PHYSIOLOGICAL DIFFERENCES BETWEEN OFFSPRING OF LONG AND SHORT MIGRATORY BROWN TROUT (SALMO TRUTTA)

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Abstract

It has been shown in various species of migratory salmonid fish that differences in migration distances can be related to several physiological and morphological traits, such as body size, aerobic scope, relative ventricular mass and gonad size. Some of these traits, e.g. fin sizes and body shape, have also been shown to be heritable. In this study we examined physiological and morphological parameters in juvenile brown trout (Salmo trutta) with different migration distances. In the first part of the study, fish was brought into the laboratory from two catchment areas in Norumsån in western Sweden, at 0.9 and 6.8 km from the river outlet, respectively. In these fish, we measured metabolic rates, using intermittent flow respirometry, blood hematocrit and hemoglobin, and relative mass of ventricle and spleen. In the second part, conducted in the field on wild fish, caught at the same locations, we measured the same blood parameters but also body size, plasma cortisol and glucose. The results from the first part showed no differences. However, results from the second part showed that the offspring of the long-migrating fish had higher plasma glucose concentration and were generally larger than their short-migrating counterparts. Since glucose is partially consumed by muscle tissue this could mean that the long-migrating fish have more glucose for their muscles during their, in comparison, longer migration. Since glucose is a parameter that is affected by a lot of factors it is difficult to pinpoint what the underlying causes of this difference could be. Suggestions include differences in development, stress response or population densities. The difference in size however is most likely explained by a potential difference in population density.

Keywords: Migration, metabolic rate, glucose, size, brown trout, offspring.

Det har visats i flera olika arter av laxartade fiskar att en skillnad i migrationssträckan kan ha en inverkan på flera olika fysiologiska och morfologiska faktorer som exempelvis kroppsstorlek, aerobic scope, relativ hjärtmassa och gonadstorlek. Vissa parametrar, som exempelvis storlek på fenor och kroppsform, som andra studier har undersökt har även visats vara äftliga. I den här studien undersökte vi fysiologiska och morfologiska parametrar i juvenil Öring (Salmo trutta) med olika migrationssträckor. I den första delen av studien hämtades fisk in till laboratoriet från två fångstlokaler i Norumsån i västra Sverige med sträckor på 0,9 och 6,8 km från utflödet. I dessa fiskar mätte vi metabolism, via intermittenter flödes respirometri, blod hematokrit och hemoglobin, relativ ventrikelmassa och mjältmassa. I den andra delen, som utfördes i fält på vida fiskar vid samma lokaler testade vi samma blodparametrar men också kroppsstorlek, plasmakortisol och -glukos. Resultatet från den första delen visade inga skillnader men den andra delen visade att avkomman från de långmigrerande fiskarna hade högre koncentrationer av plasmaglukos och var generellt större än deras kortvandrande motparter. Eftersom glukos konsumeras av muskelvävnad kan denna skillnaden betyda att de långvandrande fiskarna har mer glukos att förse deras muskler med under deras, i jämförelse, längre migrationssträckan. Eftersom glukos är en parameter som påverkas av många olika faktorer är det svårt att precisera exakt vad de underliggande orsakerna kan vara. Föreslag inkluderar en skillnad i utveckling, stressrespons eller skillnader i tätthet. Skillnaden i storlek däremot, förklaras mest troligt av en potentiell skillnad i populationstäthet.

Nyckelord: Migration, metabolism, glukos, storlek, öring, avkomma
Introduction
Salmonid migration
Migration is a common phenomenon that can be found in most, if not all, taxa including mammals, birds, insects and fish. Dingle and Drake (2007) suggests that migration can describe a couple of different notions, but one common denominator is the movement of animals on a larger scale, and the underlying cause is the need for resources. Yet there are several different ways one can categorize migration. It can be looked at from both an individual point of view or from the view of entire populations. Some species migrate as a response to a degraded quality of their current location, while other species does it regularly. Certain species migrate back and forth several times during their lifespan while others migrate only once (Dingle & Drake, 2007).

Some fish has to deal with an extra layer of environmental constraints when it comes to migration; their use of salt water versus freshwater. When fish utilize both these environments they are commonly defined as “diadromous” fish. Diadromous fish can be further divided up into catadromous fish (fish that starts out in marine environments, migrates to freshwater to grow and back to sea to spawn) and anadromous fish (fish that are born in freshwater, migrates to sea to grow and finally return to freshwater to spawn) (Gross, 1987).

The brown trout (Salmo trutta) can be an example of an anadromous species, if the circumstances are right. The brown trout has three options for migration. They can either be resident and not migrate at all or they can migrate to a nearby lake (potamodromous) or the ocean (Jonsson & Jonsson, 2011). The brown trout displays a plasticity in regards to these strategies proven by the fact that anadromous brown trout have the ability to produce non-anadromous offspring and vice-versa (Rounsefell, 1958) and what determines this is partly due to genetics and partly due to ecological factors (Jonsson & Jonsson, 1993).

Anadromous brown trout have a major obstacle, namely the change from freshwater to saltwater. In order to overcome this challenge, they go through the so called parr-smolt transformation or smoltification. Smoltification occurs within several anadromous salmonids and includes morphological, physiological as well as behavioral changes. Behavior-wise the fish becomes less territorial, less hostile towards each other and instead develops a preference for shoaling and saltwater (Folmar & Dickhoff, 1980; Iwata, 1995). Since a life in open sea is quite different from a shallow river, the physiological and morphological changes that occur during smoltification are many. The fish alters in color, the fins become brighter, its body gets a silvery shine and the dark spots along the lateral side of the fish disappear. The shape of the fish’s body changes as well. A combination off mass loss per unit of length and a sharper snout, gives the fish a thinner and more streamlined appearance (Jonsson & Jonsson, 2011). But most importantly, large changes in ion- and osmo regulation occurs to make the fish tolerant to saltwater, a must for living in the ocean (Hoar, 1988; McCormick et al., 1998).

Long and short migrating fish
One might expect to see a physiological difference between fish with different migration distances. Long-migrating fish might differ in ways that makes it easier for them to migrate further upstream. These distances inland can vary, with some studies finding migrating fish at distances between 40 meters to circa 68km from the outlet of the river and the limiting factor of migration seems to be the altitude rather than the distance (Bohlin et al., 2001).

There are studies that have found physiological differences between fish with different migration distances. An example of this is the sockeye salmon (Oncorhynchus nerka). In a river system in Canada, it was found that that the distance from the river outlet to the spawn site of the fish was correlated with a range of physiological factors including relative ventricular mass (RVM) and aerobic scope (AS), which both increased with migration distance (Eliason et al., 2011).
Morphological differences have been shown in the coho salmon (*Oncorhynchus kisutch*). A comparison between fish residing closer to the coast and further up in the same river system, revealed differences in general body-shape and fin sizes. Furthermore, the same study showed that these differences were inherited by the offspring when raised under equal environments in a laboratory setting (Taylor & McPhail, 1985), and therefore not necessarily a result of differences in development or life-history events.

When it comes to the brown trout it has been shown that several morphological and physiological aspects differ with an increasing migratory distance. Brown trout with longer migratory distances and a higher altitude of their spawning grounds are generally larger and reaches sexual maturity at an older age (L’Abée-Lund, 1991). Furthermore, with a longer migratory distance brown trout loses mass per unit length, the gonad-size of males are reduced and females produce a higher amount of eggs (Jonsson & Jonsson, 2006).

Judging from these studies, differences in migration distance can have a clear impact on several aspects of salmonid fish.

To determine AS, two parameters are needed. By subtracting the standard metabolic rate (SMR) from the maximum metabolic rate (MMR) one gets an estimation of AS. These two are commonly determined using oxygen consumption at a specific temperature (Clark et al., 2013). In the case of SMR the fish needs to be resting with no energy being allocated to any basic functions (e.g. swimming or digestion) (Chabot et al., 2016; Hulbert & Else, 2004). MMR can be considered the opposite since one must first exercise the fish until fatigued and then measure its oxygen consumption (Norin & Clark, 2016).

Measuring metabolic rates is of high interest because there is an evolutionary and ecological relevance of metabolic rates. Studies have shown that a lower metabolic rate is beneficial when food is scarce or when residing in stressful habitats (Harshman et al., 1999; Mueller & Diamond, 2001). As regards behavior, high SMR populations showed more aggression than populations with a lower estimation of SMR (Lahti et al., 2002). Similarly, atlantic salmon fry dominant to other individuals had higher SMR in comparison to the subordinate individual (Metcalfe et al., 1995). Similar results have been shown in the masu salmon (*Oncorhynchus masou*) (Yamamoto et al., 1998). Metabolic rate is associated to the growth of the fish suggested by correlations between the two found in the masu salmon (Yamamoto et al., 1998). The relationship is unclear, however, since other studies found contradicting correlations (Álvarez & Nicieza, 2005)

Even though metabolic rate is a reliable parameter to measure due to its repeatability being high (Nespolo & Franco, 2007) some external factors can still have an effect on expression of metabolic rates in some animals. Such as challenges to the immune system (Ots et al., 2001) or stress caused by social interactions (Sloman et al., 2000).

Measuring the amount of cortisol and glucose could give information about stress and the response to this stress as both these parameters are used when determining how fish perceive and handle stress (Iwama, 1998). Moreover, cortisol also plays a part in the homing behavior of other salmonid fish (Carruth et al., 2002). If looking at glucose alone there are several factors that could affect this variable. Relevant for this study would be the shifting levels throughout the smoltification (Sweeting et al., 1985) and the role glucose has in supplying different parts of the fish with energy (Blasco et al., 2001; West et al., 1993)

**Present study and aim**

Although the migration habits of the brown trout are well studied, there are fewer studies examining differences between long- and short-migrating individuals. Following that, there is even fewer studies looking at differences in their offspring. Therefore, in this study we aim to continue and expand the investigations of differences between long and short-migrating brown trout (Jonsson & Jonsson, 2006; L’Abée-Lund, 1991). We will do this by examining
parameters where others have found differences in other salmonid fish such as metabolism, tissues (Eliason et al., 2011) and body size (L’Abée-Lund, 1991) but expand by also looking at blood parameters such as hemoglobin (Hb), hematocrit (HTC), plasma cortisol and glucose, which to our knowledge has not been examined in this context before. We will examine juvenile offspring from the two migratory types to see if there is any heritability in these parameters. This study consists of two parts. In the first part fish was brought into the laboratory and tested while the second part took place at the catchment areas.

Our hypothesis is that the result will follow along the lines of previous studies with further migrants having higher AS and higher RVM. As for the rest of the parameters, predictions are hard to make since they have not, to our knowledge, been examined in the context of long versus short-migrating fish.

Materials and methods

Catch area and test fish

At two separate occasions during September of 2018, juvenile brown trout was caught via standardized electro fishing (Smith–Root LR20B, Vancouver, Washington, USA) from the river Norumsån (WGS84 decimal [lat,long]: 58.04318°N, 11.84589°E) in western Sweden, that flows out into Skagerrak (see figure 1). By using this river the risk of catching non-anadromous trout was very low since the most abundant fish species in Norumsån is known to be anadromous brown trout (Sundstrom et al., 2013). The fish were caught at two locations with distances of 900 meters and 6,8 kilometers from the river outlet and an elevation difference of approximately 34 meters. After the first occasion a decision was made to not catch small fish, roughly <10 cm in length, with the reasoning of not being able to PIT-tag them properly without injuring or killing them. Using aerated tanks, the fish were transported to the university of Gothenburg and put in aerated 10ºC aquaria with a water flow through system, gravel as bottom substrate and plastic objects as enrichment. The sides of the aquaria were covered with black plastic as not to stress the fish when working in the room. All fish were assigned to ethical ID 001548. There were technical difficulties with the lighting arrangements in the room, as the main lights did not turn completely off during nighttime. This was first discovered when the first four metabolic measurements were made, after which the lights were programmed to mimic a natural day-night cycle (12D:12L). The data from these four measurements did not seem to differ from the rest and were therefore not excluded in the analysis. The fish were fed daily with a diet of red mosquito larvae. After getting at least 48 hours to acclimatize to the laboratory environment the fish were sedated by putting them in a bucket of water with 0,5ml of 2-phenoxyethanol per liter of water. Once they were sedated their weight and length (forklength) was determined and a 12mm PIT-tag (HDX ISO 11784/11785, Oregon RFID, Portland, OR) was inserted into the abdomen of the fish via a small incision behind the left pectoral fin. The fish were then put into a well aerated aquarium to recover. These fish were used in behavioral studies during which their adipose fin partially clipped. After the behavioral studies were completed the fish were divided up into seven new aquaria with approximately 59 liters of water, each holding ca 12 fish. Four of these aquaria were for long-migrating fish, three for short-migrating fish.
Figure 1. Map describing the catchment area of all the fish used in the study.

**Equipment and setup**

Whole animal oxygen consumption rate (MO$_2$) was recorded using intermittent flow-through respirometry. Four cylindrical respirometers with a volume of 1.1 liters (radius = 35mm, height = 315mm) each were submerged in a large holding tank (length = 78cm, width = 78cm, height = 30cm) equipped with a continuous flow-through of aerated freshwater. Water was supplied from the same source that supplied the tanks holding the fish, ensuring no difference in water quality or temperature between the holding tanks and the measuring area. The respirometers were sealed with tight-fitting lids on either side, with two connectors for water inlets and outlets on each respective side. Tygon tubing was used for the water inlets/outlets as to minimize gas permeability of the respirometer system. A circulation pump was connected to each respirometer with the purpose of preventing stagnation of water and ensuring proper mixing of the water with its soluble gases within the respirometer. Another pump (flush-pump) was connected to all four respirometers and supplied each respirometer with aerated water from the larger holding tub. The flush pump could be remotely controlled (on/off) for all respirometers simultaneously. Each respirometer had an individual optode connected to the outlet of the closed inner water circuit of the respirometer that recorded oxygen concentration in the water. To record all the data, each optode was connected to a FireSting (PyroScience, Aachen, Germany) PowerLab (ADinstruments Pty Ltd, Sydney, Australia) setup which in turn was connected to a laptop with LabChart Pro (ADinstruments Pty Ltd, Sydney, Australia). Plastic barriers were set up between the respirometers to obscure the fish from each other, hindering them from stressing one another.

**Study protocol**

Prior to measuring, fish of equal size from both groups were selected. This was done because size is known to affect metabolism (Chabot et al., 2016). However, due to a complication caused by a fungal infection, some fish were replaced by a randomly selected fish from the
same aquarium just before measuring. Sample sizes were originally 14 and 13 short and long migrants, respectively. After measuring, four individuals were excluded from the analysis due to either being outliers in terms of size or their HTC values dropping below 20%, suggesting that the fish had sustained injuries leading to anemia (presumably from the PIT-tags) prior to measuring. This brought the final sample sizes to 10 and 13 for short and long migrants, respectively. Before and after testing, oxygen consumption was measured in the empty respirometers in order to acquire the “background” respiration, i.e. respiration caused by microbial oxygen consumption. After 72 hours fasting, four of the selected fish (two long-migrating fish and two short-migrating fish) were gently moved one by one via nets into a random respirometer. The measuring period lasted approximately between 44 and 48 hours based on the recommendations of Clark et al. (2013). During this time the flush-pump was shut off for ten minutes every tenth minute. During these ten minutes no aerated water entered the respirometers and oxygen concentration of the water within the respirometers declined due to the fish respiration. After the ten minutes the flush-pump started, perfusing the respirometer with aerated water and brought the oxygen saturation back up to ~100%. This meant that every 20 minutes one measurement of MO$_2$ per fish was recorded. This cycle was repeated for the entire measuring period. During this time, extra care was taken as to not disturb the experimental enclosure and to stress the fish. The only interaction with the setup was when emptying the respirometers of gas bubbles, which occurred once or twice during each recording session.

When the ~44-48 hours were over, the fish were gently moved from the respirometers into two chase tanks (radius = 17cm, height = 27.5cm) supplied with a flow-through of aerated freshwater. Two fish were manually chased in each tank, by two experimenters, until exhaustion for five minutes and then hastily put back into the respirometers. All fish were back in their respective respirometer within one minute of ending this “chase” protocol. Once back in the respirometers, MO$_2$ was measured for one hour. This time the flush-pump was shut off for ten minutes and then back on again for five minutes, totaling four measurements per fish. Once the hour had passed the fish was chased again according to the same chase protocol and were then hastily euthanized with a blow to the head. Once the fish were dead, blood and tissue samples were taken and the fish bodymass and length (standard) were determined.

**Metabolism**

To calculate the standard metabolic rate, the last five minutes of each MO$_2$ measurement was selected and the oxygen consumption slopes were calculated via a function in LabChart. The last five minutes were selected to exclude any potential measuring errors that are common when the flush-pump is turned off. All of these data points were transferred from LabChart to an excel sheet where they were compensated by subtracting the slopes from the background respiration for each respirometer, to ensure the data represented only the fish’s respiration. A graph was made that represented each measurement of MO$_2$ for the entire measuring period. If any data points looked unusual, the recording in LabChart was checked to see if it was due to apparent recording errors (e.g. optode malfunction) or deviant recordings related to the behavior of the fish. If an error was apparent the data point was removed. Using $\dot{M}_{O_2} = \frac{[(V_f - V_f) \times \Delta C_{wO_2}]}{(\Delta t \times M_f)}$ as described by Clark et al. (2013) the oxygen consumption was converted into mass specific oxygen consumption for the entire measuring duration. In this equation $V_f$ equals the respirometers total volume, $V_f$ equals the volume of the fish (presumed to be equal to $M_f$, the mass of the fish), $\Delta C_{wO_2}$ equals the slopes of the oxygen consumption and $\Delta t$ equals the amount of time when $\Delta C_{wO_2}$ was measured (in this case 10 minutes) (Clark et al., 2013). The lowest twentieth percentile of $\dot{M}_{O_2}$ for the entire measuring
period was calculated in SPSS to represent the standard metabolic rate, following the common practice when estimating SMR (Chabot et al., 2016).

To calculate the maximum metabolic rate, the steepest slopes from the post-chase recordings were selected (typically the first slope following the chase protocol) and transferred from LabChart into an excel sheet where they were compensated by the background respiration used for the SMR. Mass-specific oxygen consumption was then calculated using the same equation described earlier and represented MMR.

Aerobic scope was then calculated by subtracting SMR from MMR.

**Determination of blood parameters and tissue collection**

Immediately following euthanization, a blood sample was drawn from the caudal vein using syringes prepared with a small amount of heparin (5000 IE ml⁻¹) as an anticoagulant. The needle was gently inserted at an angle slightly above and behind the anal fin of the fish, directed towards the spine. After reaching the spine the syringe was pulled back a little to position the bevel inside of the caudal vein. The plunger of the syringe was pulled back and the syringe was carefully rotated to facilitate extraction of blood.

From the blood sample, hemoglobin and hematocrit was measured. To measure hemoglobin a small amount of blood was transferred into a HemoCue Hb 201 microcuvette and left to incubate for ten minutes, after which the cuvette was placed into a HemoCue Hb 201+ hemoglobin photometer, giving the Hb value in grams per deciliter. Hematocrit was measured by absorbing a small amount of blood into a capillary tube which was then centrifuged at 10,000 rpm for five minutes to separate blood plasma and blood cells. These tubes were then read using an analog microhematocrit reader, giving the percentage of red blood cells in the blood. If enough blood could be collected, hemoglobin and hematocrit were measured twice and the average of the two was recorded.

The heart and spleen were dissected by gently opening the abdominal and the pericardial cavity through inserting scissors into the anal cavity of the fish and cutting up towards the head, being careful not to damage any of the visceral tissue and organs. The ventricle and spleen were dissected out and any connecting tissue was removed, and the tissues were blotted dry. These organs were then weighed. Relative ventricular mass was calculated by dividing the mass of the ventricle with the body mass of the fish.

**Field tests**

In early December of 2018, 23 fish from the long-distance location and 32 fish from the short-distance location were caught via standardized electrofishing. As with the first part of the study, smaller fish (approximately <10cm) were not tested because it would not be possible to extract blood without injuring or killing them. The fish were sedated in water with 0.5ml of 2-phenoxyethanol per 1 liter of water. Once sedated the fish were weighed and measured (standardlength and forklength). Blood was extracted from the fish by the same methods described in the first part of the study. The remaining blood sample was the centrifuged at 6000 rpm for 6 minutes to separate the plasma from the red blood cells. The plasma was stored on dry ice during transport back to the university of Gothenburg and later stored in a freezer at -20º C. Cortisol and glucose levels were later measured in this blood plasma.

At the short migratory location there were more fish of larger size than at the long migratory location. After reviewing the frequency of forklength, displayed in figure 2, fish larger than 200mm were excluded from the analysis in order to avoid a bias due to size difference. This brought the sample sizes down to 20 fish from the short migratory location and 23 from the long.
Lastly, in early April of 2019 a final round of field tests were conducted. At the same two locations used earlier, a stretch of 50 meters was selected. This stretch was subjected to three rounds of standardized electrofishing after which the fish caught were put in a holding tub submerged in the river with holes on the sides to ensure waterflow. This time there was no selection on size. For each round of electrofishing the amount of fish caught was counted. However, due to a complication the first two rounds at the short migratory locations were approximations of fish caught. Following this, the fish were sedated in 1.5ml benzocaine l⁻¹ and then weighed, measured (forklength) and put in a bucket of freshwater to wake up before being released back into the river.

From the data collected via the three-pass method, population sizes could be estimated using standard procedures (Bohlin et al., 1989).

After reviewing the length distribution of the fish caught, displayed in the histogram of figure 3, fish over 110 mm in length were deemed to be of an older age group and the size analysis was therefore conducted on individuals <110 mm in length. This brought the sample sizes to 48 and 14 for the short- and long migrators respectively.
Figure 3. Length distribution of the wild fish caught in April 2019

Cortisol and glucose
Plasma cortisol was measured with the radioimmunoassay (RIA) method developed by Young (1986), but with adjustments made by Sundh et al. (2011). The samples were measured in duplicates with 10 µL plasma in each. To release the cortisol from plasma proteins 200µL of glutamate was added to each sample which were then placed in a hot water bath for 15 minutes. After which, 100µL of antibody (code: S020 Lot: 1014-180182) purchased from Guildhay Ltd (UK, no longer in business) and 250 µL of ³H-cortisol was added as a tracer. After incubating at room temperature for 120 minutes, 200µL of charcoal-dextran was added. All samples were centrifuged at 4°C, 3000 RPM for 15 minutes and the supernatant was transferred into labelled vials. This supernatant was mixed with 4,5ml of scintillation fluid which allowed for measuring radioactivity that was later translated to cortisol concentration using a standard curve.

Using a commercially available kit (Sigma-Aldrich, St Louis, USA), glucose was determined via a method adapted for a 96-well microplate reader (Schram et al., 2010). The samples were measured in duplicates, using 10µL of plasma diluted 2,5 times with distilled water. To each well, 200µL of glucose reagent was added. The samples were then incubated at room temperature for 15 minutes before the absorbance was read at a 340nm. The glucose concentration was calculated to a standard curve made with a glucose stock of 5,55mM diluted and a blank (distilled water).

Statistics
All data collected was analyzed using IBM SPSS statistics 25. Normal distribution was analyzed using Kolomogorov-smirnov. If a parameter was normally distributed an independent sample T-test was performed since the data collected came from two independent sets of measurements. If a parameter was not normally distributed it was log transformed. If still not normally distributed the non-parametric Mann-whitney U test was performed.
Results

Laboratory tests

All variables measured in the laboratory fish, represented in the box plots of figure 4, yielded no significant differences.

Field tests

The parameters tested in the field in December of 2018 are displayed in the boxplots of fig. 5. Blood plasma glucose concentration in the wild fish differed significantly between the groups (df = 35, t = -2.617, p = 0.013) after being log transformed for normal distribution. The average glucose concentration was 5.41 mmol/L for the short migrators and 6.32 mmol/L for
the long migrators, meaning that the long migrators had 16.8% more glucose in their blood plasma than the short migrators.

![Boxplot illustrating the variables tested on the wild fish in December of 2018. The asterisk indicates p<0.05.](Figure 5)

From the data collected in April of 2019 it was revealed that there was a difference in size between the fish at the two locations with an average fork length of 6.93 cm and 8.55 cm for the short and long migrators respectively (df = 60, t = -5, p = 0.000). Furthermore, there was a difference in estimated population size with 83 versus 19 for the short and long migrators respectively, see figure 6.

![Graphical illustrations of variables measured in April of 2019. The asterisk indicates p<0.05 and the error bars represents standard error.](Figure 6)

For a more detailed view of the statistics, see appendix 2.

**Discussion**

**Present study**

The first part of this study looked at differences in heart mass, spleen mass, RVM, Hb, HTC, SMR, MMR and AS between the offspring of long and short distance migrating brown trout in the laboratory, where no difference was found. The second part looked at Hb, HTC, length, population density, blood plasma cortisol- and blood plasma glucose concentrations in wild
offspring from the same river. Glucose, size and estimated population size was found to differ significantly between the groups with the long migrators having a higher concentration of glucose, larger body size and a lower estimated population size. Plasma glucose concentration is a parameter that is affected by a lot of different factors (see Polakof et al. (2012) and references within) and it can therefore be hard to draw any conclusions as to the underlying cause of this difference would be. With that said, since the measurements was taken in the same river, within 24 hours of each other and on fish of equal size, many temporal and river-specific environmental factors that could have influenced the measurements should be controlled for. Although we cannot rule out any site-specific differences that may have been present.

**Glucose, size and estimated population size**

It has been shown that plasma glucose consumption differs in various tissues of fish, with some tissues consuming more than others (Blasco et al., 2001). However, during exercise the usage of glucose in Rainbow trouts red muscle tissue increases substantially (West et al., 1993). This difference in glucose could then suggest that this is an adaptation, with the longer migrating fish having a higher plasma glucose concentration to supply their muscles with the glucose needed for their longer migratory distance towards the sea.

The difference in glucose might be due to differences in development between the groups. In Coho salmon (*Oncorhynchus kisutch*) plasma glucose concentration varies during different periods throughout the parr-smolt transformation and also in response to injections of growth hormone (Sweeting et al., 1985), which is a hormone that increases in concentration during smoltification (Prunet et al., 1989). The fact that the two migratory groups in the present study differed in plasma glucose could suggest that they might have been in different stages of the smoltification process. However, because the concentrations sometimes surged and sometimes was lower in the Coho salmon (Sweeting et al., 1985), it is not possible to say which group would be closer to being fully smoltified. A reasonable guess could be the long-migrating are closer to being smoltified since fish further up in river systems have been shown to migrate at an earlier date (Stewart et al., 2006) and would therefore presumably need to go through smoltification at an earlier time.

Another possible reason for the difference in glucose concentration may be that the two groups respond to stress in different ways. Both the short- and long-migrating wild fish had concentrations of cortisol (24.3 and 14.5 ng/ml, respectively) that were slightly above what is generally considered to be the resting values for salmonid fish (<10 ng/ml) but not quite high enough to reach the threshold for stress (40-200 ng/ml). At the same time, the blood glucose for both groups were slightly above what is considered the threshold for stress (>5mmol/l) in salmonid fish (Iwama, 1998). This would suggest that they both experienced a minor form of stress, or at the very least were not at rest. This potential stress and elevated levels of both glucose and cortisol can be due to the electrofishing, which has been shown to increase these concentrations in other fish (Barton & Dwyer, 1997; Bracewell et al., 2004). It may also be due to the anesthesia, 2-phenoxyethanol, which has been shown to elicit an upsurge in plasma glucose (Molinero & Gonzalez, 1995; Velisek et al., 2007). The difference we found might not indicate that one group is more stressed than the other since glucose alone might not be a good evidence for stress (Martínez-Porchas et al., 2009), and there was no difference in cortisol. However, since both of the groups were subjected to the same procedure and the increase in plasma glucose is supposed to supply the fish with more energy following a stressor (Iwama, 1998) it might suggest that the two groups respond in different ways when exposed to stress.

The difference in estimated population size is most likely exaggerated because the sampling was done during a period of the year when the brown trout migrates (supported by
the fact that smoltified individuals were found during sampling) and since all fish in the river needs to pass through the short distance location on their way to the sea, it is therefore logical for that location to have a higher estimated population size at that time. At the time of glucose sampling, we did not quantify population sizes but simply based on the observation that it was somewhat easier to find fish at the short distance location we suspect that there was somewhat of a difference at that time as well. With that said, a difference in density could explain both the difference in glucose and the difference in size. This is based on the fact that plasma glucose concentration is negatively affected by high densities in brook charr (Salvelinus fontinalis) in a laboratory environment (Vijayan et al., 1990) and that brown trout in high densities grow less in comparison to ones in lower densities (Bohlin et al., 2002).

**Metabolic rates**

Why did Eliason et al. (2011) find a significant difference between long and short distance migrating sockeye salmon in AS and RVM, while the present study found no such difference in the brown trout? Firstly, it may simply mean that this phenomenon is not present in the brown trout. Secondly, this might be something that arises later in the fish’s life due to developmental factors or life history events. Eliason et al. (2011) looked at adult fish while the present study looked at juveniles. It is also possible that this phenomenon is present in brown trout but it arises with greater migration distance differences. Eliason et al. (2011) had migration differences of a couple of hundred of kilometers while the present study had a difference of ~5.9km. Lastly, in the case of AS, it might be that we missed the difference. This is based on the fact that in the field tests we found a difference in plasma glucose concentration and blood sugar concentration has been shown to correspond with SMR in bony fish (Umminger, 1977), coupled with the fact that certain factors could have had an effect on the expression of metabolic rates in the laboratory fish, such as the extended laboratory confinement (Závorka et al., 2019) and potential social stress experienced in the aquaria (Sloman et al., 2000). Furthermore, the metabolic rates of the laboratory fish might also have been affected by the unfortunate fungi outbreak in the laboratory, since challenges to the immune system have shown to raise basal metabolic rates in birds (Ots et al., 2001). Naturally no fish with visible fungi infection was ever used in the study. However, since the type of fungi was unknown there was no way of knowing if a fish could have fungi internally before the it sprouted on the outside of the fish’s body. These factors may have affected the metabolic values and masked a potential difference.

**Implications for management and further studies**

These results could have implications for certain types of conservation strategies. One approach to manage populations of brown trout is to stock rivers with hatchery bred fish. This strategy is somewhat controversial since it has been shown in several species of salmonid fish that it can have a negative impact on several aspects of the wild fish populations (Hindar et al., 1991). This is taken into consideration when managing fish populations in Sweden by having regulations on fish stocking that say one should strive to make sure that stocking fish are from local origin, meaning that if you plan to stock fish in one river the fish should originate from that particular river (Fiskeriverket, 2001). If the results from the present study reflects an inherited physiological difference between long and short-migrating brown trout, then this strategy could be improved by taking into consideration from where within the river the cultured fish originate and also where they are later stocked. This would presumably better conserve any differences that would arise as a consequence of differences in migration distance. Furthermore, studies like this one gives us a deeper understanding and a more detailed view of the brown trouts life cycle, which is an important knowledge to have when considering and planning conservational efforts.
It is important to consider the fact that all the fish in this study was collected from the same river, Norumsån. Meaning that these findings might not be applicable to the brown trout as a species since one might get a different result when looking in other rivers. However, the advantages of using only this stream is that there is little environmental variety between the two sampling locations that could have affected the results and the recorded high amount of anadromous brown trout (Sundstrom et al., 2013). A suggestion on how to follow up this study could be to find a multitude of streams, all of which housing anadromous brown trout and test long- and short-migrating individuals from all streams. These results would be more applicable to the brown trout as a species. Another suggestion could be to collect fertilized eggs from the two locations and rear the offspring in identical environments, a common garden experiment, and examine the same parameters. This way, any potential influences by external factors would be circumvented.

**Conclusion**
In conclusion, the results presented in this study show that there is a difference between the short- and long-migrating fish in this system that is reflected in the plasma glucose concentration. The results also show a size difference between the groups, most likely explained by a potential difference in population density between the areas. Beyond this we did not find any differences between the two groups. These results, and results that can come from further studies like this, are useful when planning and performing conservational efforts but might also be relevant for improving current management strategies. However, as stated earlier one must be cautious when trying to draw any conclusions from the difference in glucose since there are many factors that can influence glucose concentration. The underlying cause of these differences could be any of the theories discussed above, or it can be none of them. Nevertheless, the fact remains that there is quite a clear difference which warrants further investigations into what the underlying causes could be.

**Acknowledgements**
I want to thank Johan Höjesjö for being a great supervisor, planning the project, discussing ideas and guidance during the course; Magnus Lovén Wallerius for assisting in the fieldwork, offering help and discussion; Erik Sandblom and Andreas Ekström for teaching me about flow respirometry and metabolism, helping and teaching me to process the data, helping out with experimental protocol, -setup and fieldwork; Jonathan Roques for helping out with field work, blood sampling and the measuring of blood parameters; Libor Závorka for offering help, feedback, ideas and discussion; Anders Engström for being a great coworker, discussing ideas and being a huge help during the entire project.
References


Appendix 1
Popular science summary

Appendix 2
Statistics

Presented here is a more detailed view of statistical values for all parameters tested.

**Table 1. Statistical values for all parameters tested in the laboratory.**

<table>
<thead>
<tr>
<th>Migration</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P-value</th>
</tr>
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<td></td>
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<td>12</td>
</tr>
<tr>
<td></td>
<td>Long</td>
<td>13</td>
<td>89</td>
<td>21</td>
</tr>
<tr>
<td>HTC</td>
<td>Short</td>
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<td>31</td>
<td>5,9</td>
</tr>
<tr>
<td></td>
<td>Long</td>
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<td>31</td>
<td>6,5</td>
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**Table 2. Statistical values for all parameters tested in the field in December of 2018.**

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<td>HB</td>
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<tr>
<td></td>
<td>Long</td>
<td>23</td>
<td>89</td>
<td>19</td>
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<tr>
<td>HTC</td>
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**Table 3. Statistical values for the length data collected in April of 2019**

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</thead>
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