be used as an energy source during the over-wintering period and during the return migration of Atlantic salmon to salt water. Although the roles of lipid, protein and carbohydrate during the migration of Atlantic salmon havebeen described (Jonsson *et al.* 1991, 1997, Martin *et al.* 1993), they have not been examined in Atlantic salmon kelts. Given the decline observed in total plasma NEFAs following spawning, an examination of changes in other fuels is clearly warranted.

In summary, the preceding studies provide estimates of swimming performance in three migratory forms of Atlantic salmon, smolts, upstream migrating adults and kelts. Differences observed in the swimming capabilities of upstream migrating adults and kelts indicated that Atlantic salmon experience reductions in their swimming capabilities while in fresh water. This observation was confirmed by a detailed study of upstream migrating adult salmon which demonstrated that Atlantic salmon experience significant reductions in sustained, prolonged and burst swimming capabilities during their freshwater migrations. In particular, the results of my study of upstream migrating salmon indicated that most of the change in the swimming performance of male and female salmon occurred just prior to spawning. Evidence of a combined effect of a decrease in temperature and an increase in

cross-sectional area on swimming performance was evident. Temperature was probably the largest contributor to the reduction in swimming capabilities since, the since both males and females experienced declines in their swimming capabilities, but only females experienced an increase in cross sectional area. Further investigation concerning the effects of temperature and cross sectional area on the swimming performance of wild Atlantic salmon is warranted.

Previous studies have demonstrated that lipids are an important source of fuel during the migration of Atlantic salmon (Jonsson *et al.* 1997). In particular, changes in plasma NEFAs may be a good indicator of lipid requirements in migrating fish as they are used as fuels for locomotion and as substrates for gonadal development (Ballantyne *et al.* 1996). In this regard, I observed significant reductions in total plasma NEFA levels in both male and female salmon during their freshwater migration. Analysis of specific fatty acids revealed sex-dependent differences in the requirements of specific NEFAs which were attributed to differences in the NEFA requirements of maturing ovaries and testes. However, to make definitive statements about the roles of specific plasma NEFAs in gonadal development, detailed investigations of fatty acids in the gonadal tissue would have to be undertaken.

The studies described previously have been conducted in order to provide new information concerning the migratory abilities of anadromous Atlantic salmon. However, given the lack of information previously available, there are many areas where future work can be directed. Throughout this study areas for future research have been discussed. The present study will hopefully provide a foundation on which future studies can be based and provide important references that can be drawn upon by future researchers.

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Appendix A

Measurements of muscle activity and time required by adult Atlantic salmon to ascend an artificial fishway, as described in chapter 2. Time is measured in seconds (s).

	18°C				12°C			
time	fish1	fish2	fish3	fish4	fish1	fish2	fish3	fish4
(s)	149.489	149.439	149.419	149.798	149.798	149.439	149.419	149.489
0	1821	1715	1875	1842	1942	1879	1966	1766
2	1846	1737	1863	1874	1924	1889	1964	1726
4	1820	1625	1807	1870	816	1786	1963	1805
6	1793	1669	1563	1631	816	1654	1964.2	1119
8	1723	1717	1556	1552	690	1345	1546	1118
10	1717	1601	1370	1800	690	1328	614	867
12	1759	1230	1628	1633	597	1420	596	965
14	1747	1612	1520	1258	597	1354	585	847
16	1778	1349	1541	1699	707	1123	587	1152
18	1421	1429	1606	1776	707	985	563	988
20	1423	1623	1605	1786	657	907	561	865
22	1680	1650	1442	1597	657	741	565	596
24	1637	1476	1549	1742	545	741	702.1	554
26	1691	1521	1451	1564	1899	501	722	554
28	1592	1486	1584	1592	finish	501	603	563
30	1407	1182	1522	1539		634	761	635
32	1551	1437	1508	1505		634	582	finish
34	1233	1430	1457	1294		1876	1956	
36	1526	1365	1440	1541		finish	finish	
38	1291	1650	1693	1380				
40	1227	1511	1592	1621				
42	1367	1383	1555	1422				
44	1567	1198	1163	1535				
46	1876	1123	1315	1370				
48	finish	1588	992	1384				
50		1291	1245	1281				
52		828	1954	1767				
54		881	finish	1889				
56		1456		finish				
58		1899						
		finish						

Appendix B

Slopes, intercepts and correlation coefficients for regressions of muscle activity and swimming speeds for individual male and female Atlantic salmon (chapter 4). Site 1 are estuary sampled Atlantic salmon and site 5 are salmon sampled just prior to spawning. Temperature is measured in °C.

location	sex	temp	r^2	intercept	slope
site 1	f	17.5	0.83	2332.3	-4.44
site 1	f	16.5	0.70	2293.5	-3.97
site 1	f	17.0	0.71	2342.2	-4.49
site 1	m	15.5	0.74	2180.8	-3.10
site 1	m	15.0	0.64	2123.4	-4.05
site 1	m	15.0	0.80	2281.3	-4.03
site 2	f	18.4	0.61	2183.1	-2.87
site 2	f	18.5	0.64	2118.5	-3.46
site 2	f	19.0	0.75	2121.5	-3.22
site 2	f	21.5	0.69	2259.9	-4.79
site 2	m	18.0	0.78	2284.6	-3.61
site 2	m	18.5	0.62	2175.6	-3.13
site 2	m	19.0	0.73	2355.1	-4.56
site 2	m	19.0	0.69	2294.4	-3.93
site 3	f	21.0	0.61	2106.6	-2.89
site 3	f	20.5	0.73	2217.0	-3.32
site 3	f	20.5	0.76	2240.0	-3.35
site 3	f	18.0	0.89	2365.4	-4.57
site 3	m	19.0	0.86	2144.7	-3.09
site 3	m	21.0	0.78	2194.4	-3.33
site 3	m	17.5	0.64	2076.7	-2.35
site 3	m	17.8	0.70	2155.1	-2.76
site 4	f	14.5	0.61	2454.6	-6.24
site 4	f	15.5	0.79	2802.8	-6.63
site 4	f	16.0	0.85	2387.5	-5.20
site 4	f	14.5	0.82	2547.4	-5.02
site 4	m	15.0	0.67	2267.5	-4.81
site 4	m	14.5	0.90	2320.9	-4.11
site 4	m	15.0	0.64	2267.5	-3.61
site 4	m	17.0	0.89	2338.5	-4.58
site 5	f	10.5	0.67	3246.7	-15.27
site 5	f	11.5	0.7	2865.7	-11.71
site 5	f	9.5	0.64	3186.4	-14.19
site 5	m	9.5	0.74	2615.5	-10.31
site 5	m	9.5	0.87	2733.4	-8.62
site 5	m	11.0	0.71	2462.3	-9.92

Appendix C

Swimming endurance from studies of forced swimming trials for Atlantic salmon

site	sex	speed (bl sec^{-1})	time (min)	fork length (cm)	weight (kg)	girth (cm)	temp (°C)
site1	F	2.50	200.00	56.00	1.62	26.00	18.60
site1	F	2.46	200.00	57.00	1.60	26.70	17.80
site1	F	2.76	200.00	58.00	2.00	28.40	18.00
site1	F	2.88	200.00	55.60	1.36	24.80	18.00
site1	F	2.41	200.00	58.00	2.00	28.40	18.00
site1	F	2.49	200.00	56.20	1.58	26.40	18.40
site1	F	2.82	200.00	56.70	1.80	27.80	18.60
site1	F	2.84	200.00	56.30	1.62	26.20	18.40
site1	F	2.80	200.00	50.00	1.10	23.20	18.20
site1	M	2.56	200.00	54.60	1.82	27.40	18.80
site1	M	2.64	200.00	53.00	1.56	26.40	18.60
site1	M	2.60	200.00	53.80	1.76	25.40	18.80
site1	M	2.51	200.00	55.80	1.40	26.00	13.90
site1	M	2.50	200.00	56.00	1.42	27.20	14.00
site1	M	3.01	200.00	53.20	1.24	24.00	16.90
site1	M	2.31	200.00	60.50	1.38	25.20	14.40
site1	M	2.93	200.00	54.60	1.64	27.60	18.40
site2	F	2.76	200.00	58.00	2.00	28.40	18.50
site2	F	2.82	200.00	56.80	1.80	26.00	18.50
site2	F	2.91	200.00	55.00	1.80	26.40	18.60
site2	F	3.30	200.00	48.50	1.12	24.80	18.40
site2	F	3.14	200.00	51.00	1.30	26.20	17.80
site2	F	2.84	200.00	56.40	1.50	26.00	15.20
site2	F	2.93	200.00	54.60	1.56	24.60	19.20
site2	F	2.76	200.00	58.00	2.00	28.40	18.00
site2	F	3.54	200.00	45.20	1.00	21.40	18.20
site2	F	2.79	200.00	57.40	1.80	56.80	18.20
site2	F	3.13	200.00	51.20	1.34	23.60	18.20
site2	F	2.82	200.00	56.80	1.74	26.00	18.70
site2	M	2.84	200.00	56.40	1.68	25.40	20.10
site2	M	3.08	200.00	52.00	1.44	25.80	18.60
site2	M	3.01	200.00	53.20	1.24	24.00	13.90
site2	M	2.64	200.00	60.50	1.38	25.20	14.40
site2	M	2.96	200.00	54.00	1.28	24.00	14.50
site2	M	3.03	200.00	52.80	1.10	22.60	14.40
site2	M	2.82	200.00	56.80	1.46	25.40	18.20
site2	M	2.89	200.00	55.40	1.45	25.60	17.80
site2	M	2.78	200.00	57.60	1.64	36.20	18.40
site2	M	3.08	200.00	52.00	1.40	22.80	18.00
site2	M	3.54	200.00	45.20	1.02	21.40	17.00
site2	M	2.48	200.00	64.60	1.40	25.40	14.40
site3	F	2.81	200.00	57.00	1.58	27.80	19.00
site3	F	2.94	200.00	54.50	1.62	27.00	18.80
site3	F	2.81	200.00	57.00	1.58	27.80	19.00
site3	F	2.94	200.00	54.50	1.62	27.00	18.80

Appendix C *continued*

site	sex	speed (bl sec^{-1})	time (min.)	fork length (cm)	weight (kg)	girth (cm)	temp (°C)
site3	F	2.84	200.00	56.40	1.45	27.50	19.80
site3	F	3.02	200.00	52.90	1.10	21.50	18.20
site3	F	2.81	200.00	57.00	1.50	23.80	17.90
site3	F	2.74	200.00	58.40	1.42	24.80	17.20
site3	F	2.88	200.00	55.50	1.22	25.00	18.00
site3	F	3.09	200.00	51.80	1.24	23.00	17.00
site3	F	2.91	200.00	55.00	1.42	25.00	17.50
site3	F	3.00	200.00	53.40	1.38	25.00	16.90
site3	M	3.46	200.00	46.20	0.94	22.40	17.30
site3	M	2.97	200.00	53.80	1.26	22.80	17.50
site3	M	3.05	200.00	52.50	1.34	22.40	17.30
site3	M	2.89	200.00	55.40	1.20	24.00	17.60
site3	M	3.25	200.00	55.30	1.28	26.40	18.80
site3	M	2.75	200.00	58.10	1.38	27.60	19.70
site3	M	2.89	200.00	55.30	1.28	26.40	18.80
site3	M	2.75	200.00	58.10	1.38	27.60	19.70
site3	M	2.56	200.00	62.50	1.40	26.00	18.30
site3	M	2.90	200.00	55.20	1.36	22.80	17.10
site3	M	3.05	200.00	52.50	1.24	23.00	17.50
site3	M	3.08	200.00	52.00	1.28	22.50	17.60
site4	F	1.90	200.00	73.80	3.60	33.60	16.50
site4	F	3.30	200.00	48.50	1.20	22.80	16.00
site4	F	3.48	200.00	46.00	1.00	18.80	15.20
site4	F	2.17	200.00	73.80	3.60	33.60	15.00
site4	F	2.56	200.00	62.50	1.60	26.00	14.70
site4	F	2.54	200.00	55.20	1.38	25.00	14.60
site4	F	2.72	200.00	51.50	1.10	22.80	14.90
site4	F	3.14	200.00	51.00	1.20	22.00	15.00
site4	F	3.05	200.00	52.40	1.12	22.00	15.00
site4	F	2.90	200.00	55.20	1.34	23.80	15.00
site4	F	2.82	200.00	56.80	1.58	26.40	15.00
site4	F	2.17	200.00	73.80	3.60	33.60	14.60
site4	M	3.17	200.00	50.40	1.12	23.00	14.40
site4	M	3.10	200.00	45.20	0.92	21.20	14.20
site4	M	2.59	200.00	54.00	1.26	26.00	14.20
site4	M	3.07	200.00	52.20	1.20	22.80	14.60
site4	M	2.89	200.00	48.50	1.10	21.60	14.60
site4	M	2.68	200.00	52.20	1.20	22.80	14.60
site4	M	3.07	200.00	52.20	1.20	22.80	14.60
site4	M	2.88	200.00	55.60	1.32	24.00	14.20
site4	M	2.56	200.00	62.60	1.58	25.20	15.60
site4	M	2.41	200.00	66.40	1.62	25.00	15.00
site4	M	2.67	200.00	60.00	1.76	27.50	15.40
site4	M	2.89	200.00	48.40	1.32	24.80	14.60
site5	F	1.08	200.00	73.80	3.60	33.6	10.80
site5	F	1.42	200.00	56.50	1.65	27.5	12.20

Appendix C *continued*

site	sex	speed (bl sec^{-1})	time (min.)	fork length (cm)	weight (kg)	girth (cm)	temp (°C)
site5	F	1.26	200.00	63.50	1.70	33.6	10.90
site5	F	1.28	200.00	62.50	1.85	29.0	11.70
site5	F	1.45	200.00	55.20	1.38	28.0	11.60
site5	M	1.38	200.00	58.0	1.80	28.8	11.8
site5	M	1.43	200.00	56.0	1.55	26.5	12.0
site5	M	1.44	200.00	55.5	1.65	26.0	12.0
site5	M	1.43	200.00	56.0	1.50	24.0	11.6
site5	M	1.48	200.00	54.0	1.35	22.0	11.6
site5	M	1.44	200.00	55.6	1.32	24.0	11.2
site5	M	1.92	200.00	62.6	1.58	25.2	12.6
site1	F	4.32	0.50	55.6	1.50	26.0	15.2
site1	F	4.30	0.50	51.2	1.34	23.6	15.0
site1	F	4.01	0.50	54.8	1.50	24.5	14.7
site1	F	3.46	0.50	52.0	1.40	22.8	14.6
site1	F	3.28	0.50	54.8	1.50	24.5	14.9
site1	F	4.23	0.50	52.0	1.40	22.8	15.0
site1	M	3.52	0.50	56.8	1.52	28.2	15.0
site1	M	3.62	0.50	60.8	2.10	28.0	15.0
site1	M	4.23	0.50	56.8	1.52	28.2	15.0
site1	M	3.60	0.50	55.5	1.40	23.2	14.6
site2	F	4.40	0.50	54.6	1.50	24.0	18.5
site2	F	4.73	0.50	46.5	0.96	22.0	18.5
site2	F	4.15	0.50	53.0	1.30	22.8	18.6
site2	F	4.19	0.50	52.5	1.60	24.8	18.4
site2	F	4.17	0.50	57.5	2.10	27.5	17.8
site2	F	4.10	0.50	58.5	1.78	28.5	15.2
site2	F	4.36	0.50	55.0	1.30	27.0	19.2
site2	F	4.44	0.50	54.0	1.60	22.0	18.0
site2	F	4.44	0.50	54.0	1.20	22.0	18.2
site2	F	4.36	0.50	55.0	1.45	25.5	18.2
site2	F	4.17	0.50	57.5	1.60	27.5	18.2
site2	F	4.36	0.50	55.0	1.70	27.0	18.7
site2	M	3.78	0.50	58.2	1.86	25.8	20.1
site2	M	4.12	0.50	58.2	1.86	25.8	18.6
site2	M	4.62	0.50	52.0	1.20	23.2	13.9
site2	M	4.12	0.50	58.2	1.86	25.8	14.4
site2	M	4.27	0.50	56.2	1.42	24.0	14.5
site2	M	4.12	0.50	58.2	1.86	25.8	14.4
site2	M	4.36	0.50	55.0	1.40	24.5	18.2
site2	M	4.44	0.50	54.0	1.45	25.0	17.8
site2	M	4.57	0.50	52.5	1.34	25.5	18.4
site2	M	4.29	0.50	56.0	1.45	26.0	18.0
site2	M	4.80	0.50	50.0	1.20	25.5	17.0
site2	M	4.10	0.50	58.5	1.54	26.4	14.4
site3	F	4.49	0.50	53.5	1.50	23.0	19.0
site3	F	4.25	0.50	56.5	1.54	24.0	18.8
site3	F	3.78	0.50	63.5	2.20	29.5	19.0
site3	F	3.76	0.50	63.8	3.00	31.2	18.8

Appendix C *continued*

site	sex	speed (bl sec^{-1})	time (min.)	fork length (cm)	weight (kg)	girth (cm)	temp (°C)
site3	F	4.44	0.50	54.0	1.34	23.4	19.8
site3	F	4.29	0.50	56.0	1.64	25.5	17.9
site3	F	3.76	0.50	58.5	1.40	28.5	17.2
site3	F	4.17	0.50	57.5	1.66	26.0	18.0
site3	F	4.07	0.50	59.0	1.80	28.0	17.0
site3	F	4.44	0.50	54.0	1.60	22.0	17.5
site3	F	4.44	0.50	54.0	1.40	22.0	16.9
site3	M	4.62	0.50	52.0	1.20	21.4	17.3
site3	M	4.40	0.50	54.5	1.30	24.5	17.5
site3	M	4.07	0.50	59.0	1.58	27.5	17.3
site3	M	4.21	0.50	57.0	1.54	27.0	17.6
site3	M	3.79	0.50	58.0	1.60	28.5	18.8
site3	M	3.45	0.50	58.0	1.50	25.0	19.7
site3	M	4.32	0.50	55.5	1.70	28.5	18.8
site3	M	4.57	0.50	52.5	1.40	26.0	19.7
site3	M	4.14	0.50	58.0	1.40	25.0	18.3
site3	M	4.75	0.50	50.5	1.00	25.5	17.1
site3	M	4.57	0.50	52.5	1.20	26.0	17.5
site3	M	4.29	0.50	56.0	1.60	26.0	17.6
site4	F	5.16	0.50	46.5	1.00	23.0	18.6
site4	F	3.93	0.50	56.0	1.40	25.5	17.8
site4	F	3.96	0.50	55.5	1.34	25.5	18.0
site4	F	3.93	0.50	56.0	1.65	25.5	18.0
site4	F	3.57	0.50	56.0	1.50	26.0	18.0
site4	M	3.45	0.50	58.0	1.70	28.5	18.4
site4	M	3.81	0.50	52.5	1.40	26.0	18.6
site4	M	4.75	0.50	50.5	1.34	25.5	18.4
site4	M	4.19	0.50	52.5	1.20	26.0	18.2
site4	M	4.75	0.50	50.5	1.00	25.5	18.8
site4	M	4.29	0.50	56.0	1.64	26.0	18.6
site4	M	4.00	0.50	55.0	1.34	25.5	18.8
site4	M	3.96	0.50	55.5	1.36	25.0	13.9
site4	M	3.78	0.50	63.5	2.20	29.5	14.0
site4	M	3.48	0.50	57.5	1.40	24.8	16.9
site4	M	3.57	0.50	56.0	1.60	26.5	14.4
site4	M	3.57	0.50	56.0	1.60	25.5	18.4
site4	M	3.51	0.50	57.0	1.45	26.0	16.5
site4	F	3.65	0.50	54.8	1.50	24.5	16.0
site5	F	2.44	0.50	73.8	3.60	33.6	10.8
site5	F	2.41	0.50	58.0	1.80	28.8	11.8
site5	F	2.83	0.50	56.5	1.65	27.5	12.2
site5	F	2.52	0.50	63.5	1.70	33.6	10.9
site5	F	2.24	0.50	62.5	1.85	29.0	11.7
site5	F	2.54	0.50	55.2	1.38	28.0	11.6
site5	F	2.57	0.50	54.5	1.55	27.0	11.9
site5	F	2.14	0.50	56.0	1.55	26.5	12.0
site5	F	2.16	0.50	55.5	1.65	26.0	12.0
site5	M	2.86	0.50	56.0	1.50	24.0	11.6

Appendix C *continued*

site	sex	speed (bl sec^{-1})	time (min.)	fork length (cm)	weight (kg)	girth (cm)	temp (°C)
site5	M	2.39	0.50	58.5	1.55	24.5	11.6
site5	M	2.57	0.50	54.5	1.40	23.5	11.6
site5	M	2.96	0.50	54.0	1.35	22.0	11.6
site5	M	2.52	0.50	55.6	1.32	24.0	11.2
site5	M	2.52	0.50	55.6	1.32	24.0	11.2
site5	M	2.56	0.50	62.6	1.58	25.2	12.6

Appendix D

Plasma non-esterified fatty acid values for male female Atlantic salmon

Females

	site 1		site 2		site 3		site 4		site 5		site 6		kelts	
NEFA	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
14:0	162.19	12.92	173.31	23.63	184.02	11.25	131.40	21.77	103.61	12.06	41.92	4.20	11.51	1.73
14:1	109.22	31.13	98.83	24.02	114.36	34.57	43.50	18.70	33.44	7.16	15.59	4.42	6.34	2.97
16:0	865.00	37.16	750.75	84.06	907.90	26.68	1041.53	72.64	928.04	16.05	389.71	18.59	117.86	15.38
16:1	436.82	23.46	579.69	44.18	653.05	69.86	424.13	49.77	334.98	70.65	185.43	13.12	41.96	7.32
18:0	124.42	25.18	93.57	16.15	141.35	12.78	173.34	11.38	129.10	16.31	54.08	4.06	34.01	8.26
18:1,9	835.08	2.94	861.96	168.05	974.98	6.66	945.57	20.99	1034.11	123.01	444.98	38.76	106.28	3.55
18:2,6	59.99	7.84	102.66	32.41	80.66	8.79	70.12	17.68	62.32	17.47	44.17	7.10	9.44	3.40
18:3,3	8.51	2.32	7.90	0.41	63.88	32.80	12.87	2.87	11.46	3.42	8.17	3.36	8.28	0.00
18:4,3	30.49	0.87	49.64	14.34	33.53	0.48	31.99	0.55	15.95	0.47	10.83	5.90	3.04	0.00
20:0	71.50	0.04	19.38	12.91	53.04	24.17	23.97	14.29	13.73	5.17	4.03	1.19	2.49	1.40
20:1,9	236.42	42.07	220.23	37.83	192.30	6.79	152.24	13.03	120.84	10.25	52.76	23.12	11.38	0.92
20:2,6	9.50	1.40	15.57	8.14	21.91	4.26	7.21	2.66	9.98	3.49	3.52	0.90	1.80	0.05
20:3,6	8.46	2.06	4.44	0.00	21.00	8.83	10.42	1.12	12.17	0.47	3.02	0.42	3.22	0.00
20:4,6	29.73	1.71	36.00	9.05	51.25	10.40	36.86	1.71	27.45	6.32	22.79	1.56	7.86	0.37
20:3,3	3.51	0.96	nd	nd	16.54	6.08	nd	nd	7.60	nd	1.07	0.07	1.73	0.00
20:4,3	50.87	6.70	26.40	5.23	48.49	9.58	79.34	13.58	43.64	16.60	18.01	2.92	5.21	0.29
20:5,3	523.32	37.97	666.10	100.29	567.09	15.54	595.60	30.30	278.75	34.07	239.88	16.33	54.94	16.67
22:0	80.16	44.58	39.02	21.95	160.27	4.15	5.95	2.43	5.72	4.72	7.14	3.94	5.97	3.11
22:1	95.62	17.35	49.67	13.61	74.85	1.60	75.81	15.32	50.45	9.97	28.65	15.64	1.21	0.00
22:2,6	nd	nd	16.29	8.32	15.10	2.12	10.43	0.41	10.57	3.23	7.52	1.04	0.13	0.00
23:0	16.96	4.62	nd	nd	24.47	12.82	3.85	0.00	4.34	2.59	nd	nd	0.05	0.00

Appendix D *continued*

Females

	site 1		site 2		site 3		site 4		site 5		site 6		kelts	
NEFA	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
22:4,6	8.42	1.73	1.40	0.42	32.92	18.54	12.27	0.00	15.38	0.00	4.08	0.00	3.06	0.00
22:5,6	66.13	13.34	33.44	5.31	54.90	11.18	113.47	13.59	30.92	5.36	29.23	21.76	16.01	6.85
22:5,3	85.01	31.12	49.49	9.70	91.26	9.79	147.76	10.86	93.00	42.42	36.82	5.59	10.45	3.46
22:6,3	755.79	21.82	903.51	136.50	872.42	11.78	994.33	65.90	466.17	26.97	336.57	21.51	98.93	15.31
24:0	16.62	4.52	nd	nd	25.07	2.14	1.51	0.43	nd	nd	nd	nd	1.08	0.40
total	4650.69	27.34	4793.18	590.13	5476.60	174.25	5113.85	92.72	3808.77	303.98	1971.44	101.49	541.50	64.19

nd: not detected

Appendix D *continued*

Males

	site 1		site 2		site 3		site 4		site 5		site 6		kelts	
SITE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
14:0	118.42	20.22	164.80	20.19	179.46	50.80	111.34	0.94	92.68	13.86	77.67	12.64	17.76	3.23
14:1	48.75	13.54	96.17	13.32	97.33	83.50	53.96	4.21	54.86	3.74	46.85	7.49	8.43	3.18
16:0	682.00	56.59	883.41	100.96	1005.06	55.80	808.13	36.34	507.46	21.61	473.08	36.79	177.93	31.98
16:1	379.51	60.49	499.54	28.55	333.56	54.15	447.50	43.94	364.49	25.75	293.35	51.15	51.30	17.62
18:0	91.38	16.53	120.67	19.06	118.91	10.55	131.79	25.57	101.73	15.98	88.93	14.03	66.24	26.69
18:1,9	652.03	16.99	715.48	21.96	920.14	67.55	804.04	53.32	673.07	1.55	565.20	28.72	132.55	29.96
18:2,6	68.77	14.87	106.81	18.68	80.09	33.64	88.62	10.39	79.08	6.40	58.68	12.51	22.67	7.55
18:3,3	10.96	3.65	25.36	8.30	20.97	11.20	15.13	3.72	3.84	0.71	22.57	7.75	20.69	5.18
18:4,3	21.41	8.43	46.41	8.09	43.45	17.48	43.25	12.31	11.61	7.92	24.83	6.47	18.77	2.89
20:0	14.18	1.27	53.98	21.34	13.70	1.58	39.89	26.98	50.98	5.50	34.20	11.21	1.18	0.96
20:1,9	236.77	5.32	148.45	27.20	223.95	20.35	155.19	12.84	102.70	20.71	79.02	9.93	12.50	5.78
20:2,6	14.56	6.07	17.17	1.98	12.77	2.40	11.60	1.88	13.48	nd	11.03	3.91	2.53	0.87
20:3,6	10.67	2.77	33.70	10.41	11.69	3.50	7.65	3.58	6.58	nd	11.12	1.34	5.37	1.92
20:4,6	23.04	3.66	32.05	0.18	25.70	1.73	35.58	4.91	11.46	0.07	21.21	4.70	14.37	9.17
20:3,3	2.30	0.41	5.00	1.80	5.78	5.78	nd	nd	nd	nd	6.92	0.00	2.89	0.51
20:4,3	25.31	1.91	27.97	9.63	71.51	19.41	51.70	5.84	17.92	6.18	21.70	7.93	7.47	5.69
20:5,3	440.95	56.36	529.85	19.68	415.18	31.69	424.82	41.91	247.23	10.39	270.07	54.63	59.65	27.26
22:0	80.38	17.45	88.81	17.06	6.94	1.67	78.61	14.57	67.63	4.49	50.02	21.39	6.91	5.64
22:1	74.37	20.80	34.83	7.66	89.82	22.93	38.08	8.64	15.02	nd	24.29	4.91	3.03	0.45
22:2,6	25.48	1.64	24.59	0.00	14.05	2.73	3.65	0.00	nd	nd	4.75	0.00	0.65	0.00
23:0	nd	nd	11.30	0.00	0.00	0.00	1.28	0.00	nd	nd	6.19	2.64	0.27	0.00
22:4,6	1.66	0.00	5.71	0.00	0.00	0.00	4.09	0.00	nd	nd	15.20	0.00	7.64	3.59

Appendix D *continued*

Males

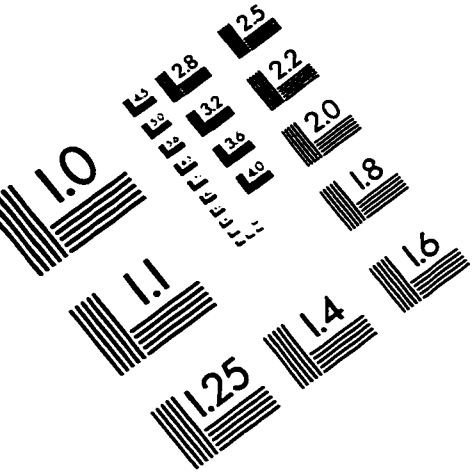
	site 1		site 2		site 3		site 4		site 5		site 6		kelts	
SITE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
22:5,6	62.69	9.13	80.75	11.31	64.02	17.11	70.23	11.81	19.25	7.60	27.29	14.63	6.87	2.09
22:5,3	68.11	1.42	49.38	21.38	127.52	20.75	53.99	21.24	26.03	4.12	38.71	20.00	16.80	12.41
22:6,3	508.49	164.97	883.51	112.86	744.84	47.68	795.65	40.68	458.91	32.63	400.70	53.89	118.81	30.89
24:0	nd	nd	6.66	2.98	nd	nd	0.68	0.01	nd	nd	3.90	3.90	1.38	0.00
total	3616.76	250.24	4477.34	30.19	4617.20	270.43	4247.83	159.02	2908.44	40.89	2653.79	200.22	756.43	152.05

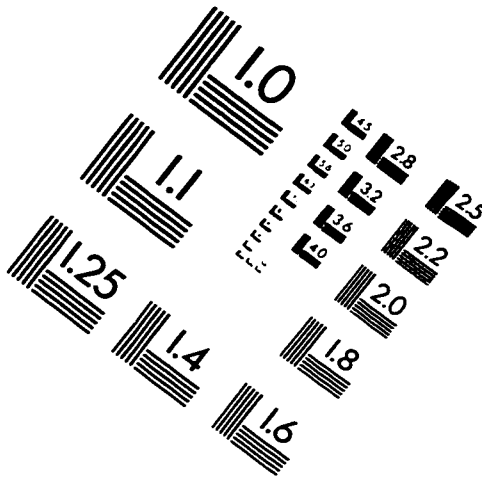
nd: not detected

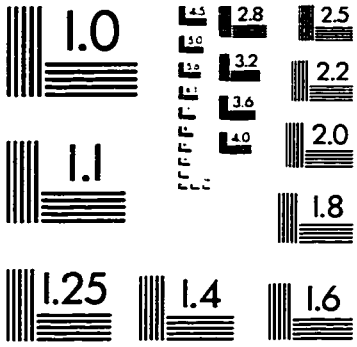
Glossary

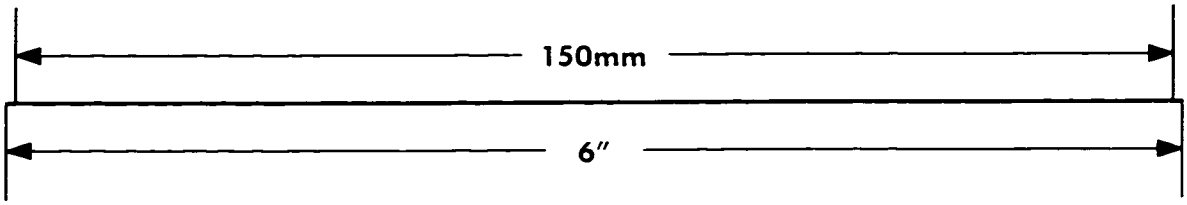
ATP	adenosine triphosphate
bl sec^{-1}	body lengths per second
EMG	electromyogram
EMG*i*	integrated electromyogram
fl	fork length
gt	girth
K	condition factor
Kelt	an Atlantic salmon which has completed spawning
MUFA	monounsaturated fatty acid
NEFA	nonesterified fatty acid
PUFA	polyunsaturated fatty acid
Smolt	salmon which have undergone smoltification
Smoltification	a process which prepares salmon for life at sea
wt	weight

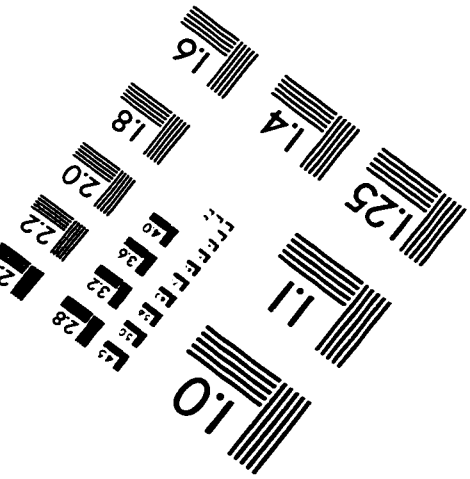
IMAGE EVALUATION
TEST TARGET (QA-3)

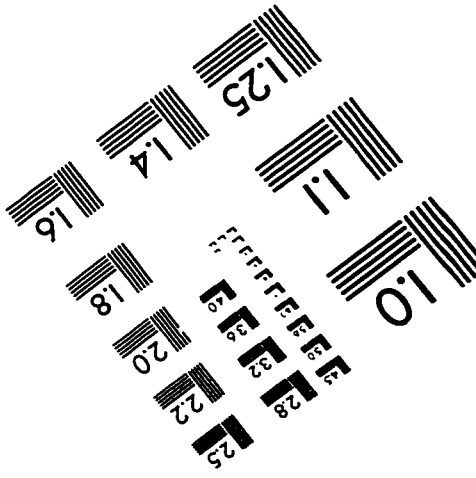












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