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Developmental plasticity of muscle cellularity and swim performance of juvenile Chinook salmon in response to temperature

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Graduate Program in Biology
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science
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DEVELOPMENTAL PLASTICITY OF MUSCLE CELLULARITY AND SWIM
PERFORMANCE OF JUVENILE CHINOOK SALMON
IN RESPONSE TO TEMPERATURE

(Thesis format: Monograph)

by

Dan Dohyung LIM

Graduate Program in Biology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

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Abstract

I investigated the influence of incubation temperature on muscle development and swim performance in juvenile Chinook salmon (*Oncorhynchus tshawytscha*). In 2011 and 2012, embryos were incubated at different combinations of temperature (7, 9, 15 ℃), before and after the onset of free swimming. High-intensity fixed velocity swim tests were performed to assess anaerobic capacity of juveniles. In 2012, these tests were done at a standardized body size (~40 mm). The mean (least-squares) logged times to fatigue of the 15 ℃-incubated fish was higher (0.623 ± 0.049 SE) than the 7 ℃-incubated fish (0.435 ± 0.048 SE) even after acclimation at a common temperature (9 ℃). This indicates a carry-over effect of incubation temperature on swim performance. However, cross-sectional fibre area and number did not correlate with individual swim performance. My study shows the importance of controlling for body size in studies linking muscle cellularity to swim performance.

Key Words

Acclimation temperature

Body size

Carry-over effect

Early development

Fixed velocity swim test

Fibre area

Fibre number

Incubation temperature

Oncorhynchus tshawytscha

Skeletal muscle development

Time to fatigue

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Introduction

Water temperature has a profound influence on many aspects of life for aquatic ectotherms. Fish are at the mercy of ambient water temperature because they are not able to regulate their own body temperature, and even changes of a few degrees Celsius (°C) can have substantial effects on their growth, swim performance, and survival (Beacham and Murray, 1989; Richter and Kolmes, 2005; Rombough, 2011; Burt et al., 2012). Numerous studies have shown that temperature determines the developmental pattern of skeletal muscles, which influences the overall growth of fish (Stickland et al., 1988; Usher et al., 1994; Johnston and McLay, 1997; Albokhadaim et al., 2007). Water temperature is also an important determinant of swim performance in fish because it ultimately controls the organism's physiological capacity for an activity. Rates of reactions in biochemical metabolic pathways, muscle contraction power output, and blood O_2 saturation levels are significantly affected by temperature (Farrell, 2002; Farrell et al., 2008; James, 2013). Temperature-modulations of muscle development may alter swim performance of fish (Koumoundouros et al., 2009). However, the effects of muscle phenotype on swim performance remain unclear despite the extensive research on the effect of temperature over the past few decades.

Water temperature experienced during the early development (herein referred to as incubation temperature) of salmon is important because it influences growth and body size of the fish. Early development encompasses the period from fertilization of eggs to emergence (a life history event when a developing salmon starts to free-swim and begins free feeding). Warm incubation temperature (≥ 10 °C) tends to increase the growth of

embryos and alevins, whereas cool incubation temperature (< 10 ℃) slows down the growth, but increases growth efficiency (utilization of energy from yolk to somatic growth) (Nathanallides et al., 1995; Weatherley and Gill, 1995). Therefore, for fish hatching from eggs of a similar size and fertilized at the same time, individuals incubated at a warm temperature hatch and emerge at a much earlier date compared to those incubated at a cooler temperature. Fish incubated at a cooler temperature would have larger body sizes and masses at hatching and emergence compared to warm-incubated individuals.

Body size (e.g., fork length) of fish is determined by the growth and proliferation of skeletal muscle fibres because skeletal muscle contributes to over 60% of the body mass in ray-finned fishes (Sänger and Stoiber, 2001; Johnston, 2006). Muscle development in salmon comprises both hyperplasia (formation of new muscle fibres) and hypertrophy (enlargement of existing muscle fibres) (Nathanallides et al., 1995; Albokhadaim et al., 2007). Unlike mammals, many teleost fish including salmonids are able to continuously generate new muscle fibres well beyond hatching, and somatic growth is a combination of hyperplasia and hypertrophy. In rainbow trout (*Oncorhynchus mykiss*), hyperplastic growth is observed until the fish reaches a fork length of approximately 55 cm, after which further growth only occurs via hypertrophy (Weatherley et al., 1980).

Incubation temperature is known to affect development of white skeletal muscle fibres, altering the muscle cellularity (number and size distribution of muscle fibres) of developing fish (Johnston and McLay, 1997; Koumoundouros et al., 2002; Johnston, 2006). White skeletal muscles are fast-twitch type muscles that constitute over 90% of

skeletal muscles. Salmonids incubated at cool water temperatures often show a greater intensity of muscle hyperplasia than those incubated at a warmer temperature (**Table 1**). In contrast, fish incubated at warm temperatures (e.g., 10 ℃ vs. 5 ℃) tend to have larger diameters of muscle fibres than those incubated at a cooler temperature until 6 weeks after emergence (Albokhadaim et al., 2007). Hypertrophy of the muscle ceases when the maximum fibre diameter (which can be up to 300 μm depending on the species) is reached (Sänger and Stoiber, 2001). At this point, further growth ceases. Since fish incubated at a warm temperature have a smaller number of muscle fibres compared to fish incubated at a cooler temperature, warm incubation temperature usually results in a smaller body size (Macqueen et al., 2008). Temperature-mediated changes in red muscle cellularity in salmonids have not been studied as extensively as the white muscle cellularity; however, one study showed that incubation temperature does not significantly influence the juvenile red muscle cellularity in rainbow trout (Albokhadaim et al., 2007).

Although there are numerous studies that showed the effect of temperature on muscle development, the patterns observed in temperature-induced muscle cellularity are not always consistent among studies, and the patterns vary among species (**Table 1**). Generally, cool incubation temperatures lead to the formation of a higher number of muscle fibres (greater intensity of hyperplasia) than the fish incubated at warm temperatures when examined at a consistent developmental stage (e.g., hatching and emergence). On the other hand, fish incubated at warm temperatures show larger but fewer number of muscle fibres than those incubated at cool temperatures (greater intensity of hypertrophy). However, cool incubation temperatures sometimes result in

Table 1. A summary of studies that investigated the effects of low and high incubation temperatures on skeletal muscle cellularity of juvenile fish.

Species	Intensity of Hyperplasia	Intensity of Hypertrophy	Acclimation at common temperature	Authors
Salmo salar	$T_{low} > T_{high}$	$T_{low} < T_{high}$	N/A	Nathanallides et al., 1995
S. salar	$T_{low} > T_{high}$	$T_{low} > T_{high}$	N/A	Johnston and Mclay, 1997
S. salar	$T_{low} > T_{high}$	$T_{low} < T_{high}$	N/A	Usher et al., 1994
S. salar	$T_{low} > T_{high}$*	$T_{low} < T_{high}$	Eyed stage†	Macqueen et al., 2008
S. salar	$T_{low} > T_{high}$	$T_{low} > T_{high}$	Hatching†	Albokhadaim et al., 2007
Oncorhynchus mykiss	$T_{low} > T_{high}$	$T_{low} < T_{high}$	N/A	Matschak et al., 1998
Rutilus meidingeri	$T_{low} > T_{high}$	$T_{low} > T_{high}$	Hatching†	Steinbacher et al., 2011
Gadus morhua	$T_{low} > T_{high}$	$T_{low} < T_{high}$	Hatching†	Galloway et al., 1998
Clupea harengus	$T_{low} < T_{high}$	$T_{low} < T_{high}$	Hatching†	Johnston et al., 1998
Mugil capito	$T_{low} < T_{high}$	$T_{low} < T_{high}$	Hatching†	Sänger and Stoiber, 2001

*Fish incubated in 4 different temperatures (2, 5, 8, and 10 ℃), but the highest rate of hyperplastic growth (number of muscle fibres/cross-section area) observed at 5℃, not 2°C. †Carry-over effects in muscle cellularity. T_{low}, lower incubation temperature; T_{high}, higher incubation temperature.

greater intensities of both hyperplastic and hypertrophic muscle growth in juvenile

Atlantic salmon (*Salmo salar*) and rainbow trout (Johnston and McLay 1997;

Albokhadaim et al., 2007). Species such as Atlantic herring (*Clupea harengus*) and

mullets (*Mugil capito*) show completely opposite patterns. In these species, higher

intensities of the muscle hyperplasia are seen in individuals incubated at a warm

temperature, while more prominent hypertrophy is seen at a cooler incubation

temperature (Johnston et al., 1998; Sänger et al, 2001). Temperature-induced differences

in muscle cellularity can persist to the adult stage (Macqueen et al., 2008; Steinbacher et

al., 2011). Rainbow trout embryos incubated at different pre-hatch temperatures (5 and

10 ℃) and transferred to a common temperature (5 ℃) at hatching showed differences in

muscle cellularity (greater hyperplasia and hypertrophy in 5 ℃ incubated fish

[Albokhadaim et al., 2007]). The differences in muscle structures persisted up to 21

weeks after emergence. Further, fish initially incubated at 5 ℃ were more actively

engaged in food seeking activity compared to those incubated at 10 ℃. This suggests that

differences in muscle morphology are linked with other phenotypes, such as foraging

behaviours. Similarly, in another study with Atlantic salmon embryos incubated at 4

different temperatures (2, 5, 8, 10 ℃) and introduced to a common temperature (8 ℃) at

the eyed stage (a point of developmental stage where eyes of the embryos become

visible), differences in muscle cellularity were still present 3 years after hatching. The

lasting effect (i.e., carry-over effect) of temperature on muscle cellularity means that

temperature may also affect other phenotypes that are likely related to muscle cellularity,

such as swim performance.

Water temperature has a substantial influence on the swim performance of fish, such as endurance and swimming speed (Ojanguren and Brana, 2000; Martins et al., 2011, Burt et al., 2012). The effect of temperature on swim performance can be categorized into three types: 1) the immediate effects of the temperature experienced during swimming (swim temperature); 2) the effect of acclimation temperature; and 3) the effects of temperature experienced during the early development of fish (incubation temperature). Among the three types of the temperature effects on swim performance, fish are most sensitive to the swim temperature (James, 2013). In the genus *Oncorhynchus*, the optimum temperature for the best swim performance tends to be around 15 ℃, meaning that swim performance increases until 15 ℃, then decreases at warmer temperatures (Webb, 1995; Hammer, 1995). However, the thermal optima vary among species and populations of salmon (Lee et al., 2003). Temperatures below a thermal optimum slow down the rates of chemical reactions and cell membrane fluidity. This results in reduced power of muscle fibre contractions. For example, a 10 ℃ decrease in water temperature results in a 50% reduction of maximum muscle contractile velocity, which in turn reduces the overall swim speed by 30% (James, 2013). On the other hand, temperatures above the thermal optimum would result in the collapse of cardiac function because of the exponential increase in oxygen demand.

In cold-acclimated or cold-incubated individuals, fish undergo various physiological changes (e.g., increased metabolic capacity, changes in muscle cellularity) to compensate for the physiological consequences of prolonged (i.e., more than a week) cold temperatures, and these changes provide improved swim performance in cold-acclimated or cold-incubated individuals. For example, a 10 ℃ drop in the acclimation

temperature resulted in increased mitochondrial density in red muscle fibres of zebrafish (*Danio rerio*) as the fish showed a 50% and 20% increase in citrate synthase and cytochrome oxidase activities in the red muscles, respectively (McClelland et al., 2006). Consistent with these findings, scup (*Stenotomus chrysops*) and rainbow trout acclimated at cold temperatures (10 °C for scup and 2 °C for rainbow trout) showed higher red muscle contraction force, heart muscle contraction force, and maximum swimming speed than the scup and trout acclimated at higher temperatures (20 °C and 12 °C, respectively [Swank and Rome, 2001; Rome, 2007; Klaiman et al., 2011]). Rainbow smelt (*Osmerus mordax*) acclimated at 5 °C showed faster maximum swimming speed and faster white muscle contraction velocity (2-fold increase) than fish reared at 20 °C (both temperature groups acclimated for 6 weeks) when both temperature groups were swum at a common temperature (10 °C) (Woytanowski and Coughlin, 2013). This supports the hypothesis that cold temperature acclimation leads to increased swim performance. The effect of incubation temperature on swim performance does not seem to differ from the effect of acclimation temperature in some species. Sockeye salmon (*Oncorhynchus nerka*) embryos incubated at 12 °C showed higher swim performance than fish incubated in warmer temperatures [14 °C and 16 °C (Burt et al., 2012)]. A similar pattern was seen in European sea bass (*Dicentrarchus labrax*) incubated at temperatures ranging from 15 °C to 28 °C. Fish incubated at 15 °C showed the highest swim performance with the highest mitochondrial density compared to the fish incubated at higher temperatures (Koumoundouros et al., 2009). Therefore, the effects of acclimation or incubation temperature on swim performance are generally the opposite to the effects of swim temperature (Koumoundouros et al., 2009; Sfakianakis et al., 2011; Burt et al., 2012).

Cold-incubation does not always increase the swim performance of fish. Zebrafish incubated at 31 ℃ were better swimmers than those incubated at 22 ℃ (Sfakianakis et al., 2011). A similar trend was also observed in Atlantic herring. Fish incubated at 12 ℃ until first feeding showed higher burst swim speed compared to the fish incubated at 5 ℃ when these fish were tested at the same temperature (Sänger and Stoiber, 2001). This shows that the influence of incubation temperature on swimming performance also varies among species as seen in the temperature-modulation of muscle cellularity.

Although the effects of incubation temperature on muscle cellularity and swim performance were found in previous studies, little is known about the direct influence of muscle cellularity on swim performance of salmon (**Fig. 1**). Therefore, my study examines the relationship between the temperature-modulation of muscle cellularity and swim performance of juvenile salmon. Muscle cellularity is expected to influence swim performance because the power output of muscle depends on the size of the skeletal muscle. Larger fibres generate higher contractile force by forming more actin-myosin cross linkages. Each muscle fibre in skeletal muscle comprises bundles of thin parallel threads known as myofibrils. Each myofibril consists of thick and thin filaments consisting of myosin and actin, respectively. Typically in striated skeletal muscle, a myosin filament is surrounded by six actin filaments and this unit of arrangement is repeated in parallel, forming a unit of sarcomeres. Fueled by ATP hydrolysis and the presence of Ca^{2+} released from the sarcomere, myosin heads pull the attached actin filaments (cross bridge formation) resulting in contraction of a myofibre. These series of
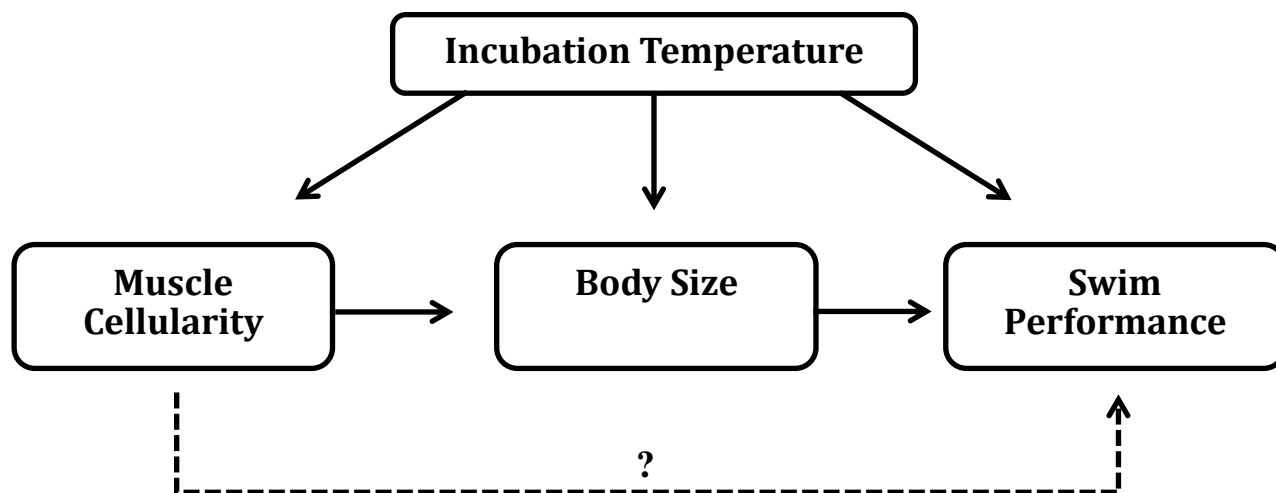
Fig. 1. Study rationale and question. Incubation temperature influences muscle cellularity, body size, and swim performance of juvenile salmon. However, the influence of muscle cellularity, independent of body size, on swim performance of Pacific salmon remains relatively unknown.

events are known as the cross bridge cycle. The contractile force in skeletal muscle is determined by various factors such as concentrations of Ca^{2+}, concentrations of parvalbumin (calcium binding protein), and the muscle cross-sectional area because larger muscle fibres form more cross bridge formation (Russell et al., 2000).

Since fibre size influences the contractile force of muscle, temperature modulation of muscle cellularity should have an effect on swim performance. Juvenile sea bass acclimated at 15 ℃ (vs. 20°, 25°, and 28 ℃) not only had higher maximum swim speed , but also showed a 2% larger red muscle cross-sectional area and a higher number of red myofibres when compared to fish with similar muscle sizes among the temperature groups (Koumoundouros et al., 2009). Their study, however, did not test for the effect of temperature on the cellularity of white muscle fibres, which is a crucial component of burst or high intensity swim performance. Temperature acclimation also tends to affect cardiac muscle cellularity and its physiological properties in rainbow trout (Klaiman et al., 2011). Rainbow trout acclimated at 4 ℃ showed hypertrophy and hyperplasia of spongy myocardium in heart muscle and increased activity of Mg^{2+}-ATPase compared to the trout acclimated at 20 ℃. This could possibly lead to a higher contractile force of the cardiac muscle cells in fish acclimated to cold water. Muscle hypertrophy induced by regular exercise training also improves the swim performance of fish. In juvenile gilthead sea bream (*Sparus aurata*), swim exercise at a consistent current velocity over 4 weeks resulted in the hypertrophy of white muscle fibres compared to the control group (Ibarz et al., 2011). The hypertrophy induced by training is responsible for improved muscle strength and performance (Houlihan and Laurent, 1987).

Swim performance in juvenile salmon is an important determinant of their survival because better swimmers are more likely to evade predation, which is a significant cause of juvenile mortality (Bradford, 1995). Swim performance can be categorized into three distinct phases: sustained, burst (sprint), and prolonged swimming. During the sustained swimming phase, fish can swim for over 200 min, and red muscle fibres (slow-twitch oxidative) are recruited (Adams et al., 1999). During this swim phase, a fish uses its median and/or paired fins to hover in the water column, hold a position, and perform slow, steady swimming (Webb, 1995). Burst swimming involves the use of mainly white (fast-twitch, glycolytic) muscles, and it lasts no more than 20 seconds. To achieve maximum propulsion, the fish uses its whole body and caudal fin. Burst swimming can be visually distinguished from sustained swimming—it usually starts with a C-shaped body form where the head and tail of the fish are at one side, then a sprint is followed by an S-shaped body propulsion (Eaton et al., 1977; Johnson et al., 1998). Prolonged swimming involves the recruitment of both red and white muscle and it usually lasts from 20 to 200 seconds (Deslauriers and Kieffer, 2012). Since fish use their whole body to generate propulsion, especially during burst swimming, body size has a substantial effect on swim performance (Hammer, 1995). The relationship between swim performance (Y) and body length (X) is described by:

$$Y = aX^b,$$

Here, X is usually measured as a fork length and Y is either measured as the time to fatigue (s) or critical swim speed (cm/s) with the scaling factor a and the exponent b, which tends to be approximately 0.5. As swim performance depends on body size, it is important to control for body size when testing for swim performance. This can be done

by testing fish at a standardized size and/or testing fish at a same velocity relative to body size.

Swim performance tests usually involve the use of a swim flume or tunnel, where fish are placed and allowed to swim against a generated measurable flow. There are well-established methods for assessing the swimming capacity and stamina of fish—the incremental velocity test and the fixed velocity test (Farrell, 2008). The incremental velocity test is conducted by exposing the fish to increasing velocity increments for fixed durations (usually 30 to 60 min) until the fish fatigues. Swim performance in this test is measured as critical swimming speed (also referred as U_{crit}). Therefore, higher U_{crit} values mean better swimmers. The incremental velocity test is the most widely used swim test, but determining a U_{crit} can be a very time consuming process ($\geq$ 10 hours per trial) compared to the fixed velocity test (~ 1 hour per trial). Although fish in incremental velocity test perform prolonged swimming which incorporates both aerobic and anaerobic metabolism, incremental velocity test mainly measures aerobic swimming capacity (e.g., migration, foraging, and routine swimming [Reidy et al., 2000]).

The fixed velocity test measures swim duration (time to fatigue) at a constant current velocity. Most fish would cease swimming within 15 min [usually 7 to 8 body lengths/s (McDonald et al., 1998, 2007)]. Therefore, fish that swim for a longer duration are better swimmers. Fish in the fixed velocity test will also show a prolonged swim performance. However, this type of swim test focuses more on determining the anaerobic swim capacity (i.e., burst swimming) of individuals than the incremental velocity test because fish in the fixed velocity test swim at much higher intensity and for a shorter duration. In addition, the fixed velocity swim test results in higher depletion of anaerobic

fuels (i.e., glycogen, phosphocreatine, and ATP) compared to the incremental swim test. This also indicates that swimming is heavily fueled by anaerobic metabolism (McDonald et al., 1998). This type of swim test is highly reproducible after the recovery of muscle fatigue and the anaerobic fuels (McDonald et al., 2007). Overall, the fixed velocity test is more ecologically relevant than the incremental velocity test. In streams, predator avoidance or escaping behaviour mostly involves burst swimming behaviour (Nadeau et al., 2009; Deslauriers and Kieffer, 2012).

Swim performance, muscle cellularity, and growth are not only influenced by temperature, but also by parental phenotypes. Tierney et al. (2009) found that the juveniles of moribund spawning female sockeye salmon had significantly lower U_{crit}, as well as higher plasma lactate concentration (waste product from anaerobic swim performance) than the juveniles of healthy females, showing that the physiological state of females affects progeny, at least until their emergence. Maternal effects on swim performance are also seen in juvenile sockeye salmon, where individual burst swim performance varied among different maternal identities, independent of body sizes (Burt et al., 2012). In this study, large variation in mean time to fatigue (up to 8 seconds) was observed among families. For comparison, the variations in mean time to fatigue of fish incubated at different rearing temperatures were smaller than the parental effects (up to 2-3 seconds). This indicates parental effects can confound the effects of incubation temperature on swim performance. In contrast, Nadeau et al. (2009) did not find any maternal effect on the burst swim performances of sockeye juveniles, although they observed a significant maternal effect on juvenile body mass. Interestingly, the two previous studies examined the same population of sockeye salmon from Weaver Creek,

BC, and used the same swim test protocol. The results from the studies may be different because the fish were examined at different developmental stages. Burt et al., (2012) sampled their fish at emergence (~ 1 month post hatching) and Nadeau et al. (2009) tested their fish at approximately 3 months post hatching, when maternal effects presumably fade.

Parentage also has an effect on muscle cellularity of progeny. Johnston and McLay (1997) found that muscle cellularity of Atlantic salmon at emergence varied among families although they could not confirm that the differences were driven by a maternal effect. Overall, it is important to consider the parental identities as nuisance parameters when comparing juvenile phenotypes (i.e., swim performance, muscle cellularity) because they are likely to contribute to the variation among individuals. Growth of Chinook salmon is strongly influenced by parental phenotypes (Su et al., 1996). During early development, maternal effects tend to play a much larger role than paternal effects on progeny growth. Heath et al., (1999) found that there was a significant positive correlation between the female body size and progeny size, but this relationship became negligible after emergence. Conversely, there was a significant sire effect on progeny size after emergence.

Study Objective and Hypothesis

A majority of Pacific salmon species are currently exposed to higher water temperatures than in the past. Fraser River experienced a net increase of > 1.8 °C in peak summer water temperature over the last 40 years (Mathes et al., 2010). Lake Ontario also experienced a significant increase in winter water temperature from 1950 to 2000 (> 1.0

°C) with more frequent extreme temperature events in the late 90's (Casselman, 2002). In order to increase the general awareness on the impacts of climate change on Pacific salmon, it is necessary to understand how temperature influences the phenotypes (e.g., muscle cellularity and swim performance) of developing individuals. Although studies have shown that temperature has a strong influence on muscle development and swim performance of the juvenile salmon, no study has simultaneously examined the effect of temperature on muscle cellularity and swim performance of individuals while accounting for their family effects. It is also still unclear whether the differences in muscle cellularity contribute to the differences in swim performance. Therefore, my study investigates the influence of incubation temperature on muscle development and swim performance of juvenile Chinook salmon, and whether swim performance could be explained by muscle cellularity. This is the first study to compare the relationship between muscle cellularity and swim performance of Chinook juveniles at a controlled body size. This study would also clarify whether fibre number and size actually contributes to the swim performance of individuals at a controlled body size.

I hypothesized that incubation temperature will have a lasting effect on swim performance of juvenile Chinook salmon via temperature-induced modulation of muscle cellularity. I tested this hypothesis by achieving the several objectives. 1) Measure differences in muscle cellularity in juveniles incubated at different temperatures while controlling for parental effects. 2) Determine whether the differences in muscle cellularity persist when individuals are transferred to a common rearing temperature. 3) Investigate how swim performance is affected by incubation temperature. 4) Determine whether muscle cellularity influences the swimming performance of juveniles. I predicted

that the individuals incubated at a lower temperature regime would show a greater number of smaller muscle fibres. The hyperplasia and hypertrophy of the muscle fibres in fish incubated at a lower temperature should also result in higher swim performance compared to the fish incubated at a warmer temperature. I also predicted to find maternal effects on body size and muscle cellularity (fibre areas and fibre numbers) at emergence, and that these effects would be diminished after emergence.

Materials and Methods

Study System

I studied Chinook salmon sampled from Credit River, ON. They are anadromous Pacific salmon species introduced to Lake Ontario tributaries in the 1970s. They spawn and inhabit the river until they and migrate to the lake within a year of hatching (Healey, 1991). After spending up to several years of their adult life in Lake Ontario, they return to their native streams to spawn in fall (generally late September to early October). Hatching occurs around February and newly hatched fish (i.e., alevins) remain mostly inactive in the gravel beds of natal streams and rely on their yolk sac for nutrition. Thus, fish in their early development are vulnerable to the immediate environmental conditions they inhabit, (e.g. water temperature). In streams, incubation temperature may range between 0 to 16 ℃ (Beacham and Murray, 1989). In the Credit River, incubation temperature ranged between 0 to 9.9 ℃ from 9 November 2012 to 4 February 2013 (Thorn, 2013). Juvenile salmon were used according to the Animal Use Protocol approved by the University of Western Ontario Animal Use Subcommittee (2007-043, see Appendix).

Gamete Collection

On 6 October 2011 and on 1 October 2012, sexually-mature Chinook salmon were sampled (10 females and 5 males) from approximately 10 m downstream of the Streetsville Dam in the Credit River, ON (43°34'39.58"N, 79°42'8.57"W). The fish were collected in conjunction with the Ontario Ministry of Natural Resources via electrofishing. No unhealthy fish, as determined by visual inspection, were used in the

study. Approximately 250 mL of eggs and 30 mL of milt were taken from each fish by gently compressing the abdomen. The gametes were transported in a cooler (maintained at approximately 4 ℃) to Western University, London, ON, on the same day.

Gamete Fertilization

Upon arrival of the gametes to the laboratory, 40 eggs from each female were placed into a dry Petri dish, and were fertilized with a few drops of milt from a male. After a gentle swirl, fertilized eggs were placed into an embryo incubation cup (polyvinyl chloride (PVC) cylinder, 5 cm in diameter and 6 cm in length with 1 mm^2 plastic mesh at the bottom). Immediately after fertilization, the eggs in the incubation cups were rinsed with approximately 30 mL of pathogen-free water and were placed in diluted Ovadine[®] solution (50 mL of Ovadine to 10 L of dechlorinated water at respective incubation temperatures) for 60 minutes to disinfect against potential pathogens. After disinfection, the Ovadine solution was poured off and the eggs were rinsed with fresh water and were placed into incubators at 7 ° C, 9 ℃, or 15 ℃ (see *Experimental Design for more details*). This range of incubation temperatures includes the upper tolerance limit for the survival of Chinook salmon embryos and alevins (~15 ℃) (Beacham and Murray, 1989) and the developmental temperature minimizing mortality (~9 ℃) (Richter and Kolmes, 2005). (Murray and McPhail, 1988). Fish were also incubated at 7 ℃ because it was a temperature well below the and above the maximum and minimum temperature threshold, respectively.

Experimental Design

The gametes of Chinook salmon were cross fertilized in a half-sibling breeding design (2 females to 1 male; Lynch and Walsh, 1998). A total of 10 families ($N = 120$ individuals per family) were created. Therefore, 40 fertilized eggs from each family were incubated at three temperature treatments: 7 (7.17 ± 0.12 SD) ℃, 9 (9.41 ± 0.77 SD) ℃, and 15 (15.13 ± 0.21 SD) ℃ in 2011; 7 (7.44 ± 1.00 SD) ℃, 9 (9.45 ± 0.28 SD) ℃, and 15 (15.56 ± 1.44 SD) ℃ in 2012. The incubation cups were placed into flow-through Marisource® vertical incubation stacks. Embryos were checked daily to note and remove deceased individuals (white, opaque individuals). Emergence timing of each family was predicted based on using an established equation by Jensen et al. (2009):

$$y = 5.2409 \text{ x } 10^{8}/(t + 19.0721)^{3.9184},$$

where y = number of days from fertilization to emergence and t = mean incubation temperature. Emergence was assessed via visual inspection (when ≥ 50% of the fish were at equilibrium, and swimming in the water column in an upright position). Although the predicted timing and actual timing were similar in families at 15 ℃, families at lower incubation temperature emerged earlier (by at least 7 days) than the predicted dates.

At emergence, four individuals from each family at each temperature were lethally sampled for body morphometry measurements (mass, fork length [FL]), and muscle cellularity (2011 only; see below). Fish were euthanized with a tricaine methanesulfonate (MS-222) overdose (300 mg of MS-222, 300 mg of $NaHCO_3$ in 1.5 L of water).

After the sampling at emergence, half of the live remaining individuals (maximum 18 individuals) in each family at 15 ℃ and 7 ℃ were transferred to 9 ℃ to test for carry-over effects of early development temperature until the termination of the experiment. The experiment was terminated at 300 accumulated thermal units (ATU; mean incubation temperature [℃] × number of days post emergence) for the 2011 experiment. The termination at 300 ATU post-emergence was selected to ensure a similar amount of development post emergence. In 2012, the experiment was terminated when fish reached 40 mm of FL. The 5 different temperature treatments were: 7 ℃ (7-7 ℃), 7 to 9 ℃ (7-9 ℃), 9 to 9 ℃ (9-9 ℃), 15 to 9 ℃ (15-9 ℃), and 15 to 15 ℃ (15-15 ℃) (**Fig. 2**). At emergence, individuals were transferred from the embryo incubation into fry incubation cups immersed in rearing tanks to allow the fry more space for free swimming and feeding. Each fry cup was prepared by cutting 10.2 cm-diameter PVC pipe into 30 cm long pieces. The fry cups also consisted of a pair of circular "windows" (4 cm in diameter) on the walls with 1 mm^2-hole plastic mesh screen to allow water flow, but to keep the fish inside the cups. A piece of plastic mesh screen was also fitted and glued at the bottom of each fry cup with water-proof silicone. Fish were fed to satiation daily with fine ground salmon feed (Corey Aquafeeds, Corey Nutrition Company, Fredericton, NB).

In 2011, at 300 ATU after emergence, individuals from each family were sampled (n = four per family) for a swim performance test (see Fixed Velocity Swim Test). Upon the completion of swim tests, individuals were transferred to separate fry cups (family and individual identity labelled). After allowing five hours to recover from the swim test, individuals were euthanized with MS-222 for the measurement of FL and muscle cellularity analyses. To confirm that the swim tests did not affect muscle cellularity, four

Fertilization: half-sib design

(10 Females X 5 Males)

| 7 ºC | 9 º C | 15 ºC |

Emergence [a]   Emergence [a]   Emergence [a]

| 7 ºC | 9 ºC | 15 ºC |

Termination [a,b]   **Termination** [a,b]   **Termination** [a,b]
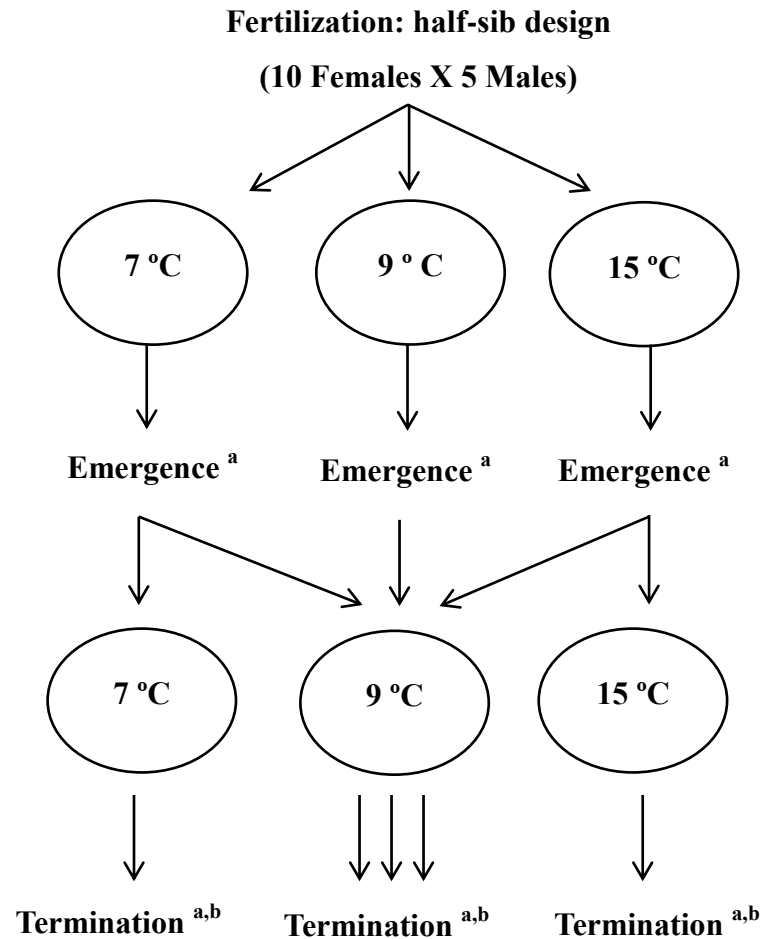
Fig. 2. Schematic of experimental design and sampling points from fertilization to termination. Termination was at 300 ATU (accumulated thermal units: mean incubation temperature × number of days) after emergence for the 2011 experiment, and at a fork length of 40 mm for the 2012 experiment. [a] Body morphometry (e.g., fork length and mass) and muscle cross-sections sampled for muscle cellularity analysis, [b] Swim performance measured.

individuals which were not tested for swim performance from each family were sampled for muscle cellularity analyses.

In 2012, individuals incubated at different temperatures were sampled at a common body length to control for effects of body size on muscle cellularity and swim performance. Fish were sampled when their fork length reached 40 mm not only to control for the effect of body size on muscle cellularity and swim performance, but also to assure a complete absorption of yolk sac by the juveniles. Incomplete absorption of yolk sac may cause a drag while swimming, altering the swim performance of the fish. In order to predict the date when fish would reach

40 mm at 9 °C, the following equation was established from the growth data of Credit River alevins and juveniles provided by Thorn (2011, unpublished data):

$$y = 21.2994 + 0.4183x + 0.0038\ x^2,$$

where y = FL of an individual, and x = number of days it would take for an individual to reach the FL of y (i.e., 40 mm) from hatching. Once the predicted date was reached in 2012, all individuals in each family were transferred to a Petri dish. Beneath the Petri dish, a ruler was placed to calibrate the scale in each photograph. Photos of individuals

were taken from directly above the Petri dish with a Canon G10 digital camera and then were analyzed via Image J software to measure FL of all fish in each family.

Muscle Cellularity Analyses

 Immediately after euthanasia, cross-sections (~5 mm thick) were dissected with a microtome blade at the anterior insertion point of the dorsal fin. Each cross-section was

placed in a plastic embedding cassette (Sigma-Aldrich®) and was fixed in 10% neutral

buffered formalin (Fisher Scientific®) for 46 hours at 4 ℃. The cross-sections were then

dehydrated in a series of 70% to 95% ethanol and infiltrated with paraffin wax (Stickland

et al., 1988; Johnston and McLay, 1997) using a Leica ASP6025 tissue processor.

Processed cross-sections were transferred to a paraffin bath and each section was

embedded in a paraffin block using a Leica EG1150 tissue embedding centre. The

anterior plane of the cross-section always faced the bottom of the rectangular mold for

sectioning consistency. Embedded cross-sections were cut into 5 μm thin sections with a

rotary microtome (Leica RM2245) and placed on slides. After an overnight dehydration

of the slides at 45 ℃, cross-sections on slides were deparaffinised and stained with

haematoxylin and eosin (HE) for microscopy using a Leica ST5010 Autostainer. HE

staining is the most widely used staining method in histology because nuclei and

cytoplasm are easily differentiated (**Fig. 3**). Haematoxylin is basic and it binds to DNA

and RNA, showing a bluish-purple colour of the stained nuclei. On the other hand, eosin

is acidic, and binds to most proteins in the cytoplasm (e.g., muscle fibres). Cross-sections

were photographed with an Infinity 2 digital camera and a dissecting microscope (Meiji

EMZ 13TR*)* and a light microscope (Nikon Eclipse 50*i*).

Epaxial cross-sectional area (**Fig. 3**) was determined by manually tracing the

epaxial muscle areas using Infinity Analyze software on the digital photos taken on the

dissecting microscope. This software allowed mouse-tracing and calculations of the

cross-sectional areas from the digital photos. To estimate the total fibre number and the

mean fibre cross-sectional area in the epaxial cross-section, the first procedure was to

draw a vertical line extending from the centre of the dorsal fin insertion
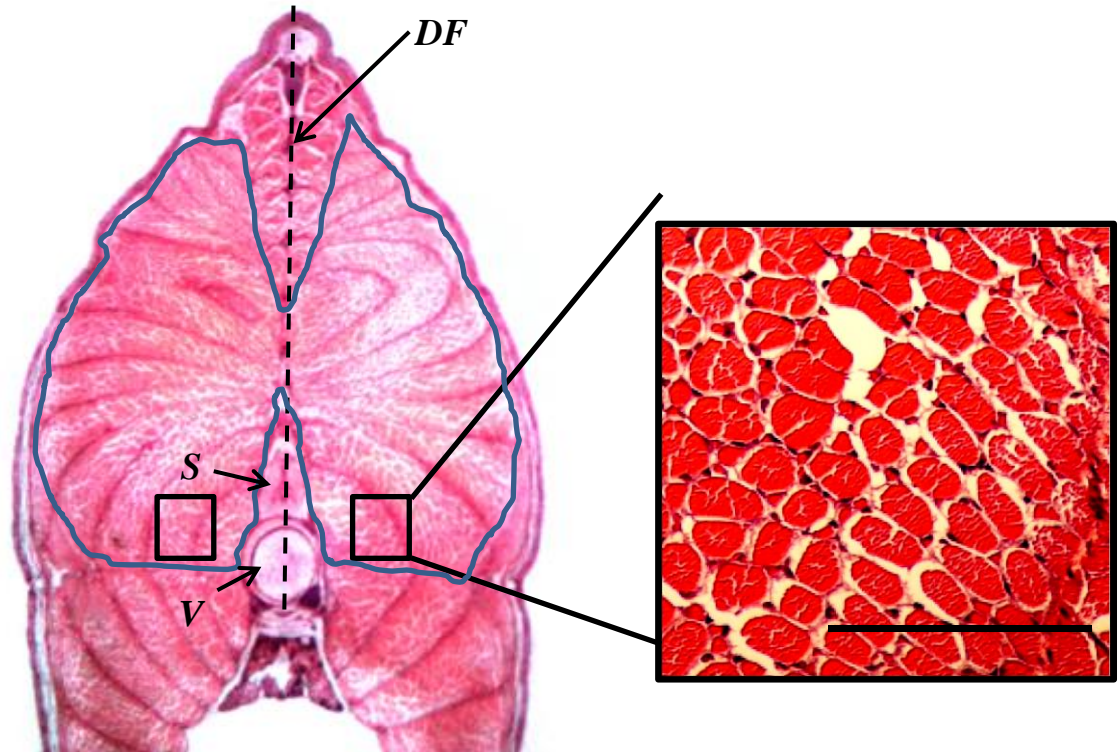
Fig. 3. Cross-sections of white skeletal muscle fibres of fish at emergence.
Epaxial cross-sectional area is outlined in blue line. Locations of the square grids 300 μm from the reference line (dashed); *S*, spinal cord; *V*, vertebrae; *DF*, dorsal fin base. The dashed vertical line is a transect from the centre of the dorsal fin base to the centres of the spinal cord and vertebrae. Muscle fibres in each square grid were analyzed for cellularity. The scale bar in the micrograph indicates 100 μm. Fibre cross-sections were stained in red colour with eosin, while haematoxylin stained fibre nuclei and calcified structures with dark purple to black colour.

using NIS-Elements imaging software (V 2.30) on the photos taken on the light microscope at a 20x objective setting. Then, a pair of square grids (200 μm by 200 μm) was drawn horizontally 300 μm to the left and right from the intersection of the vertical line and the top of the vertebrae using Image J software (**Fig. 3**). The fibre number was determined by:

(epaxial cross-sectional area/80,000 μm^2) × number of fibres from the two grids,

where the fibre number per grid were manually counted with the "Cell Counter" application in Image J software. All stained fibres that were in contact with the right and/or top lines of the grid were included in the count; however, fibres that touched left and/or bottom lines of the grid were excluded in the count. This accounts for any fibres that are touching the lines of the grid while performing the fibre count. Fibre cross-sectional area was analyzed instead of the fibre diameters, because the formalin fixation and staining process resulted in the distortion of fibre cross-sections, making it difficult to measure the fibre diameters. In order to obtain the fibre area, each photograph was converted to black and white (from RGB colour to 8-bit), then the "Threshold" function was applied to enhance binary contrast. The total number of black pixels and white pixels in each grid was quantified by using the "Analyze Particles" function.

Fixed Velocity Swim Test

All fish were fasted for 48 hours prior to the swim test to avoid the possible effects of specific dynamic action on swim performance (Alsop and Wood, 1997; Thorarensen and Farrell, 2006). A day prior to the swim test, digital photos of individuals were taken to measure the FL of individuals in each family. The fork length of each fish

was determined via Image J software. A fixed velocity swim test (McDonald et al., 1998; Ojanguren and Brana, 2000) was performed to evaluate the swimming capacity of an individual. An open-top recirculating swim flume of 100 L in total volume was used for swim test trials. The flume was 11 cm in width, 20 cm in height, and 74 cm in length (McDonald et al. [1998] for more details on flume design and specifications). Approximately an hour prior to the swim test, the flume was filled with the water from the rearing tank to ensure a similar temperature and water chemistry.

Four fish were taken from each family and were transferred to the flume using a dip net. Once the fish were in the flume, the top of the flume except for the last 15 cm from the back mesh was covered with a black PVC panel to minimize the visual stimulation of the fish while swimming. Fish in the flume were then acclimated for 40 min at a current of 0.5 fork length/s. After the acclimation, current velocity was gradually increased to 8 fork lengths/s over a 2 min period. Generally, 8 fork lengths/s is known to be the speed at which most juvenile salmonids will fatigue in < 5 min (McDonald et al., 1998; Nadeau et al., 2009; Burt et al., 2012). Time to fatigue was measured from the time when individuals started to perform prolonged swimming at 8 fork lengths/s to the point of their fatigue. A fish was considered to be fatigued when it ceased swimming, lost equilibrium, and rested against the rear mesh screen of the swim flume even after a light physical stimulation with blunt-ended plastic rod. Therefore, the time to fatigue represents a swim performance of individuals that can be sustained for a short period of time (< 15 min), but longer than a startling response that can only last for a few seconds. Individuals that did not swim were classified as 'non-swimmers' and they were not included in the data analyses. Fish that did not fatigue at 15 min were noted as 'did not

fatigue' and were removed from the swim flume. However, since they swam for at least 15 min, their times to fatigue were marked as 900 seconds and the data were included in the data analyses.

Data Analyses

All statistical analyses were performed using the R statistical computing software, version 3.0.1 (2013). Differences among treatments were considered to be significant when $p < 0.05$. Prior to each statistical analysis, normality of the data was assessed with Q-Q plots. Under normality, the data points would not deviate from the line on the Q-Q plot by visual inspection. In addition, residual plots were done to ensure that for each value of the independent variables, x, variance in y (dependent variable) remained approximately constant. For all the analyses of covariance (ANCOVA) with significant results, η^2 values were determined to examine effect sizes (lsr package in R):

$$\eta^2 = SS_{effect} / SS_{total},$$

where SS_{effect} = sum of squares for the effect, and SS_{total} = total sum of squares. Therefore, a η^2 value shows the proportion of variance in a dependent variable contributed by an independent variable (e.g., temperature treatment) in a statistical model (Tabachnick and Fidell, 2007). In the analysis that included two or more independent variables, η^2 value may not be an accurate indicator of an effect size because SS_{total} includes variances explained by other independent variables. Therefore, partial η^2 values (η^2_p) were used:

$$\eta^2_p = SS_{effect} / (SS_{effect} + SS_{error}),$$

where SS_{error} = error variance.

Muscle Cellularity

In the 2011 data, differences in the total fibre number, the fibre area, and fork length among temperatures were tested via one-way ANOVA followed by a Tukey's post hoc test. ANCOVA could not be done in 2011 because there was no overlap in fork length among treatments. In 2012, ANCOVA based on the following equation was performed to control for the effect of fork length:

fibre number or fibre area = TRT + fork length + TRT*fork length,

where treatment (TRT) was an independent factor. In the analyses, the interaction between the temperature and fork length was checked first. When there was no significant interaction, the interaction term was dropped (Tabachnick and Fidell, 2007), and overall effects of fork length and temperature were examined.

Swim Performance

Time to fatigue is expected to increase exponentially with increasing fork length (McDonald et al., 1998; Burt et al., 2012) and there is often a large variation in the times to fatigue among individuals; therefore, the time to fatigue values were log-transformed prior to analysis. In the 2011 data, regression analyses of time to fatigue vs. fork length of each treatment were done to examine the effect of fork length on time to fatigue (slopes of the regression lines) and the relative swim performance among treatments (elevation of the regression lines). Without the overlap of fork length in fish among all temperature treatments, ANCOVA could not be done.

In the 2012 data, ANCOVA was performed in order to control for the effects of FL on TTF:

time to fatigue = TRT + fork length + TRT*fork length,

The interaction between time to fatigue and fork length among the treatments was tested first. When there was no significant interaction, the interaction term was dropped and overall effect of fork length and temperature was tested.

Least squares mean (ls-mean) of the logged times to fatigue in each treatment was determined. The ls-mean calculates the mean logged times to fatigue by adjusting each individual's time to fatigue to the overall mean fork length of all treatments.

Using the 2011 data, ANCOVAs were used to compare the muscle cellularity (total fibre number and fibre area) of tested and non-tested individuals within each temperature treatment to confirm that the swim trials did not have any effect on muscle cellularity:

X_i = SWIM + fork length + SWIM*fork length,

where X_i = muscle cellularity parameter (fibre number or fibre area), and SWIM = factor that represented a tested group and a non-tested group. Fork length is a covariate. The significance of the interaction was checked first. Fork lengths were also compared among SWIMs using ANCOVA to confirm that the fork length was not a contributing factor to muscle cellularity, by setting fork length as a dependent variable, using muscle cellularity (fibre number or fibre area) as a covariate:

$$\text{fork length} = \text{SWIM} + X_i + \text{SWIM}* X_i$$

Effects of Muscle Cellularity on Swim Performance

Using the 2012 data, ANCOVAs were performed to test the effects of fibre number and fibre area on swim performance of individuals in each temperature:

$$\text{time to fatigue} = X_i + \text{TRT} + \text{fork length} + X_i*\text{TRT} + \text{fork length}*\text{TRT}.$$

Individuals with fork length ranging from 30.9 to 40.9 mm were selected to minimize the effect of the fork length, which resulted in smaller sample size ($n = 55$) compared to the 2012 swim performance sample size. Non-significant interaction terms with were dropped from the statistical equation (Tabachnick and Fidell, 2007).

Parental Effects

An unconditional means model (Singer, 1998) was used to assess parental effects (lme package in R) on fork lengths, muscle cellularity, and swim performance. The proportion of variation in fork length, the fibre number, the fibre area, and time to fatigue contributed by sire and dam in each treatment was determined using:

$$y = \mu + S + S(D) + \varepsilon,$$

where y is either the fork length, the fibre number, the fibre area, or time to fatigue, $\mu =$ the population mean, $S =$ effects contributed by sire, $D =$ effects contributed by dam nested within sire, and $\varepsilon =$ effects contributed by the unexplained variance in the model.

Since the parental effects were observed from the unconditional model (over 70%
in some cases: refer to the results), treatment effects were tested while controlling for the
parental effects in a linear mixed model (MCMC glmm package in R):

$$y = \mu + T + FL + S + S(D) + \varepsilon,$$

where y is either the fibre number, the fibre area, or time to fatigue, μ = the population
mean, T = effect of temperature treatment (fixed effect), FL = fork length, S and D =
same as defined above (random effect), ε = any effect contributed by unexplained
variable in the model.

Results

Fertilization Success and Mortality

In both the 2011 and 2012 experiment, median survival rates of individuals from fertilization to termination were generally very high except for the 15-15 ℃ treatment fish in 2012 (**Table 2**). High mortality seen in the 15-15 ℃ treatment showed the symptoms of coagulated yolk disease, likely due to thermal stress (Wedemeyer, 1996); therefore, they were not included in the 2012 data analyses.

Muscle Cellularity

The effect of incubation temperature on muscle cellularity at emergence was visually noticeable (**Fig**. **4**). In fish sampled at emergence in 2011 (sample sizes summarized in **Table 3**), there were significant differences in the fibre number (ANOVA, $F_{2, 159} = 164.25$, $p < 0.001$, $\eta^2 = 0.73$) and the fibre area (ANOVA, $F_{2, 159} = 210.21$, $p < 0.001$, $\eta^2 = 0.67$) among temperature treatments. Fish incubated at 9 ℃ had the highest mean cross-sectional fibre number when compared to fish at 7 ℃ (post hoc Tukey test, $p = 0.003$) and 15 ℃-incubated fish (post hoc Tukey test, $p < 0.001$; **Fig. 5 A**). The fibre area of both 7° and 9 ℃-incubated fish were approximately 60% larger than at 15 ℃ (post hoc Tukey tests, $p < 0.001$; **Fig. 5 B**). There was no significant difference in the fibre area between fish incubated at 7 ° and 9 ℃ (post hoc Tukey test, $p = 0.403$).

Table 2. Median fertilization success, hatching success, and survival rates of individuals at sampling points from each family. Unfertilized eggs were not considered as deceased eggs, and were excluded from all calculations.

Sampling Year	Temperature Treatment (°C)	Fertilization Success (range)	Hatching Success (range)	Survival at Emergence (range)	Survival from Emergence to Termination (range)
2011	15-15	0.95 (0.40 – 1.00)	0.95 (0.11 – 1.00)	0.88 (0.00 – 1.00)	1.00 (0.00 – 1.00)
	9-9	0.98 (0.50 – 1.00)	0.98 (0.55 – 1.00)	0.98 (0.52 – 1.00)	1.00
	7-7	0.98 (0.10 – 1.00)	98 (0.21 – 1.00)	0.98 (0.68 – 1.00)	1.00 (0.68 – 1.00)
	15-9				0.99 (0.79 – 1.00)
	7-9				1.00 (0.68 – 1.00)
2012	15-15	0.93 (0.02 – 1.00)	0.99 (0.82 – 1.00)	0.97 (0.00 – 1.00)	0.42 (0.00 – 0.88)
	9-9	0.93 (0.45 – 0.98)	0.97 (0.82 – 1.00)	0.94 (0.00 – 1.00)	1.00 (0.97 – 1.00)
	7-7	0.84 (0.12 – 0.95)	0.98 (0.85 – 1.00)	0.97 (0.74 – 1.00)	1.00 (0.00 – 1.00)
	15-9				0.93 (0.69 – 1.00)
	7-9				1.00 (0.92 – 1.00)
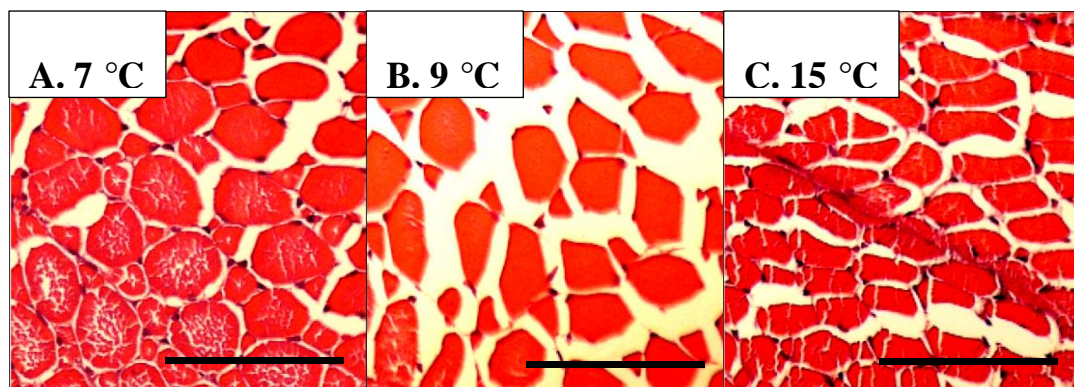
Fig. 4. Muscle cross-section of fish incubated at (A) 7 °C, (B) 9 °C, and (C) 15 °C and sampled at emergence in 2011. The black solid bars in the micrographs indicate 100 μm. Fibres were stained in red colour with eosin, while haematoxylin stained fibre nuclei and calcified structures with dark purple to black.

Table 3. Sample sizes in each treatment at each sampling point for muscle cellularity analyses and swim performance tests. Sample size in muscle cellularity analyses group in 2012 was smaller than the swim performance group as individuals with similar body lengths (±1 mm of fork length) were selected from the swim performance group.

Sampling Year	Sampling Point	Temperature Treatment (°C)	*n*	
			Muscle Cellularity	Swim Performance
2011	Emergence	15	63	*NA*
		9	62	*NA*
		7	37	*NA*
	Termination	15-15	34	21
		15-9	40	26
		9-9	103	54
		7-9	25	13
		7-7	34	15
2012	Emergence	15	45	*NA*
		9	38	*NA*
		7	33	*NA*
	Termination	15-9	11	36
		9-9	17	35
		7-9	14	36
		7-7	13	32

NA: Not applicable; no swim test performed at emergence
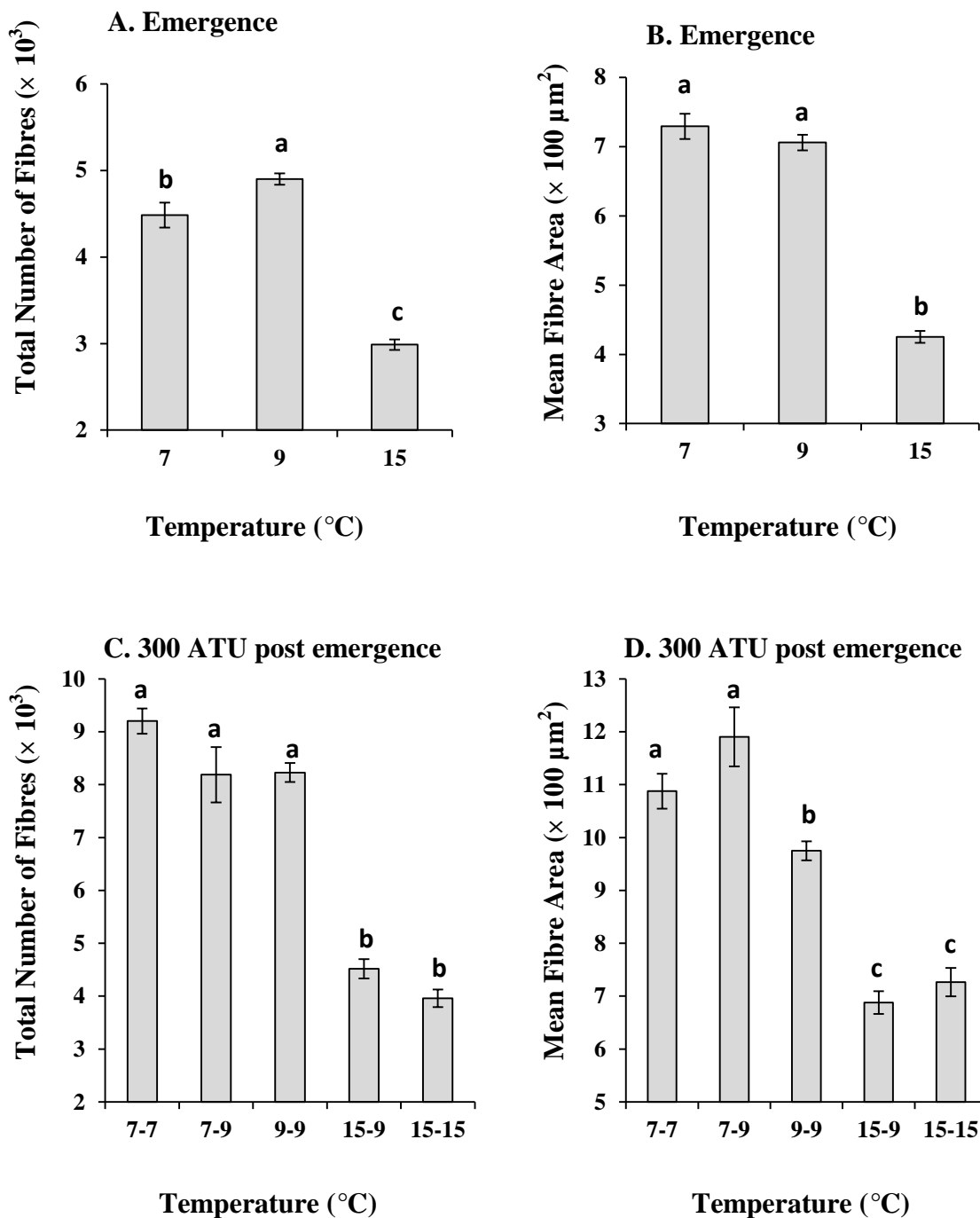
Fig. 5. Effect of incubation temperature on fibre number and fibre area of fish at emergence in the 2011 experiment. (**A**) Estimated total cross-sectional fibre number of fish sampled at emergence, (**B**) mean fibre cross-sectional area of fish sampled at emergence, (**C**) estimated total fibre number of fish sampled at 300 ATU post emergence, (**D**) mean fibre cross-sectional area of fish sampled at 300 ATU post emergence. Data are presented as the mean ± SE (*n* shown in **Table 3**). Different letters indicate statistical differences ($p < 0.05$) among treatments based on Tukey's HSD post-hoc tests.

In the 2011 experiment terminated at 300 ATU, incubation temperature had significant effects on both fibre number and fibre area (ANOVA, $F_{4, 231} = 58.56$ for fibre number, $F_{4, 231} = 45.92$ for the fibre area, $p < 0.001$ for both tests, $\eta^2 = 0.50$ for both tests; **Figs. 5C, D**). There was no difference in the fibre number between the fish initially incubated at 7 °C (7-7 and 7-9 °C treatments) and 9-9 °C treatments, while the fibre number of the fish initially incubated at 15 °C (15-9 and 15-15 °C treatments) were significantly less than rest of the treatments (post hoc Tukey tests, $p < 0.001$). The mean fibre areas were significantly different among fish with different initial treatments (post hoc Tukey tests, $p < 0.001$). The mean fibre areas of 7-7 and 7-9 °C treatment fish were the largest, whereas the fish in 15-9 and 15-15 °C treatment had the smallest mean fibre areas. The transfers of fish to 9 °C at emergence did not change fibre numbers or areas.

Water Temperature and Body Size

There was a significant effect of incubation temperature on the fork length of individuals at emergence for both 2011 and 2012 data (ANOVA, $F_{2, 159} = 335.63$, $p < 0.001$, $\eta^2 = 0.81$ for the 2011 data [**Fig. 6 A**]; ANOVA, $F_{2, 113} = 539.46$, $p < 0.001$, $\eta^2 = 0.91$ for the 2012 data [**Fig. 6 B**]) and at 2011 termination (ANOVA, $F_{4, 231} = 545.45$, $p < 0.001$, $\eta^2 = 0.90$; **Fig. 6 C**). A higher incubation temperature resulted in smaller fork length at emergence and at termination. In 2012, 9 °C-incubated fish were slightly (by 1.9%), but significantly (post hoc Tukey test, $p = 0.04$) longer than the 7 °C-incubated fish, which was an opposite trend from the 2011 experiment observation. In 2012, the fork length of individuals at termination were not significantly different among different temperature treatments (ANOVA, $F_{3, 51} = 1.22$, $\eta^2 = 0.07$, $p = 0.308$; **Fig. 6 D**).
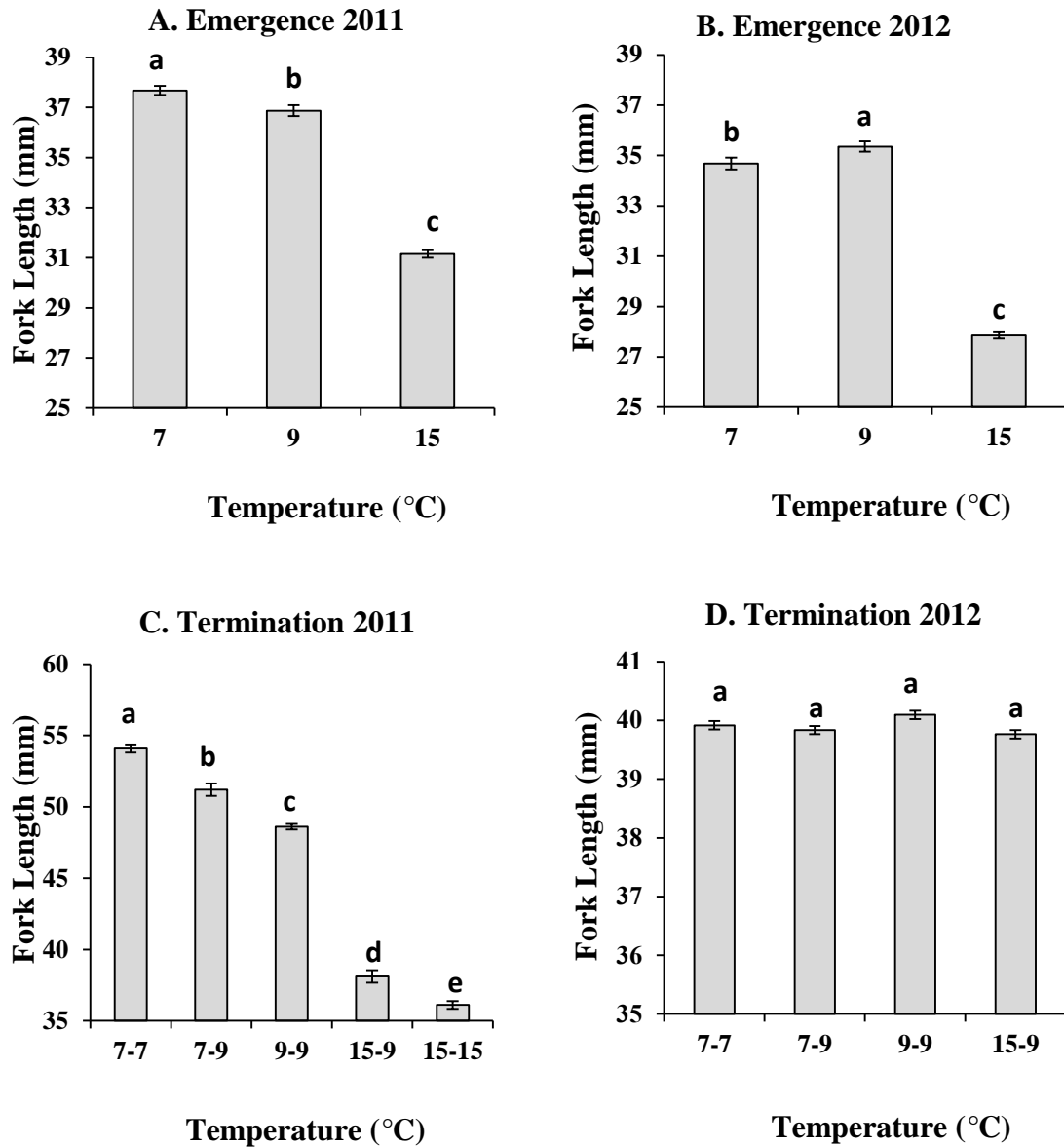
Fig. 6. Effect of incubation temperature on the body size of fish at different developmental stages. Fork length at (A) emergence and at (B) 300 ATU post emergence for fish sampled in 2011; fork length at (C) emergence in 2012 and at (D) terminal sampling point in 2012 where fork length was controlled. Data are presented as the mean ± SE. Sample sizes for each treatment are shown in **Table 3**. Different letters indicate statistical differences ($p < 0.05$) among treatments based on Tukey's HSD post-hoc tests.

Muscle Cellularity at a Common Fork Length

At termination at a common fork length (mean of 39.9 mm) in 2012, there were no differences in the fibre number among treatments **(Fig. 7 A)**, and differences in the fibre area among treatments were much reduced **(Fig. 7 B)**. The effect of fork length on fibre number did not depend on the treatment (ANCOVA, fork length*TRT: $F_{3, 52} = 0.27$, $p = 0.844$). Fork length did not affect fibre number ($F_{1, 50} = 1.24$, $p = 0.270$) and the fibre number did not differ among treatments ($F_{3, 50} = 1.03$, $p = 0.366$). The effect of fork length on the fibre area did not differ among treatments (ANCOVA, fork length*TRT: $F_{3, 52} = 1.97$, $p = 0.130$). Also, there was no effect of fork length on fibre area ($F_{1, 50} = 0.01$, $p = 0.937$). On the other hand, a gradual increase in cross-sectional fibre area was seen as incubation temperature decreased, resulting in a significant effect of incubation temperatures on fibre areas ($F_{3, 50} = 3.03$, $p = 0.038$, $\eta^2_p = 0.50$). The fibre area of the fish in the 7-7 °C treatment was significantly larger (approximately by 12%) than the fish in the 15-9 °C treatment (post hoc Tukey test, $p = 0.048$).

Swim Performance

In 2011, regression analyses of the 15-9, 9-9, and 7-9°C treatments showed a positive linear relationship between fork length and log-transformed times to fatigue, while no significant relationship between fork length and time to fatigue was found in the 15-15 and 7-7 °C treatments (**Table 4 and Fig. 8**). There was a large variation in fork length among individuals from different treatments and fork length did not overlap among all treatments. It was also confirmed that swim tests did not influence the fibre number nor
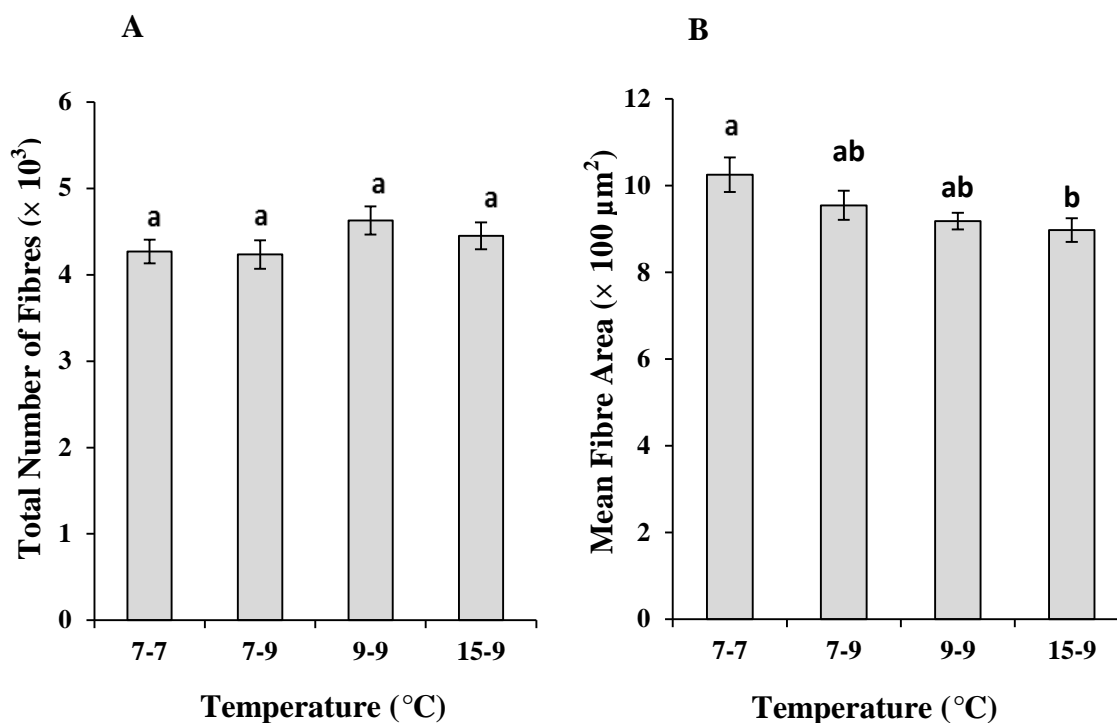
Fig. 7. Effect of temperature on cross-sectional fibre number and area at termination of the 2012 experiment. (A) Estimated total fibre number of the individuals, **(B)** mean fibre cross-sectional area of fish sampled at 300 ATU post emergence. Data are presented as the mean ± SE. Sample sizes for each treatment are shown in **Table 3**. Different letters indicate statistical differences ($p < 0.05$) among the temperature treatments based on Tukey's HSD post-hoc tests.

Table 4. A summary of linear regression analyses of fixed velocity swim test at different temperatures. The logged values of time to fatigue (y) increases with fork length (x).

Temperature Treatment (°C)	Equation	r^2	df	t	p
15-15	$y = 0.0262x + 2.222$	0.007	20	1.229	0.233
15-9	$y = 0.1097x - 1.226$	0.271	25	3.307	0.028
9-9	$y = 0.1101x - 2.031$	0.212	53	3.756	< 0.001
7-9	$y = 0.2037x - 7.268$	0.368	12	2.534	0.028
7-7	$y = 0.0717x - 0.276$	0.095	14	1.165	0.265
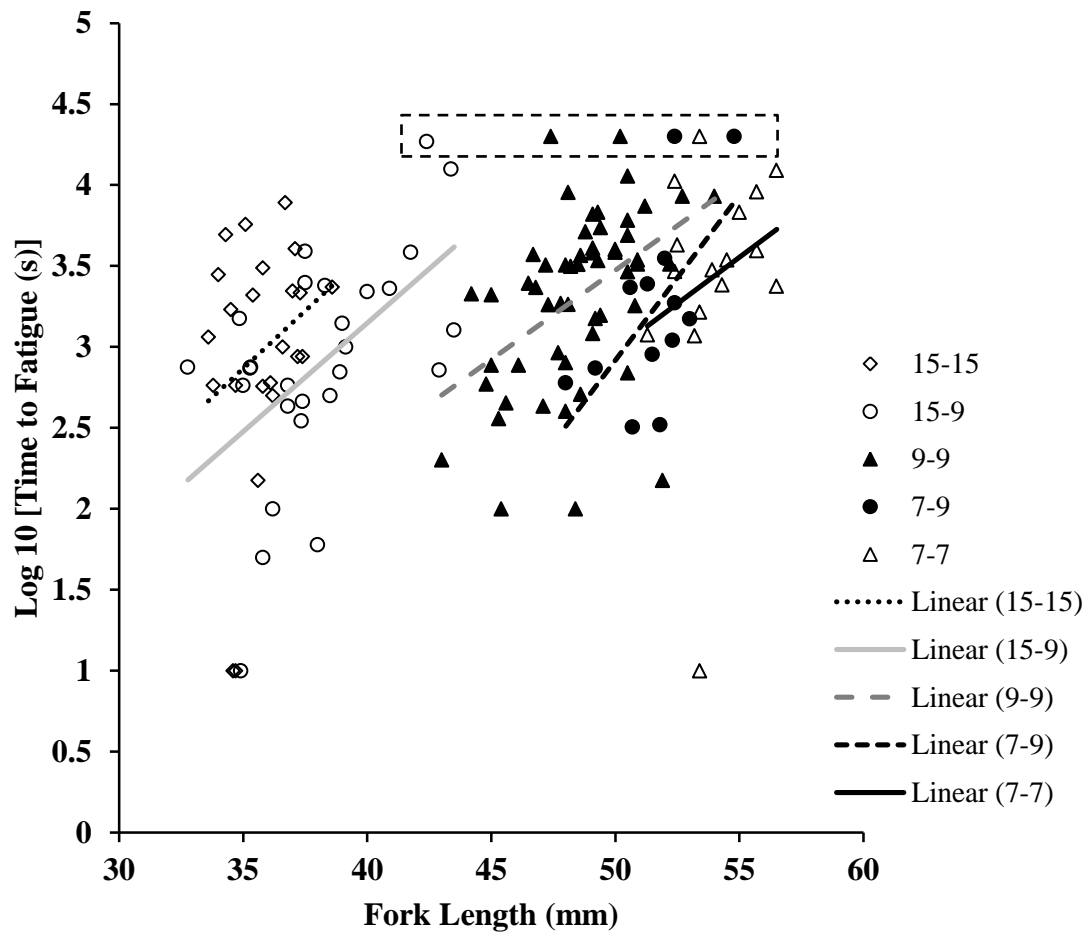
Fig. 8. Swim performance of Chinook salmon from different temperature treatments at 300 ATU post emergence in 2011. Diamonds: 15-15 ℃, Open circles: 15-9 ℃, Closed triangle: 10-9 ℃, closed circle: 7-9 ℃, open triangle: 7 ℃. Regression lines were plotted according to each temperature treatment (Black dotted: 15-15 ℃, Grey solid: 15-9 ℃, grey dashed: 9-9 ℃, black dashed: 7-9 ℃, black solid: 7-7 ℃). Individuals that did not fatigue are shown the box with dashed outline. Sample size for each treatment is shown in **Table 3**.

the fibre area of individuals (**Table 5**) and there was no significant difference in the fork length between tested and non-tested groups in any treatment.

In 2012 swim performance tests, the effect of fork length on time to fatigue did not differ among treatments (ANCOVA, TRT*fork length: $F_{3, 135} = 1.48$, $p = 0.221$). Overall, the fork length had a significant effect on time to fatigue ($F_{1, 130} = 37.61$, $p < 0.001$, $\eta^2_p = 0.54$) and time to fatigue differed significantly among treatments ($F_{3, 128} = 37.21$, $p < 0.001$, $\eta^2_p = 0.50$, **Fig 9**). In particular, fish in the 15-9 °C treatment swam for significantly longer durations (ls-mean = 0.623 ± 0.049 SE) than those in the 7-9 °C treatment (ls-mean = 0.435 ± 0.048 SE; post hoc Tukey test, $p = 0.020$) and the 7-7 °C treatment (ls-mean = 0.325 ± 0.051 SE; post hoc Tukey test, $p < 0.001$). The swim performances of the 9-9 °C treatment individuals were not significantly different from fish in other treatments.

Muscle Cellularity and Swim Performance

In 2012, the effect of the fibre number on time to fatigue did not depend on the treatments (ANCOVA, fibre number*TRT: $F_{3, 43} = 3.12$, $p = 0.805$). The effect of fork length on time to fatigue also did not differ among treatments (fork length*TRT: $F_{3, 43} = 2.52$, $p = 0.070$). In this analysis of fewer individuals (**Table 3**), fork length did not have an overall effect on time to fatigue ($F_{1, 49} = 2.00$, $p = 0.163$) nor did the treatment ($F_{3, 49} = 1.67$, $p = 0.186$). On the other hand, the effect of fibre area on time to fatigue differed among treatments (fibre area*TRT: $F_{3, 43} = 2.82$, $\eta^2_p = 0.72$, $p = 0.050$). However, there was no significant effect of fibre area on time to fatigue within each treatment (**Table 6**).

Table 5. A summary of ANCOVAs performed to compare the fork lengths and muscle cellularity between tested and non-tested individuals in 2011. There was no significant difference in muscle cellularity or fork length between the two groups.

Temperature Treatment (°C)	Comparison parameters between tested vs. non-tested individuals		
	Fork Length	Mean Fibre Area	Total Number of Fibres
15-15	$F_{1,31}=1.98, p=0.169$	$F_{1,31}=1.87, p=0.181$	$F_{1,31}=1.01, p=0.323$
15-9	$F_{1,37}=0.00, p=0.945$	$F_{1,37}=1.98, p=0.168$	$F_{1,36}=0.32, p=0.571$
9-9	$F_{1,100}=0.00, p=0.979$	$F_{1,100}=0.94, p=0.334$	$F_{1,100}=0.51, p=0.476$
7-9	$F_{1,22}=0.64, p=0.431$	$F_{1,22}=0.17, p=0.688$	$F_{1,22}=0.86, p=0.856$
7-7	$F_{1,31}=0.64, p=0.431$	$F_{1,31}=0.39, p=0.534$	$F_{1,31}=0.50, p=0.484$
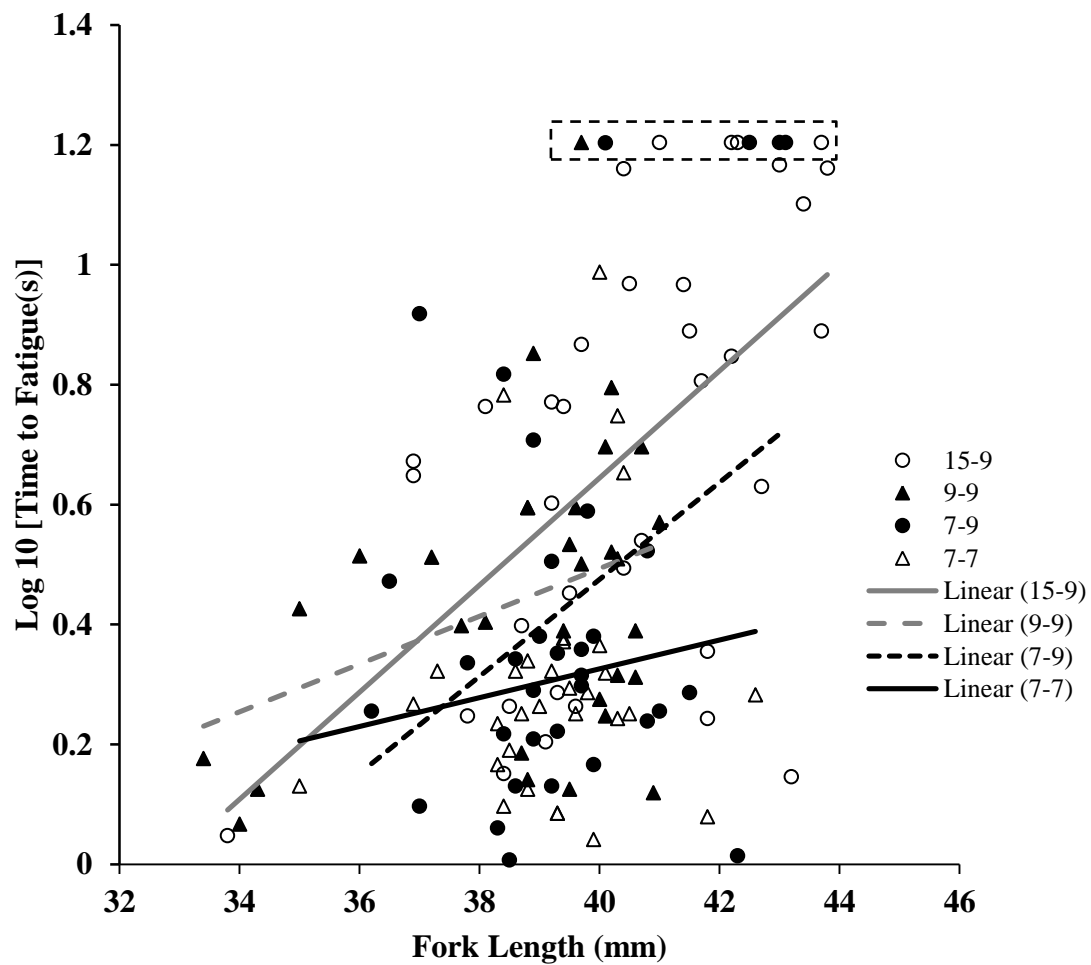
Fig. 9. Incubation temperature effects on the swim performance of Chinook salmon. The 15-9 ℃ fish had a significantly longer time to fatigue than the 7-9 ℃ and 7-7 ℃ fish. Open circles: 15-9 ℃, Closed triangle: 9-9 ℃, closed circle: 7-9 ℃, open triangle: 7-7 ℃. Regression lines were plotted according to incubation temperature, and are shown for illustrative purposes only. Individuals that did not fatigue are shown the box with dashed outline. Sample sizes for each treatment are shown in **Table 3**.

Table 6. A summary of ANCOVAs performed in each treatment to compare the effect of mean fibre area on swim performance of juvenile Chinook salmon sampled in 2012.

Temperature Treatment (°C)	Effects of Mean Fibre Area to Time to Fatigue	
	F	p
15-9	$F_{1,8} = 1.56$	0.091
9-9	$F_{1,14} = 1.34$	0.607
7-9	$F_{1,11} = 4.61$	0.055
7-7	$F_{1,10} = 1.44$	0.257

Parental Effects

At emergence, 14 to 76% of the variation in fork lengths was attributable to maternal effects in 2011 and 2012. The maternal effect at emergence was more apparent in all treatments than the maternal effect at termination. Generally, the maternal effect on fork length was higher than the paternal effect in all treatments except for the 15 ℃ treatment in 2011 (**Table 7**).

Although maternal effects on fork length were seen in 15-15, 15-9, and 9-9 treatments at termination in in 2011, this trend was not seen at termination in 2012. Parental identities also affected the fibre number, the fibre area, and time to fatigue in some treatments; however, the effects were not consistent. Since there were signs of parental effects on fork length, muscle cellularity, and swim performance of juveniles, GLMMs were run on the 2012 data.

The GLMM results were consistent with the results in the ANCOVA (**Table 8**). Both tests showed that time to fatigue increased with warmer incubation temperature, although the GLMM analysis showed the times to fatigue in all treatments were significantly different from each other.

Table 7. Proportions of the variances in fork length, total fibre number, mean fibre area, and time to fatigue contributed by sire and dam. Proportions that are >10% are in bold.

Sampling Year	Sampling Point	Temperature Treatment (°C)	Fork Length		Total Fibre Number		Mean Fibre Area		Time to Fatigue	
			Dam	Sire	Dam	Sire	Dam	Sire	Dam	Sire
2011	Emergence	15	**0.144**	**0.535**	0.036	<0.001	<0.001	<0.001	NA	NA
		9	**0.221**	<0.001	**0.111**	**0.352**	<0.001	<0.001	NA	NA
		7	**0.720**	<0.001	**0.350**	<0.001	<0.001	<0.001	NA	NA
	Termination	15-15	**0.550**	<0.001	**0.266**	<0.001	**0.502**	<0.001	**0.141**	0.013
		15-9	**0.171**	<0.001	0.055	<0.001	<0.001	<0.001	<0.001	<0.001
		9-9	**0.306**	<0.001	**0.108**	<0.001	<0.001	<0.001	**0.401**	<0.001
		7-9	0.013	0.013	<0.001	<0.001	0.011	0.011	0.026	0.026
		7-7	<0.001	<0.001	0.028	<0.001	**0.107**	0.038	<0.001	0.089
2012	Emergence	15	**0.345**	<0.001	NA	NA	NA	NA	NA	NA
		9	**0.757**	<0.001	NA	NA	NA	NA	NA	NA
		7	**0.660**	<0.001	NA	NA	NA	NA	NA	NA
	Termination	15-9	<0.001	<0.001	**0.568**	**0.171**	<0.001	**0.137**	<0.001	<0.001
		9-9	<0.001	0.056	<0.001	**0.774**	<0.001	**0.378**	**0.189**	<0.001
		7-9	<0.001	**0.443**	<0.001	<0.001	<0.001	<0.001	<0.001	**0.336**
		7-9	<0.001	0.227	<0.001	0.004	<0.001	<0.001	<0.001	<0.001

NA: Not applicable; no swim test performed at emergence. In addition, muscle cellularity analysis in 2012 emergence was not done due to variations in fork lengths among treatments.

Table 8. Comparison of the results from ANCOVAs and GLMMs on data from the 2012 experiment at termination. Parental effects were accounted as nuisance parameters in GLMM.

Measured Parameters	Analysis Type	Differences among treatments	Relevant Fig.
Total Fibre Number	ANCOVA	NS	Fig. 7 A
	GLMM	NS	Fig. 7 A
Mean Fibre Area	ANCOVA	SD (15-9 > 7-7)	Fig. 7 B
	GLMM	SD (15-9 > 7-7)	Fig. 7 B
Time to Fatigue	ANCOVA	SD (15-9 > 7-9; 15-9 > 7-7)	Fig. 9
	GLMM	SD (All treatments)	Fig. 9

SD: Significant difference between or among treatments, NS: no significant difference observed.

Discussion

Water temperature showed a positive effect on the swim performance of the juvenile Chinook salmon. Fish incubated at 15 ℃ and swum at 9 ℃ showed a significantly higher swim performance than the fish incubated and swum at 7 ℃ (**Fig. 9**). These findings are consistent with the results of previous studies (Hammer, 1995; Lee et al., 2003), in which swim performance increased with water temperature up to 15 ℃ over the temperature range of 5 to 20 ℃ in sockeye salmon. This is because the amount of metabolic fuels required for fast contraction of muscle fibres, such as glycogen and ATP are more abundant at a higher temperature, increasing fibre contraction velocity (Johnston and Lucking, 1978; Larsen et al., 2001). In addition, increased water temperature increases the cardiac output levels, increasing the oxygen uptake and swimming velocity of fish (Klaiman et al., 2011; Woytanowski and Coughlin, 2013).

Incubation temperature especially plays a pivotal role in determining the swim performance of juvenile Chinook salmon. My results show that the swim performance of the juvenile salmon increases with the temperature experienced early in life. In the 2012 swim performance tests, the fish incubated at 15 ℃ swam for longer than the fish incubated at 7 ℃, even after they were acclimated and tested at a common temperature (9 ℃) after emergence (**Fig. 9**). This shows that warmer incubation temperature results in the higher swim performance of the juveniles. A similar trend is seen in the 2011 swim test results, though statistical comparison was not possible due to non-overlapping body sizes (**Fig. 8**). In the 2011 swim test, the time to fatigue values of the fish incubated at 15 ℃ seemed to be higher than the fish incubated at 9 ℃ and 7 ℃ when they were all

acclimated and tested at 9 ℃, because the elevation of the regression line of the 15-9 ℃ treatment was higher than the 9-9 ℃ and 7-9 ℃ treatments.

The observed effect of incubation temperature on swim performance of the juveniles was consistent with previous studies on other species. Atlantic herring (*Clupea harengus*) incubated at 12 ℃ showed faster burst swimming speed compared to those incubated at 5 ℃, when the fish were acclimated and tested at a common ambient temperature (~8 ℃)(Johnston et al., 2001). Similarly, Sfakianakis et al. (2011) found that zebrafish embryos incubated at a high temperature (31 ℃) showed higher swim performance (U_{crit}) than those incubated at 22 ℃ when acclimated and tested at a common temperature (26.5 ℃). In another study, zebrafish incubated at 32 ℃ showed higher swim performance compared to the fish incubated at 27 ℃ when the fish were acclimated and tested at 34℃ (Scott and Johnston. 2012). In addition to their finding, fish incubated at 22 ℃ also showed higher swim performance than the 27 ℃-incubated fish, indicating incubation of fish at both high and low temperature extremities for normal growth could lead to increased swim performance in adult zebrafish. The authors found that higher swim performance at higher incubation temperature was associated with an increase in fibre area and number, showing a relationship between muscle cellularity and swim performance of the zebrafish.

However, there are also studies that found an opposite effect of temperature on swim performance, perhaps due to a different experimental design. Scup (*Stenotomus chrysops*), European sea bass (*Dicentrarchus labrax*), and rainbow smelt (*Osmerus mordax*) acclimated at a cold temperature showed higher swim performance compared to

the control group when they were tested at a common temperature (Swank and Rome, 2001; Rome, 2007; Koumoundouros et al., 2009; Woytanowski and Coughlin, 2013). My study investigated the effect of incubation temperature (temperature experienced from fertilization to emergence) on swim performance of the juveniles. This is different from the majority of the previous studies with opposing results, which focused on the effect of temperature on swim performance of more developed fish (i.e., fish at post emergence). Therefore, fish at different developmental stages may have different responses to temperature. Fish during their early development undergo rapid somatic growth (especially during embryonic stage, where growth rate is 30 to 50% faster than the growth in juvenile stage [Rombough, 2011]). Developing fish also show higher expression of transcription factors responsible for muscle formation (Xie et al., 2001), higher synthesis rates of muscle protein, and higher levels of stress hormones, such as cortisol, than juveniles or adults (de Jesus and Hirano, 1992; Rombough, 2011). Such differences in growth rate, gene expression, and hormone levels between fish during early development and those at later developmental stages may explain for the different effects of water temperature on swim performance. In fact, all previous studies that showed consistent results with my study examined the effect of incubation temperature during the early development of fish. Although the opposing effect of temperature on swim performance was found in many previous studies, they also suggest that the temperature modulation of swim performance is likely due to the alteration of muscle cellularity (Johnston et al., 2001; Batty and Blaxter, 1992; Koumoundouros et al., 2009; Burt et al., 2012).

Despite the claimed effects of muscle cellularity (i.e., fibre size and fibre number per cross-sectional area) on swim performance in previous studies (Johnston et al., 2001; Koumoundouros et al., 2009; Scott and Johnston, 2012), my results show that fibre number did not correlate with swim performance in juvenile Chinook salmon after controlling for the effects of body size (**Fig. 7 A**). Although fish incubated at 7 ℃ (7-7℃) had larger fibre areas than the fish incubated at 15 ℃ and transferred to 9 ℃ (15-9 ℃) (**Fig. 7 B**), the larger fibre (thus more cross bridge formation) did not seem to contribute to swim performance as the time to fatigue of the 7-7 ℃ treatment fish were the lowest. Possibly, the variation in swim performance among the treatments was due to the temperature modulations of other physiological parameters. Glycogen, ATP, and phosphocreatine (PCr) are the main metabolic fuels for the contraction of white muscle fibres in salmonids and their availabilities in muscle fibres can become limiting factors in the swim performance of salmon (Milligan, 1996; McDonald et al., 1998). The influence of temperature on the availability of muscle glycogen in salmonids is currently not well known. On the other hand, liver glycogen seems to be influenced by temperature in coho salmon (*Oncorhynchus kisutch* [Larsen et al., 2001]). Fish acclimated at 2.5 ℃ had 40% less glycogen in their liver when compared to the fish acclimated at 10 ℃. Future studies should investigate the effect of temperature on muscle glycogen levels of juvenile salmon as they can be the limiting factor of the swim performance. The ATP levels in muscle fibres may not explain the differences in the swim performance, because most previous studies show that cold-acclimation of fish actually increases the ATP synthase activity in both white and red muscles (thus, increased levels of ATP in muscles), which in turn

increases their contractile velocities (Guderley and Blier, 1988; Johnson and Bennett, 1995; Woytanowski and Coughlin, 2013).

A major determinant of swim performance is the body size of fish (McDonald et al., 1998, 2007; Burt et al., 2012). As expected, a positive linear relationship between the body size and the swim performance is confirmed in my results (**Figs 8 and 9**) even though there was a large variation in the time to fatigue values unexplained by body size within each treatment (the variation ranging from 60 to 70%). The variation in the time to fatigue at individual treatment level is likely caused by the differences in swim behaviour (i.e., propensity to swim [McFarlane and McDonald, 2002]). Since the body size was experimentally and statistically controlled, the differences in the swim performance among treatments were not caused by the variation in body size.

Body size not only influences the swim performance, but it also shows a strong positive relationship with muscle cellularity: higher cross-sectional fibre number and larger mean fibre area are seen in larger fish (**Fig. 5 and 6**). Since fish incubated at lower temperatures were larger individuals, it is perhaps obvious to see more and larger muscle fibres from these fish compared to the fish at incubated higher temperatures (e.g., 7-7 ℃ treatment in the 2011 experiment). The differences in muscle cellularity among treatments were much reduced when fish were compared at a common body size (**Fig. 7**) in 2012. However, larger mean fibre area was still seen in the 7-7 ℃ treatment fish compared to the 15-9 ℃ fish (**Fig. 7 B**). This may suggest that fish incubated at a lower temperature (i.e., 7-7 ℃ treatment) show higher intensity of muscle hypertrophy than the fish incubated at warmer temperatures. A gradually decreasing pattern in mean fibre area with increasing temperature (**Fig. 7 B**) also show that incubation temperature may

influence the hypertrophic growth of muscle fibres in Chinook salmon. Larger mean fibre area at low incubation temperatures was observed in previous studies, although they also observed differences in cross-sectional fibre numbers (Johnston and McLay, 1997; Albokhadaim et al., 2007; Steinbacher et al., 2011). I believe the insignificant difference in fibre areas among fish transferred and acclimated at 9 ℃ (i.e., 15-9 ℃, 9-9 ℃, and 7-9 ℃) was due to low sample size. With higher sample size, I expect to see a significant difference in fibre areas among these treatments as well. The standardization of the body size shows the differences in cross-sectional fibre numbers among treatments are largely due to the different body size determined by temperature. Therefore, my results show that incubation temperature influences the proliferation and growth of muscle fibres (**Fig. 5**), which in turn, would result in different muscle cellularity and body sizes at a similar developmental age (e.g., 300 ATU) as shown in **Fig. 6**. However, the numbers of the muscle fibres remain consistent when fish are examined at a similar body size regardless of the differences in incubation temperature.

Many previous studies did not experimentally control for body size (i.e., sampling of fish at a standardized body size) while examining muscle cellularity, and this likely led them to different results from my study as they observed the effect of temperature on fibre numbers (**Table 1**). The consequences of not controlling for body size can also be seen in the results of my study. When body size was not considered as a covariate in the 2011 muscle cellularity analysis (**Fig. 5**), larger fibres and more number of fibres in fish incubated at lower water temperature was seen, which was consistent with previous studies (Johnston and McLay, 1997; Albokhadaim et al., 2007; Macqueen et al., 2008). However, the results are much different when the fork length was both

experimentally and statistically controlled, as no difference in fibre number was observed, and the differences in fibre areas were much reduced among treatments (**Fig. 7**). This shows the importance of experimentally controlling for body size when comparing muscle cellularity and any variable influenced by body size.

It is also important to control for the developmental age of fish when examining the effect of temperature, because temperature alters the developmental rate of fish. The controlling of the developmental age can be done by examining fish at a constant developmental stage which can be easily defined (e.g., hatching, emergence, and ATU). Some previous studies used different timing of sampling (i.e., certain days after emergence instead of using degree days or ATU) for the comparison of muscle cellularity that might have led them an alternative interpretation of their results (Nathanallides et al., 1995; Albokhadaim et al., 2007). Incubation period until hatching and emergence of fish at 7 ℃ was twice as long as the incubation period of fish at 15 ℃. Therefore, sampling fish at 3 weeks post emergence instead of a certain ATU post emergence results in different muscle cellularity and body size among treatments due to the differences in the amount of thermal units received (Albokhadaim et al., 2007). In my study, when the fish were tested for their swim performance in 2012, individuals at different treatments were not at the same developmental age. Thus the differences in the developmental ages might be responsible for the differences seen in swim performance among the treatments. However, it is impossible to control for both developmental age and body size simultaneously in an experiment.

The rapid development of fish at a high incubation temperature results in an earlier emergence than the fish incubated at a lower temperature. The earlier emergence

of fish offer a longer free swimming duration from emergence to termination than the late-emerging fish at a lower temperature. In the 2012 experiment, fish incubated at 15 ℃ spent the longest time from emergence to termination as free swimming individuals compared to fish in other treatments (**Table 9**). Therefore, the longer free swimming duration may have provided a training effect on swim performance. However, when I compared the duration of free swimming period among treatments (days spent from post emergence to termination), free swimming duration did not increase with swim performance (**Fig. 10**). This observation suggests that any differences in free swimming durations do not affect the swim performance (i.e., no training effect).

Although the fish incubated at the highest temperature showed the best swim performance (**Fig. 9**) and the fastest development (e.g., earliest hatching and emergence time), they also showed the lowest growth efficiency (i.e., smaller fork length at emergence and termination [**Fig. 6**]) and higher mortality rate compared to the fish at the lower temperatures (in 2012, [**Table 2**]). This indicates that the optimal incubation temperature for maximum survival/growth efficiency and swim performance could be different for Chinook salmon. In previous studies, incubation temperature above 12 ℃ has been shown to decrease fertilization success, growth efficiency, and survival during the early development of salmon (Beacham and Murray, 1989; Richter and Kolmes, 2005). In my study, a higher mortality in the 15-15 treatment in the 2012 experiment was also seen (**Table 2**). Therefore, higher swim performance in the 15-9 treatment fish might be a trade-off for the reduced survival/growth efficiency at higher temperatures.

Table 9. A summary of free swimming periods for each treatment in the 2012 experiment (Fig. 9). The transfers of the fish incubated at 15 ℃ and 7 ℃ to a common temperature (9 ℃) were done at emergence.

Treatment (℃)	Free Swimming Period	Swim Performance Rank	Transfer (emergence)	Termination
15-9	33	1st	15 November, 2012	18 December, 2012
9-9	12	2nd	28 December, 2012	9 January. 2013
7-9	21	3rd	26 January, 2013	16 February, 2013
7-7	28	4th	26 January, 2013	23 February, 2013
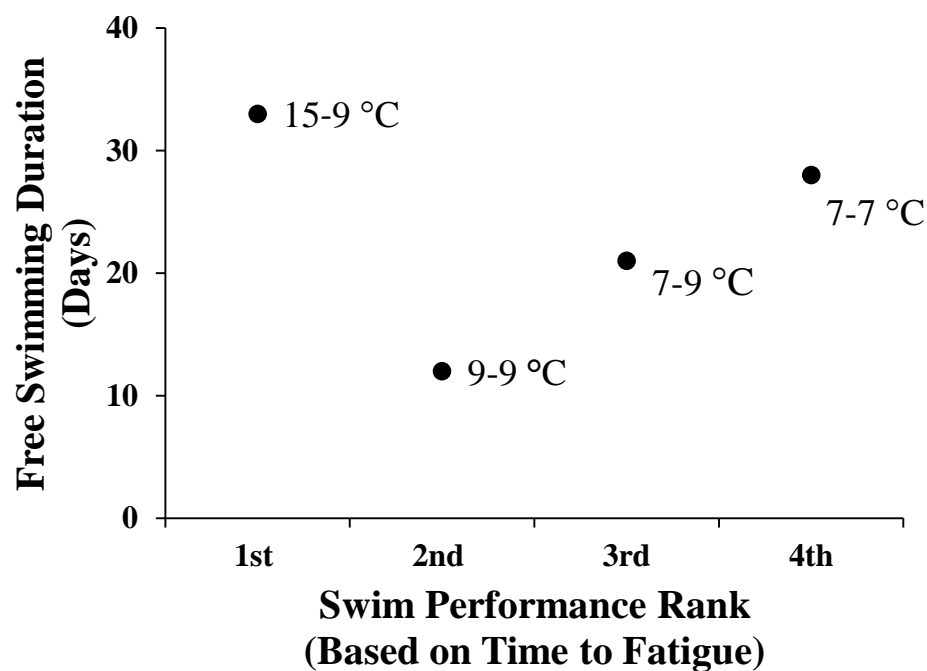
Fig. 10. The relationship between free swimming duration and swim performance rank of juvenile Chinook salmon. There was no effect of free swimming duration (thus no training effect) on swim performance. Free swimming duration represent periods that juvenile spent in fry cups from emergence to termination. Swim performance rank was based on time to fatigue (**Fig. 9**)

Trade-offs between survival/growth efficiency and swim performance were seen in previous studies. A population of Atlantic silverside (*Menidia menidia*) in Nova Scotia had higher growth than the population in South Carolina, whereas the South Carolina population showed higher burst swim performance than the Nova Scotia fish (Conover and Schultz, 1995). Burt et al. (2012) also found that juvenile sockeye families with higher swim performance had higher mortality rates, showing a trade-off between swim performance and survivorship when developing sockeye salmon were introduced to a high incubation temperature (16 ℃). Although my study did not show a trade-off between swim performance and mortality among families (**Table 7**), the results suggest a potential trade-off between swim performance and survival/growth efficiency at different incubation temperatures in individuals because fish incubated at a temperature that is not ideal for the best growth and survival (i.e., 15 ℃) were the best swimmers.

As expected, the effects of incubation temperature on muscle cellularity, body size, and swim performance persisted after the transfer at emergence (**Figs. 5 C, 5 D, 6B, and 9**). Therefore, temperature experienced during the early development is important because it can influence the survival and fitness of individuals at later life stages. Swim performance (especially burst swimming) and body size are related to predator avoidance, dominance, and survival (Taylor and McPhail, 1985; Johnsson, 1993; Berejikian et al., 1996; Lorenzen, 1996; Sogard, 1997). Some studies show that even a 2 ℃ difference in incubation temperature may alter the rate of protein synthesis and proliferation of muscle precursor cells (Johnston and Cole, 1997; Johnston, 2006), consequently influencing the proportion between white and red muscle fibres and swim performance of juvenile fish

(Koumoundouros et al., 2009). My study also shows that incubation temperature could modulate the swim performance of the juveniles.

Although parentage influenced the body size, muscle cellularity, and swim performance of juveniles, the effect of temperature on these parameters persisted after controlling for parentage (**Table 8**). In both 2011 and 2012, there seemed to be a high maternal effect on fork length at emergence (**Table 6**). This was consistent with previous studies on Chinook salmon, which showed a higher maternal effect than paternal effect on the growth of offspring until emergence (Heath and Blouw, 1998; Heath et al., 1999). Although parental effects on muscle cellularity and swim performance were observed in some treatments, the observed parental effect did not show a clear pattern. Johnston and McLay (1997) also found that muscle cellularity of Atlantic salmon alevin varied among families, but the effects of temperature on muscle cellularity was much stronger than parental effects.

Body size and swim performance are positively related with social dominance and aggressive behaviours (Johnsson, 1993; Berejikian et al., 1996; Sloman et al., 2000; Johnston et al., 2001; Harwood et al., 2002). Although the effect of temperature on growth and swim performance is seen clearly, the effect of temperature on social dominance remains unknown. Individuals with higher social dominance and aggressiveness could be at an advantage for feeding and territory acquisition, which is usually associated with survivorship and greater lifetime fitness (Quinn, 2005). So far, at least one study showed that incubation temperature could affect behavioural phenotype of juvenile salmonids. Juvenile Atlantic salmon incubated at 5 °C showed higher food-seeking behaviour than the fish incubated at 10 °C (Albokhadaim et al., 2007). Since very

little is known on the effect of incubation temperature on social dominance, such a hypothesis can be tested in future studies.

Future studies also should focus on examining the mechanism underlying the effects of incubation temperature on muscle development and swim performance in fish. Several studies show possible underlying cellular/molecular mechanisms for the temperature modulation of the muscle cellularity and swim performance. Incubation temperature tends to influence the expression levels of the transcription factors associated with muscle development, which might possibly alter muscle cellularity of individuals. Myogenic regulatory factors are a family of transcription factors that are responsible for differentiation and determination of muscle precursor cells to mature muscle fibres. Higher expression levels of the myogenic regulatory factors are therefore considered to be associated with higher levels of fibre formation and proliferation (Johnston et al., 2011). Past studies show that rainbow trout incubated at 4 °C had higher and more prolonged expression of the myogenic regulatory factors than the fish incubated at 12 °C, which also resulted in larger body size of the fish incubated at the low temperature at hatching or at emergence than the fish incubated at the high temperature (Xie et al., 2001). In addition, Atlantic salmon reared at 5 °C showed higher myogenin (one of the myogenic factors) levels with more pronounced hypertrophy and hyperplasia than the fish reared at 10 °C (Albokhadaim et al., 2007). Therefore, higher fibre counts and larger fibre area in the 7 °C-incubated Chinook salmon could be partially due to increased expression levels of the myogenic regulatory factors. However, the relationship between expression levels of myogenic regulatory factors and muscle cellularity was not investigated in Chinook salmon, and should be tested in a follow-up study. The

temperature-modulated variation in the swim performance of zebrafish tends to be associated with different expression levels of the genes involved in energy production and utilization, and blood vessel modulation (Scott and Johnston, 2012). At a higher incubation temperature (32 °C), the expression levels of these genes were significantly higher than at a low incubation temperature (22 °C). Therefore, modulation of gene expression is likely to be related to swim performance.

Conclusion

This study investigated the developmental plasticity of muscle cellularity and swim performance of juvenile Chinook salmon in response to temperature, and tested for a relationship between muscle cellularity and swim performance. The results revealed new findings that fibre number did not differ among incubation temperatures when body size was controlled while the differences in fibre areas were reduced, but still observed. This finding also led to a conclusion that the differences in swim performance of individuals at different incubation temperatures are likely determined by physiological parameters other than muscle cellularity. These findings are novel and pertinent because most previous studies neglected to experimentally control for the effect of body size on muscle cellularity and swim performance. Therefore, my study shows the importance of a study design, especially for the control of body size. I also showed the importance of incubation temperature, because it has a lasting effect on the body size by modulating muscle development, and influences swim performance of individuals.

Swim performance is an important fitness-related trait associated with predator avoidance, prey capture rate, and social dominance (Sloman et al., 2000; Johnston et al.,

2001). My results showed warm incubation temperature in Chinook salmon may have positive effects on the swim performance of the juveniles, even though negative effects were seen on the growth efficiency (e.g., body size at emergence and 300 ATU) and survival (**Table 3**) of the juveniles. This was the first study to show that warm incubation temperature does not always have a detrimental effect on developing Pacific salmon, unlike the effects seen on juvenile sockeye salmon (Burt et al., 2012). This suggests that Chinook salmon may be less vulnerable to warm incubation temperature than sockeye salmon, and that the response to developmental temperature may vary among Pacific salmon species.

Despite the finding of the effects of incubation temperature on swimming performance and growth (via the variation muscle growth), the differences in swim performance could not be explained by the differences in muscle cellularity and the underlying physiological mechanism at the cellular level still remains unknown. Therefore, the follow up study should be integrative research, which investigates the effect of incubation temperature from the molecular and cellular level, physiological level, and ecological and evolutionary level. Expression levels, timing, and duration of the myogenic regulatory factors can be examined at the molecular and cellular aspect of the proposed future study. At the physiological level, the availability and abundance of the endogenous fuels, metabolites, and the associated enzymes (e.g., glycogen, ATP, ATPase) can be examined. Lastly, at the ecological and evolutionary level, the effect of incubation temperature on social dominance can be examined while controlling for the parentage. Studies that investigate the integrative effect of incubation temperature on Chinook salmon will provide a better understanding of the lasting effect of temperature on the fish.

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Appendix

Letters of Approval by Animal Use Subcommittee: next 3 pages

AUP Number: 2007-043-05
PI Name: Morbey, Yolanda
AUP Title: Evolutionary Biology And Ecology Of Early Development In Salmon

Official Notice of Animal Use Subcommittee (AUS) Approval: Your new Animal Use Protocol (AUP) entitled "Evolutionary Biology And Ecology Of Early Development In Salmon

" has been APPROVED by the Animal Use Subcommittee of the University Council on Animal Care. This approval, although valid for four years, and is subject to annual Protocol Renewal.2007-043-05::5

1. This AUP number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this AUP number.
3. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Copeman, Laura
on behalf of the Animal Use Subcommittee
University Council on Animal Care

Western 🛡️

2007-043-05::5:

AUP Number: 2007-043-05
AUP Title: Evolutionary Biology and Ecology of Early Development in Salmon

Approval Date: 07/28/2011

The YEARLY RENEWAL to Animal Use Protocol (AUP) 2007-043-05 has been approved.

1. This AUP number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this AUP number.
3. Purchases of animals other than through this system must be cleared through the ACVS office.
   Health certificates will be required.

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Kinchlea, Will D
on behalf of the Animal Use Subcommittee

Western

2007-043-05::6:

AUP Number: 2007-043-05
AUP Title: Evolutionary Biology and Ecology of Early Development in Salmon

Yearly Renewal Date: 08/01/2013

The YEARLY RENEWAL to Animal Use Protocol (AUP) 2007-043-05 has been approved, and will be approved for one year following the above review date.

1. This AUP number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this AUP number.
3. Purchases of animals other than through this system must be cleared through the ACVS office.
   Health certificates will be required.

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Kinchlea, Will D
on behalf of the Animal Use Subcommittee

Vita

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Scholarly Activity:

Presentations   Lim, D.D., Morbey, Y.E., and Milligan, C.L. (2013). "*Effects of rearing temperature on muscle cellularity and swim performance of developing Chinook salmon.*" Oral Presentation at the Canadian Society of Zoologists annual meeting, May 15th, 2013, University of Guelph, Guelph, Ontario.

                  Lim, D.D., Morbey, Y.E., and Milligan, C.L. (2012). "*Developmental plasticity of muscle cellularity and swim performance of juvenile Chinook salmon in response to temperature.*" Poster presented at the Biology Graduate Research Forum, Oct 13th, 2012, University of Western Ontario, London, Ontario.