Katja Anttila

SWIMMING MUSCLES OF WILD, TRAINED AND REARED FISH

ASPECTS OF CONTRACTION MACHINERY AND ENERGY METABOLISM
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SWIMMING MUSCLES OF WILD, TRAINED AND REARED FISH
Aspects of contraction machinery and energy metabolism

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Abstract

Billions of reared fish are released to the wild to compensate e.g. for the loss of natural populations. However, the efficiency of the releases is low. It has been proposed that one of the factors affecting the low survival rate of reared fish is their low swimming capacity.

The molecular, metabolic and structural characters of muscle fiber define the swimming capacity of fish. Swimming capacity is related to the ecological competence of the fish, including the ability to complete long migrations and catch prey. One of the aims of the current study is to compare the properties of muscles of reared and wild salmon. The second aim of the study is to alter the muscular parameters of reared fish closer to those of wild fish by means of training.

The muscular differences between wild, reared and trained fish are analyzed with immunological, histochemical and electron microscopic methods. The main focus is on the dihydropyridine and ryanodine receptors. These receptors are involved for example in the initiation, force and velocity of muscle contraction.

According to the results, the level of receptors is higher in the muscles of wild as compared to reared fish. The aerobic ATP production capacity is also higher in the wild fish. However, with training both the level of receptors and oxidative capacity of reared fish increase. Moreover, the swimming capacity is enhanced in trained fish, and there is a connection between the level of receptors and swimming capacity of fish. Training also affects the migration pattern of fish which starts to resemble more that of wild fish.

In conclusion, the results of the current study show that the performance of fish as a whole depends on functional parameters at cellular level. For the first time, it is shown that the level of receptors involved in muscular contraction is low in muscles of reared fish. However, the muscular properties are not definite. It is now shown that with training, both the muscular and migration parameters of reared fish approach those of wild fish. This will most probably increase the survival probability of trained, reared fish in the future.

Keywords: dihydropyridine receptor (DHPR), ryanodine receptor (RyR), survival, swimming capacity
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Oulu

Tiivistelmä
Kalojen kasvatus ja istutus takaisin luontoon on yksi tärkeimmistä keinoina säädetä ja palauttaa kalakantoja vesistöihin. Maailmanlaajuisesti puhutaan miljardien kalojen istutuksista vuosittain. On kuitenkin hyvin tunnettu tosiasia, että kasvatetut kalat eivät selviä luonnossa yhtä hyvin kuin villit lajikumppaninsa. On arvioitu, että vain alle 5 % istutetuista kaloista selviää lisääntymisikään asti hengissä.

Eräs tekijä, joka voi vaikuttaa kalojen selviytymiseen, on kalojen lihaskunto. Kasvatettujen kalojen uintikyvyn on todettu olevan heikko villeihin lajikumpanneihin verrattuna. Luonnossa kaloilta kuitenkin vaaditaan puhtaita uintikykyyä. Saalistukseen, peidoinniseen ja viemään vaaditaan suurta uintikykyä. Eräs tämän työn päätavoitteista on määrittää, miten kasvatettujen ja villien kalojen molekulaariset, aineenvaihdunnalliset ja rakenteelliset ominaisuudet poikkeavat toisistaan, jotta voidaan arvioida mitkä todellisesti vaikuttavat kalojen uintikykyyn ja sitä kautta selviytymiseen. Toisaalta kasvatettujen kalojen lihasten molekulaaristen tekijöiden tasoja pyritään nostamaan harjoittelun avulla lähemmäksi villien vastaavaan ja täten vaikuttamaan kasvatettujen kalojen uintikykyyn ja sitä kautta lopulta selviytymiseen.


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Oulu, March 2009 Katja Anttila
### Abbreviations

- **ATP**: adenosine triphosphate
- **ATPase**: adenosine triphosphatase
- **BCIP/NTB**: Bromo-4-Chloro-3-Indoly1 Phosphate Mono(-Toluidinium) Salt/Nitro Blue Tetrazolium
- **BL**: body length
- **CF**: condition factor
- **CICR**: calcium induced calcium release
- **CV**: coefficient of variation
- **DCCR**: directly coupled calcium release
- **DHP**: dihydropyridine
- **DHPR**: dihydropyridine receptor
- **EC**: excitation contraction
- **LDH**: lactate dehydrogenase
- **MHC**: myosin heavy chain
- **MS 222**: tricaine methanesulphonate
- **NAD**: nicotinamide adenine dinucleotide
- **NADH**: nicotinamide adenine dinucleotide reduced form
- **PST**: plastic streamer tag
- **Ry**: ryanodine
- **RyR**: ryanodine receptor
- **SDH**: succinic dehydrogenase
- **SDS-PAGE**: sodium dodecyl sulphate-polyacrylamide gel electrophoresis
- **SR**: sarcoplasmic reticulum
- **U_{crit}**: critical swimming velocity
- **VGCC**: voltage-gated calcium channel
List of original papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


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1 Introduction

Salmon is one of the most stocked fish species in the world. For example, in 1999 48 million salmon were released into the wild in the northern Atlantic area (ICES 2000a, b). However, only a minor fraction of the stocked fish survives until maturity. It has been shown that in reared fish, the survival ratio is less than three percent (Jonsson et al. 2003). Wild salmon, on the other hand, have a substantially greater probability to survive (Jutila et al. 2003; Kallio-Nyberg et al. 2004; Jokikokko et al. 2006). There are apparently several reasons for the lower survival rate of reared fish as compared to the wild ones, but the current rearing practices could have a major effect. In the hatcheries, fish live in a sheltered environment lacking predators and with unlimited food resources. Moreover, the water habitat hardly represents their natural habitat (Olla et al. 1998; Einum & Fleming 2001; Jonsson & Jonsson 2006). These differences could lead to reared fish stocks having poorer initial survival skills (Olla et al. 1998). However, attempts have been made to enhance the survival rate of reared fish. For example, fish have been exposed to predators to increase their predator avoidance skills (Mirza & Chivers 2000; Vilhunen & Hirvonen 2003). Moreover, fish are reared in environments mimicking natural conditions in order to make the behavior of fish more natural (Braithwaite & Salvanes 2005). These previous studies have, however, neglected the swimming capacity of fish even though it forms the basis for e.g. catching pray and escaping predators.

Previously it has been shown that the swimming capacity of reared fish is substantially lower when compared to wild fish (Rimmer et al. 1985; McDonald et al. 1998b; Basaran et al. 2007). This forms one of the reasons for the survival difference between wild and reared fish (McCormick et al. 1998). In the current study, one of the purposes is to extend the comparisons of swimming capacity to the cellular level by comparing the muscular variables of wild and reared juvenile salmons. An analysis at the molecular level is highly essential in order to estimate the factors affecting the competency of muscles and, further, survival. In this study, the focus is on the dihydropyridine (DHP) and ryanodine (Ry) receptors; two of the key proteins involved in muscle contraction (Lamb 2000; Fill & Copello 2002). The reason for studying these receptors is that in both fish and mammals (e.g. rodents), the level of receptors correlates positively with muscle contraction velocity (Akster et al. 1985; Kandarian et al. 1992; Golden et al. 2003), and in rodents also with contraction force (Määttäri & Järvillehto 2005). This study is, however, the first one to compare the level of receptors between
wild and reared salmon. Moreover, the connection between the level of receptors and the swimming capacity of fish is analyzed for the first time.

The aim is to diminish the measured differences at the molecular level between wild and reared fish by training the reared fish and, thus, possibly increase their survival probability in the future. During training, the water flow velocity in rearing tanks is increased and the water flow habitat thus begins to bear more resemblance to the natural habitat. However, careful testing of the training program before the survival analysis is essential since different fish species and even different fish populations of the same species respond differently to the same training protocol (Nelson et al. 1994). In the current study, one of the purposes is to find out the most beneficial training regimens for different fish species. The effect of the training program on the migration pattern, one factor affecting survival, is thereafter analyzed.
2 Review of the literature

The morphological, histological and cellular structures as well as histochemical characteristics of the swimming muscles of fish basically define how actively the fish can swim. The swimming capacity of the fish, on the other hand, relates to the ecological swimming behavior. Fish with high swimming capacity can e.g. escape from predators more easily. Moreover, fish with high swimming capacity and high activity level of muscles can accomplish migrations of hundreds of kilometers. Thanks to high swimming capacity, the fish might thus have a higher probability to survive in nature.

2.1 Structure and function of fish swimming muscles

The swimming musculature of fish is mainly composed of two different muscle types: white and red. White muscle forms the bulk of the fish (80–100%) arranged into segmental myotomes with separating connective tissue, i.e. myosepta, between the myotomes. The three-dimensional structure of myotomes is complex, with diverse folding along the longitudinal axis as illustrated in Fig. 1 (Bond 1979; Altringham & Ellerby 1999).

Fig. 1. Lateral view of musculature of whitefish (Coregonus lavaretus). Three myotomes have been completely removed anteriorly and posteriorly to two intact central myotomes. On the surface of the folded white muscle the red muscle runs parallel to the body axis. Scale bar, 1 cm.
The white muscle runs helically to the body axis. This enables a greater effect of the muscle on the change of body curvature during swimming as compared to red muscle, which runs parallel to the body axis (Rome & Sosnicki 1991). Red muscle is situated beneath the lateral line as a superficial zone of fibers. The cross-sectional proportion of red fibers increases towards the tail of the fish (Nag 1972). There also seems to be differences in the contraction kinetics of both muscle types from the anterior to posterior part of the fish. For example, twitch contraction time increases along the length of the fish (Altringham & Ellerby 1999). Moreover, the activity of aerobic enzymes increases in the red muscle towards the tail of the fish (Martínez et al. 2003). The volume of red muscle is related to the swimming activity of the fish. In benthic species (e.g. burbot, *Lota lota*), the proportion of red fibers is minor. With increasing swimming activity the proportion of fibers increases (Bond 1979; Altringham & Ellerby 1999).

In some species (e.g. mirror carp, *Cyprinus carpio*), there is also a thin layer of pink muscle fibers between red and white muscle. The contraction velocity and histochemical properties of the pink muscle are intermediate between the properties of red and white muscle. The activity pattern of muscle types follows the swimming velocity. The recruitment order of muscle types with increasing swimming velocity is red<pink<white (Johnston 1980). Interestingly, the function of pink muscle is not that significant in some fish species, e.g. in salmonids the functional importance of pink muscle is minor (Davison 1997).

The ultrastructure of both red and white fibers resembles the respective types found in mammals. Sarcolemma surrounds the fibers having invaginations, i.e. T-tubules towards the interior of the fibers (Franzini-Armstrong & Porter 1964; Nag 1972). These membrane structures contain voltage-sensitive sodium channels and voltage-gated calcium channels (VGCC). The channels mediate the action potential coming from motoneurons along and inside the fiber. T-tubules are connected to the sarcoplasmic reticulum (SR) via interaction of VGCC and ryanodine (Ry) receptors. Together the T-tubules and SR form so-called triads (Fill & Copello 2002). Inside the muscle fiber the myofibrils are divided into functional units, i.e. sarcomeres, in a manner similar to mammals. However, in fish the T-tubules are situated at the Z-line, whereas in mammals they locate at the I-A band (Fig. 2) (Franzini-Armstrong & Porter 1964; Nag 1972). The sarcomeres are mainly composed of actin and myosin, the units responsible for the contraction of muscle.
In both muscles types, Ca\textsuperscript{2+} released from SR binds to the troponin-tropomyosin complex. The following change in the conformation of the complex allows the connection of myosin and actin together and, thus, mediates the contraction. Although the actions of the component of the muscle contraction machinery are the same in both muscle types, the isoforms of myosin heavy chain (MHC) differ (Karasinski 1993; Martinez et al. 1993), for example. The differences in MHC-profile are connected to the contraction velocity of the muscles (Coughlin et al. 2001). In white muscle the myosin-actin connection-release cycle, as determined by myosin ATPase activity, acts faster than in red muscle (Nag 1972), which most probably influences the whole contraction velocity of the muscle. Besides differences in myosin isoforms, red and white muscles also differ in diameter, mitochondrial content, lipid deposit, capillary density, myoglobin concentration and activity of the enzymes involved in the energy metabolism. In Table 1, the differences between muscle types are summarized. The differences in energy metabolism as well as in myosin heavy chain profile and even in the orientation of muscles are associated with the utilization of different muscle types in different swimming events. Red, oxidative fibers are used in long-lasting, cruising swimming modes, whereas white fibers are activated when swimming velocity
and tail-beat frequency increases, e.g. in escape responses (Altringham & Ellerby 1999; Gibb & Dickson 2002). Although the contraction velocity and metabolism differs between red and white fibers, the contraction mechanism itself remains the same.

Table 1. The physiological differences between red and white muscle fibers of fish (Johnston 1980; Love 1980).

<table>
<thead>
<tr>
<th>Character</th>
<th>Red</th>
<th>White</th>
</tr>
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<tbody>
<tr>
<td>Diameter</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Capillary density</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Mitochondrial density</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>T-tubular density</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>SR volume</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Lipid concentration</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Protein concentration</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Myoglobin concentration</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Oxidative enzyme activity</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Succinic dehydrogenase activity</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Cytochrome oxidase activity</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Lipase activity</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>β-oxidation activity</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Lactate clearance rate</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Glycolytic enzyme activity</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>ATPase activity</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Contraction velocity</td>
<td>Low</td>
<td>High</td>
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</tbody>
</table>

2.2 Excitation contraction coupling

The contraction of the muscle is regulated with the release of calcium from the sarcoplasmic reticulum (SR). This calcium release occurs via excitation contraction (EC) coupling. An action potential coming from α-motoneurons evokes a depolarization that spreads to T-tubules. The change of voltage in the tubules alters the conformation of dihydropyridine (DHP) receptors (main subunit of VGCC), and as a result of functional interaction (i.e. coupling) ryanodine receptors are activated. Calcium is released from SR via the activated Ry receptors into cytoplasm, which is the process that ultimately initiates the muscle contraction (Fig. 3) (Lamb 2000; Fill & Copello 2002).
Fig. 3. Model of excitation contraction (EC) coupling. The dihydropyridine receptors (DHPR) and ryanodine receptors (RyR) interact during EC coupling leading to release of Ca^{2+} from sarcoplasmic reticulum. Figure is modified from Jurkat-Rott et al. (2006).

The activation of Ry receptors can occur via two different mechanisms. First, according to electron microscopic studies (Block et al. 1988; Franzini-Armstrong et al. 1999), VGCCs and Ry receptors are situated in corresponding positions in T-tubules and SR (Fig. 4). Thus, the change in the conformation of DHP receptors also alters the conformation of Ry receptors via direct mechanical linking whereby Ry receptors open (directly coupled calcium release, DCCR) (Sutko & Airey 1996; Lamb 2000; Fill & Copello 2002). It has been speculated that there might also be some external proteins involved in the mechanical coupling (e.g. triadin, calmodulin and FK Binding Protein) and that the presence of these proteins is essential for the proper function of VGCC and Ry receptors (Sutko & Airey 1996; Fill & Copello 2002; Dulhunty 2006). Moreover, some subunits of VGCCs and Ry receptor as a whole are indispensable for the DCCR (Flucher et al. 2005; Schredelseker et al. 2005), and DCCR is thus mainly regulated by the VGCCs and Ry receptors.
Fig. 4. Triad, composed of two sarcoplasmic reticulum (SR) and T-tubule membranes. Voltage-gated calcium channels (VGCC) occupy the T-tubules. The ryanodine receptors (RyR) are situated in the T-tubule-SR gap in corresponding positions relative to VGCC tetrads. The intramembranous portion of the RyR passes through the membrane of SR, forming the channel for Ca\(^{2+}\). The figure is modified from Block et al. (1988).

The second mechanism for activation of Ry receptors also occurs via the main subunits, i.e. DHP receptors of VGCCs, but more indirectly. The DHP receptors can also act as L-type (long lasting) calcium channels. In this case, the depolarization of T-tubules opens the DHP receptors. Through the opened receptors Ca\(^{2+}\) ions flow from the extracellular space into the fiber (Lamb 2000; Fill & Copello 2002). Ry receptors are activated in micromolar Ca\(^{2+}\) concentration (Koulen et al. 2001; Fill & Copello 2002), and the influx of Ca\(^{2+}\) ions thus achieves the calcium release from SR (Ca\(^{2+}\) induced Ca\(^{2+}\) release, CICR) (Lamb 2000; Fill & Copello 2002). In skeletal muscle, the influx of calcium through DHP receptors is considered to be too slow and small in magnitude to accomplish the CICR from SR. The Ca\(^{2+}\) ions for the CICR mechanism could thus also come from the inside of SR as a result of DCCR. However, according to
Johnston (1980), the contractile responses of both red and white muscle of fish are at least partly dependent on the extracellular Ca\textsuperscript{2+}.

It has been proposed that Ca\textsuperscript{2+} released through CICR could intensify and maintain the calcium flow from SR induced by DCCR (Sutko & Airey 1996). On the other hand, some Ry receptors are inhibited in millimolar calcium concentrations, and eventually the accessory Ca\textsuperscript{2+} release leads to inactivation and could be part of the relaxation phase (Sutko & Airey 1996; Koulen et al. 2001; Fill & Copello 2002). It has been suggested that calcium release could occur through both DCCR and CICR especially in the swimming muscles of fish (O’Brien et al. 1995; Fill & Copello 2002).

### 2.2.1 Voltage-gated calcium channel

VGCC is a voltage-dependent calcium channel (~430 kDa) in T-tubules. It is composed of a pore-forming and voltage-dependent DHP receptor, also called \( \alpha_{1S} \) subunit, and auxiliary subunits (\( \beta_{1a}, \alpha_\delta, \) and \( \gamma_1 \)) (Coronado et al. 2004; Flucher et al. 2005). In mammals, the structure and function of the VGCC has been studied extensively. However, the studies on VGCCs in the swimming muscles of fish are few. There are some studies about the sequence and molecular physiology and interactions of the channel and its subunits in fish muscles (Grabner et al. 1991; Schredelseker et al. 2005). In the study of Grabner et al. (1991), the sequence of the channel in the white muscle of carp was determined. It was proposed that the auxiliary subunits of VGCC in fish muscles differ from those in mammals. However, in a recent study of Schredelseker et al. (2005), it was noted that the antibodies against mammalian subunits also recognized the subunits in fish muscles. It is therefore most likely that the general structure of the channel has been quite conservative during evolution. Additional support for this proposal arises from the sequence of the DHP receptor, since the functionally indispensable domains of the subunit share 90% homology in fish and mammals (Grabner et al. 1991). Moreover, in frogs these parts are almost identical to their mammalian homologs (Zhou et al. 1998).

In the skeletal muscles of both fish and mammals, the VGCCs are organized in the T-tubules as arrays of four receptors known as tetrads. These tetrads are connected to the Ry receptors in SR. Thus, in skeletal muscles, there are four DHP receptors per one Ry receptor. The arrangement of the tetrads and the corresponding positions of ryanodine receptors are under direct regulation. It has been proposed that e.g. ankyrin or triadin are involved in this junctional
organization (Sutko & Airey 1996). However, it has been shown that skeletal muscles of dyspedic mouse, which lack Ry receptors, do not contain tetrads (Sutko & Airey 1996; Dulhunty 2006). Therefore, the presence of both receptors is indispensable for the proper organization of tetrads. The tetrads and Ry receptors form the mechanical connection between T-tubules and SR, thus mediating DCCR (Fill & Copello 2002). However, not all Ry receptors are connected to tetrads. In the muscles of fish, there are twice as much DHP receptors as Ry receptors. Since DHP receptors are situated as a four-receptor tetrad complex, half of the Ry receptors are unconnected (Block et al. 1988). These unconnected Ry receptors are thought to mediate the CICR (Sutko & Airey 1996; Fill & Copello 2002).

In rat muscles, studies of the activation pattern of VGCCs have shown that the L-type calcium current through CICR peaks at 50–100 ms (Beam & Knudson 1988). Therefore, it is thought that CICR through VGCCs is not fundamental for muscle contraction (Sutko & Airey 1996; Fill & Copello 2002). In fish swimming muscles, the time to peak tension in white, fast-contracting muscles is 12–14 ms, whereas in red, slow-contracting muscles it is 59–92 ms (Akster et al. 1985). Calcium could, therefore, come both through DCCR and CICR, especially in fish red muscles.

The main subunit of the VGCC is the DHP receptor. It has been thought that the DHP receptor is mainly responsible for both DCCR and CICR (Tanabe et al. 1990; Dulhunty 2006). The DHP receptor is composed of four domains (I–IV) each containing six transmembrane segments. The S4 segments of each domain are positively charged and thus react to the depolarization of the T-tubule. It has been shown that each of the segments moves outward as a response to the change of voltage. This movement leads both to the opening of the DHP receptor and coupling between Ry and DHP receptor (Coronado et al. 2004; Dulhunty 2006; Millar et al. 2007).

Previously it was observed that the domains II-III of DHP receptors are indispensable for DCCR (Tanabe et al. 1990). However, according to more recently published investigations the proper function of EC coupling requires additional subunits besides the domains II-III (Coronado et al. 2004; Flucher et al. 2005; Schredelseker et al. 2005; Dulhunty 2006). In zebrafish of the strain red<sup>ts25</sup> lacking β<sub>1a</sub> subunits, the VGCCs are not arranged as arrays of tetrads and the DCCR is thus missing (Schredelseker et al. 2005). Moreover, it has been suggested that the β<sub>1a</sub> subunit is involved in both the DCCR and the CICR process (Coronado et al. 2004; Flucher et al. 2005).
In addition to the DHP receptor and $\beta_{1a}$ subunit, the VGCCs contain $\alpha_2\delta$ and $\gamma_1$ subunits. However, it has been shown that the presence of these subunits is not necessary for EC coupling. The $\alpha_2\delta$ subunit seems to be involved in the inactivation process of calcium current through the DHP receptor since the deletion of the subunit accelerates the activation process. It is thought that the $\alpha_2\delta$ subunit does not regulate the inactivation process by itself, but alters the properties of the DHP receptor. The $\gamma_1$ subunit also participates in the inactivation process of the DHP receptor. It enhances the voltage-sensitivity of inactivation and limits the Ca$^{2+}$ current through both DHP and Ry receptors (Flucher et al. 2005).

### 2.2.2 Ryanodine receptor

The ryanodine receptor (RyR) (~500 kDa) is composed of two distinct parts: a large cytoplasmic ‘foot’ structure and a small transmembrane part. The 3D reconstruction reveals that the ryanodine receptor is square in shape and is in interaction with the tetrad of DHP receptors (review of Wang et al. 2004). The studies on Ry receptors in the swimming muscles of fish have focused on the molecular characteristics and cloning of the receptor (O’Brien et al. 1995; Franck et al. 1998; Morrissette et al. 2000; Koulen et al. 2001). According to these studies, the muscles of fish contain two ryanodine receptor isoforms: $\alpha$- and $\beta$-RyR. Moreover, at least $\alpha$-RyR is divided into fast and slow populations (Morrissette et al. 2000). According to immunological studies, the $\alpha$-RyR and $\beta$-RyR receptors correspond to the mammalian RyR1 (skeletal muscle RyR) and RyR2 (cardiac muscle RyR), respectively. On the other hand, mRNA sequence studies suggest that $\beta$-RyR is homolog to RyR3 (RyR expressed in mammalian skeletal muscles at low levels), and studies are nowadays based on this analogy (Sutko & Airey 1996; Fill & Copello 2002).

According to evolutionary studies, $\alpha$- and $\beta$-RyR have had a dual expression pattern throughout the evolution of vertebrates (O’Brien et al. 1993). However, there are some differences in the characteristics of the receptors in different species. The Ry receptor isoforms of fish muscle differ, for example, in their ryanodine binding capacity and Ca$^{2+}$ release rates from those of other vertebrates (Olivares et al. 1991; Koulen et al. 2001). Moreover, the amino acid sequences of $\alpha$-RyR isoforms of different species are only 72–77% identical (Franck et al. 1998).
Fish swimming muscles express both α- and β-isoforms in an equal manner (O’Brien et al. 1993; Sutko & Airey 1996; Koulen et al. 2001). However, in these studies the different muscle types were not separated from each other and the studies were performed merely with white muscle fibers. Franck et al. (1998) and Morrissette et al. (2000) found that in red muscle merely one Ry receptor isoform is expressed: α-RyR-slow. White muscle on the other hand expresses both α-RyR-fast and β-RyR at the same densities.

There are also significant functional differences between RyR isoforms. Only α-RyR is inhibited at millimolar Ca\(^{2+}\) concentration while β-RyR does not exhibit such a property (O’Brien et al. 1995; Sutko & Airey 1996). Moreover, β-RyR has a greater opening probability when activated with calcium. β-RyR also seems to be more sensitive to Ca\(^{2+}\) than α-RyR (Sutko & Airey 1996). These differential properties of RyR isoforms provide several pieces of indirect evidence that β-RyR could mediate the CICR while α-RyR mediates the DCCR (Sutko & Airey 1996; Morrissette et al. 2000). Moreover, it has been shown that in cn/cn muscles, which express only the β-RyR isoform, DCCR does not work and the EC coupling is achieved merely by CICR (review of Sutko & Airey 1996). Furthermore, in mammalian striated muscles, which express almost exclusively the α-RyR/RyR1 isoform, the calcium release occurs via DCCR (Fill & Copello 2002). It has been proposed that those Ry receptors that are not connected to DHP receptor tetrads (β-RyR) could mediate the CICR process while the rest (α-RyR) of the receptors mediate DCCR (Sutko & Airey 1996; Fill & Copello 2002).

The Ry receptors do not mediate calcium release alone. In the recent review of Dulhunty (2006) the protein-protein interactions during the EC coupling process are illustrated. There seems to be an increasing number of proteins involved in the process. The luminal Ca\(^{2+}\) transduction machinery, for example, is actually composed of Ry receptors, calsequestrin, triadin, and junctin complex. In this system calsequestrin binds Ca\(^{2+}\) inside the SR and modulates Ry receptor activity. Triadin and junctin anchor calsequestrin into the Ry receptor and are thus involved in the regulation of the activity of the Ry receptor (Fill & Copello 2002; Dulhunty 2006).

2.2.3 Functional relevance of dihydropyridine and ryanodine receptors in the contraction capacity of the muscle

Dihydropyridine and ryanodine receptors mediate the release of calcium from SR initiating the muscle contraction (Lamb 2000; Fill & Copello 2002). In mammals
it has been noted that the expression of receptors is also connected to the contraction capacity of the muscle. Zhong et al. (2001) showed that in the cardiac muscle of diabetic rat, the relative level of ryanodine receptors is reduced. Simultaneously they observed an impaired function of SR, i.e. the Ca\(^{2+}\) release rate was decreased, and muscle contraction velocity was thus slower. A similar relationship between the level of receptors and contraction velocity is also observed with DHP receptors. Castration leads to the reduced expression of DHP receptors in the muscles of rat and increases the time to peak shortening (Golden et al. 2003). On the other hand, biomechanical unloading increases the expression of DHP receptor mRNA and decreases the time to peak shortening (Kandarian et al. 1992). Moreover, Mänttäri & Järvilehto (2005) observed that in the muscles with a high level of DHP receptors, blocking the function of receptors had a more significant reducing effect on the force of the contraction than in those muscles where the relative level of receptor was lower. There seems thus to be a connection between the level of receptors and force and velocity of the contraction. However, the contraction characteristics of muscle do not depend solely on the DHP and Ry receptors, but also on additional parameters, e.g. myosin heavy chain profile.

Additional support for the connection between muscle contraction capacity and the level of receptors regardless of the myosin heavy chain composition is provided by training experiments. Ørtenblad et al. (2000) showed that the total number of Ry receptors in human hind leg muscle increased after high-intensity training, while no changes were seen in myosin heavy chain profile. Similar results with respect to DHP receptors were noted in the study of Mänttäri et al. (2006), where low-intensity training modified the DHP receptor content of mice muscles. Since it is well known that muscle contraction capacity increases after training at least in mammals (Kraemer et al. 1995; McCall et al. 1996), one interpretable factor could be an increased number of DHP and Ry receptors. A similar relationship is also observed after testosterone treatment in mice (Anttila et al. 2008).

In fish muscles, the studies comparing the contraction of the muscle and the level of receptors are few in number. In fish muscles, the comparison of muscle contraction capacity with receptor content is merely based on the properties of different muscle types. In the study of Akster et al. (1985) it was shown that white muscles contain a higher density of feet structures, i.e. ryanodine receptors than red muscle. Moreover, the T-tubules in white muscle are more often connected to the SR (Akster 1985). Since the white fibers contract faster than the red ones
(Altringham & Ellerby 1999), there seems to be a connection between the contraction velocity and the level of receptors in fish muscles as well. Similar differences between muscle types have also been shown in mammals (Mänttäri et al. 2001; Reggiani & te Kronnie 2006; Anttila et al. 2007). However, studies comparing the swimming capacity of the fish with the level of receptors in muscles do not exist. Moreover, although the effects of training on both muscle competence and level of DHP and Ry receptors have been extensively studied in mammals, no experiments have been done with fish despite the fact that the receptors constitute such a relevant part of muscle function.

### 2.3 Effects of training on fish swimming muscles

Although the effects of training on the receptor content in fish muscles have not previously been studied there are several other investigations on the effects of training on the performance and capacity of muscles. In Table 2, the effects of training on muscular fitness are summarized according to training types. In general, the swimming capacity of fish seems to increase with training (Houlihan & Laurent 1987; McDonald et al. 1998b; McFarlane & McDonald 2002; McClelland et al. 2006, van der Meulen et al. 2006), which is most probably connected to the altered characteristics of muscles. For example, the diameter of muscle fibers (Hinterleichter et al. 1992; Gruber & Dickson 1997; Martin & Johnston 2006), mitochondrial content (Sänger 1997), capillary density (Davie et al. 1986; Sänger & Pötscher 2000) and the rate to recovery (Young & Cech 1993b) after training increase.

It has been shown that trained fish have higher food conversion efficiency (Davison 1997, van der Meulen et al. 2006), which could be one reason for the increased size of fibers. Moreover, the concentration of myofibrils has been shown to increase (Sänger 1997). The higher amount of components involved in the contraction machinery could thus partly explain the higher critical swimming velocity \( (U_{crit}) \) of trained fish especially after maximal training (Holk & Lykkeboe 1998; McClelland et al. 2006). On the other hand, the aerobic swimming performance increases after endurance training (van der Meulen et al. 2006). This change is most probably connected to the increased number of mitochondria (Sänger 1997) and oxidative enzyme activities (Davie et al. 1986; McClelland et al. 2006), which diminish lactate accumulation. The lower concentration of lactate may enable longer lasting swimming events and faster recovery after training.
The responses to training seem to vary between different kinds of training programs used. Moreover, it has been shown that the responses differ between different fish species or even different populations of the same species (Nelson et al. 1994).

### Table 2. Some effects of high-intensity (H) and endurance (E) training on fish.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Training type</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endurance ↑</td>
<td>E</td>
<td><em>Salmo gairdneri</em></td>
<td>Houlihan &amp; Laurent (1987)</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td><em>Salmo salar</em></td>
<td>McDonald et al. (1998b)</td>
</tr>
<tr>
<td>Critical swimming velocity (U_{crit}) ↑</td>
<td>H</td>
<td><em>Salmo gairdneri</em></td>
<td>Holk &amp; Lykkeboe (1998)</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td><em>Danio rerio</em></td>
<td>McClelland et al. (2006)</td>
</tr>
<tr>
<td>Maximal oxygen consumption ↑</td>
<td>H</td>
<td><em>O. tshawytscha</em></td>
<td>Gallaugher et al. (2001)</td>
</tr>
<tr>
<td>Aerobic capacity ↑</td>
<td>E</td>
<td><em>Salmo gairdneri</em></td>
<td>Davie et al. (1986)</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td><em>Danio rerio</em></td>
<td>van der Meulen et al. (2006)</td>
</tr>
<tr>
<td>Mitochondria number ↑</td>
<td>E</td>
<td><em>L. cephalus</em></td>
<td>Sänger (1997)</td>
</tr>
<tr>
<td>Activity of oxidative enzymes ↑</td>
<td>H</td>
<td><em>Danio rerio</em></td>
<td>McClelland et al. (2006)</td>
</tr>
<tr>
<td>Activity of β-oxidation enzymes ↑</td>
<td>H/E</td>
<td><em>P. virens</em></td>
<td>Johnston &amp; Moon (1980a)</td>
</tr>
<tr>
<td>Myoglobin concentration ↑</td>
<td>E</td>
<td><em>Danio rerio</em></td>
<td>van der Meulen et al. (2006)</td>
</tr>
<tr>
<td>The proportion of red muscle ↑</td>
<td>E</td>
<td><em>Danio rerio</em></td>
<td>van der Meulen et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td><em>Morone saxatilis</em></td>
<td>Young &amp; Cech (1993b)</td>
</tr>
<tr>
<td>Capillary density ↑</td>
<td>E</td>
<td><em>Salmo gairdneri</em></td>
<td>Davie et al. (1986)</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td><em>C. chalcoides</em></td>
<td>Sänger &amp; Pötcher (2000)</td>
</tr>
<tr>
<td>Recovery rate ↑</td>
<td>E</td>
<td><em>Morone saxatilis</em></td>
<td>Young &amp; Cech (1993a)</td>
</tr>
<tr>
<td>Lactate dehydrogenase activity ↑</td>
<td>H</td>
<td><em>Salmo gairdneri</em></td>
<td>Pearson et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td><em>Danio rerio</em></td>
<td>McClelland et al. (2006)</td>
</tr>
<tr>
<td>Glycolytic enzymes (white muscle) ↓</td>
<td>E</td>
<td><em>S. fontinalis</em></td>
<td>Johnston &amp; Moon (1980b)</td>
</tr>
</tbody>
</table>

#### 2.4 Wild versus reared fish

In addition to differences between trained and non-trained fish, there are also differences in the muscles between wild and reared fish. In previous studies it has been shown that wild fish have a higher concentration of proteins and myosin in their muscles (Carpene’ et al. 1998; McDonald et al. 1998b). Moreover, the lipid content is considerably smaller in wild fish as compared to reared ones (McDonald et al. 1998b; Grigorakis et al. 2002). It has been speculated that high lipid content may disturb the swimming performance of fish (McDonald et al. 1998b). Moreover, the performance of muscles affects the swimming capacity of fish (Martínez et al. 2003). In the wild fish, both sprint performance (McDonald
et al. 1998b; Basaran et al. 2007) and endurance capacity (Rimmer et al. 1985) have thus been noted to be superior as compared to reared fish. However, there is a lack of studies concerning calcium handling capacity in swimming muscles of wild fish.

The inefficient function of muscles could be one of the reasons affecting the survival of reared fish in nature. In the wild, there is no longer a constant supply of food and protection from predators as in the hatcheries (Olla et al. 1998). These basic events require high performance of the muscles, which seems to be reduced in reared fish. Lower swimming performance of reared fish (Rimmer et al. 1985; McDonald et al. 1998b; Grigorakis et al. 2002) could thus reduce their survival probability. Indeed, in several previous investigations it has been recorded that the survival of reared fish is much lower compared to wild fish (Einum & Fleming 2001; Kallio-Nyberg et al. 2004; Jokikokko et al. 2006). In the study of Jonsson et al. (2003) the survival rate of wild salmon was about 8.9%. However, in reared salmon the probability is merely 0.2–2.9% (Jonsson et al. 2003). Jutila et al. (2003) found a similar result, the marine survival rate of wild salmon being 5.7 times higher as compared to reared salmon.
3 Aims of the study

Releasing reared fish to nature is an extremely large business at present. Billions of fish are released worldwide to supplement and restore initial populations, for example. However, the probability of reared fish to survive in nature is poor. This means a very low efficiency of the releases. In the present study, the differences in the functional variables involved in the muscle contraction capacity of swimming muscles of wild and reared salmon are compared in order to find out if these variables could explain the observed lower swimming capacity and, further, lower survival rate of reared fish. Moreover, an attempt to improve the muscle contraction capacity of reared fish closer to that of wild fish is done by means of training. The effect of training on the migration pattern, one of the factors affecting the survival of fish, is also analyzed. The specific aims of this thesis are:

1. To study the differences in the relative level of DHP and Ry receptors in swimming muscles of wild and reared salmon. Moreover, the water flow velocities of habitats are compared to evaluate the significance of flow velocity on the differences between wild and reared fish (I)
2. To extend these comparisons to the electron microscope level and to analyze the energy metabolism of wild and reared salmon in order to evaluate the prerequisites for effective contraction mechanism (II).
3. To enhance the level of DHP and Ry receptors in swimming muscles of reared salmon by means of training. Different training velocities and durations are tested in order to find the most effective training protocol enhancing the contraction capacity (III).
4. To study the effects of training on fish species having dissimilar swimming patterns in order to find out if the swimming pattern of fish affects the responses of training. Moreover, the connection between the level of receptors and swimming capacity of fish is investigated (IV).
5. To train the salmon with a previously tested protocol (III) before releasing to the wild. The aim is to investigate how training affects the muscles of fish and whether the changes affect migration pattern of fish (V).

The hypothesis of the thesis is that the relative level of receptors is connected to the muscle contraction capacity and that these are higher in wild salmon as compared to reared salmon. However, a proper training program may alter the level of receptors in muscles of reared fish closer to that of wild fish and thus affect the swimming capacity of fish.
4 Materials and methods

The details of the studies are described in the original papers. The experiments were performed in accordance with the Animal Ethics Committee of the University of Oulu (license no. 083/04).

4.1 Animals

This study was performed in collaboration with the Finnish Game and Fisheries Research Institute, which provided the fish for the experiments. Juvenile reared salmon (*Salmo salar* L.) aged 0+ (fingerlings) and 1+ (yearlings) for the comparisons between reared and wild fish (I-II) were obtained from Paltamo fish farm, Finland (64°24’ N, 27°31’ E) (Fig. 5). Wild salmon from the same age groups were caught by means of electrofishing from the River Simojoki (2005) (65°38’ N, 25°00’ E) (Fig. 5). In 2006 only the muscles of wild and reared yearlings were compared (II). The water flow velocities of the habitats were measured from the site of catching. For the training studies (III-IV) salmon (aged three years), brown trout (*Salmo trutta* m. *fario*, aged two years) and whitefish (*Coregonus lavaretus* (L.) s. str., aged two years) were maintained at Taivalkoski fish farm, Finland (65°34’ N, 28°15’ E) (Fig. 5). The salmon for the study investigating the effect of training on the migration pattern of fish (V) were obtained from Paltamo (year 2007) and from Taivalkoski (year 2008). In the hatcheries, the natural water temperature and photoperiod was maintained. Fish were fed commercial fish pellets (Bio-Optimal, Bio-Mar, Finland and Royal Response, Rehuraisio, Finland). Food was offered to the point where fish stopped eating. For the training studies the amount of food was 1.5 higher in the trained fish as compared to the non-trained, control ones to ensure that there is enough food to compensate the possible increased food consumption of the fish after training. The food was offered twice per day, before and after the training.
4.2 Training protocols

For the training studies (III-IV), fish were anesthetized with 100 mg l\(^{-1}\) tricaine methanesulphonate (MS 222) (Sigma, USA), weighed, and measured. The fish were divided into distinct groups according to length. The fish were allowed to recover and adapt to the rotation current aquarium system for two weeks before training. During the training, the fish were swimming against one of the three water flow velocities used in the studies; 1.0, 1.5, or 2.0 body lengths per second (BL s\(^{-1}\)) for six hours per day five days per week (modified from the study of Jørgensen & Jobling 1993). For the rest of the time, the fish swam against a current velocity of 0.5 BL s\(^{-1}\), which is the minimum current velocity used in regular rearing tanks in Finland. The training period also varied between the groups (2, 4 or 6 weeks). The experiments thus consisted of nine different training groups. The control fish swam in the tanks against a water flow velocity of 0.5 BL s\(^{-1}\).

For the migration behavior study (V), the fish were anesthetized with Benzocaine (0.04 g ml\(^{-1}\), Fagron GmbH, Germany) and weighed, measured and marked with Carlin tags. The fish were divided into two distinct groups: one served as a control group, the second one as a trained group. The fish were allowed to recover from the tagging for three weeks before the onset of training.
The training was performed with a water flow velocity of 1.5 BL s\(^{-1}\), six hours per day, five days per week for two weeks. The control fish swam against a flow velocity of 0.5 BL s\(^{-1}\). After the training, the fish were released into the River Simojoki, Iso-Tainikoski Rapids (65°52′ N, 25°35′ E) (Fig. 5).

### 4.3 Swimming capacity

For analyzing the swimming capacity of fish, three different tests were used. The critical swimming velocity (\(U_{\text{crit}}\)) was measured according to Young & Cech (1993a) (IV). Briefly, during the test the water flow velocity that the fish swam against was increased stepwise until the fish were fatigued. The critical swimming velocity was calculated according to Young and Cech. To analyze the time for 50% of the fish to become fatigued, the method described by Houlihan & Laurent (1987) was used (IV). The swimming endurance of the fish in the migration behavior study (V) was analyzed by allowing the fish to swim in water flow tunnels against a water flow velocity of 4.1 BL s\(^{-1}\). The water flow velocity was increased progressively from zero to 4.1 BL s\(^{-1}\) during 30 seconds. The fatigue time analysis began after the water flow velocity had reached the set value. The time point at which the fish were no longer able to swim against the current and became fatigued was measured.

### 4.4 Condition factor and size of fish

After catching (I-II) or training (III-IV), the fish were killed by cervical dislocation. For the migration study (V), ten salmon were killed before the training started. Moreover, ten trained plus ten control salmon were killed after the training. After three days of migration in the river, ten control, ten trained and ten wild salmon were killed. The total length from the nose to the end of the tail and the weight of the fish were measured in order to calculate the Fulton’s condition factor (CF) (formula from Fulton (1904)).

\[
CF = \frac{(\text{mass} \times \text{length}^{-3}) \times 100}{\text{grams}},
\]

where mass is in grams and length is in centimeters.
4.5 Muscle cryo-sections

After cervical dislocation, the fish were frozen with liquid nitrogen and stored at -80°C before handling. Blocks of muscle caudally from the middle point of adipose fin and tail were removed and cut with cryostat microtome at -20°C into cross sections (14 μm). For detecting the relative amount of dihydropyridine and ryanodine receptors in the sections, one set of sections was fixed in cold acetone. The sections were incubated for 90 minutes in fluorophore-labeled dihydropyridine (20 nM) and ryanodine (0.5 μM) (ST-BODIPY®, Molecular Probes, Netherlands) solutions as described in Mänttäri et al. (2001). Control sections were preincubated in nonfluorescing DHP (nifedipine) or Ry (dandrolone) receptor blocker solutions for 10 minutes before adding the labeling solutions (Larsson et al. 1998). For analyzing the relative level of receptors in the samples, the fluorescence intensity in the sections was measured. The sections were scanned with confocal laser scanning microscope (LSM-5 Pascal, Zeiss, Germany) by using excitation at 543 nm for DHP and 488 nm for Ry receptors. The LSM 5 Pascal software 3.2 (Zeiss, Germany) was used for analyzing the fluorescence intensity in the sections.

The activity of aerobic enzymes in the muscles was also measured from the cryo-sections. Another set of sections was stained for succinic dehydrogenase (SDH) (I-V) activity as described by (Nachlas et al. 1957) and for NADH reductase activity (II) with the modified method of Vacca (1985).

The activity of phosphorylase (I-V) in the muscle sections was measured according to a method modified from Dubowitz and Pearse (1960). Lactate dehydrogenase (LDH) activity was measured after incubation of sections in substrate solution containing: NAD⁺ 30.5 mmol l⁻¹, lithium lactate 30.5 mmol l⁻¹, Nitro Blue Tetrazolium 1.2 mmol l⁻¹, N-methylglucamine 200 mmol l⁻¹ (II). The analysis of capillary density (II) in the cryo-sections was performed according to the method described by Andersen (1975). For measuring the relative activity of enzymes, the average intensities of the staining were analyzed with LSM 5 Pascal software 3.2 (Zeiss, Germany). The same software was also used for counting the density of capillaries. The proportion of red muscle of the lateral musculature was calculated from SDH-stained cryo-sections. The measurement of areas of red and white muscle was performed with ImageJ 1.41 (Wayne Rasband, National Institutes of Health, USA).
4.6 Tissue oxygen consumption

The O2 consumption and, thus, the activity of cytochrome c oxidase in red and white muscle of reared and wild salmon was measured with Clark-type platinum O2 electrode system (Bachofer GmbH, Germany) (II). Blocks of muscles, removed caudally from adipose fin, were homogenized in homogenization buffer as described in (II). The oxygen consumption rate was measured according to Saarela et al. (1989). Pico ADC-16 High resolution data logger to PicoLog software for Windows (Pico Technology LTD, UK) was used to detect the change in O2 concentration as a function of time.

4.7 Transmission electron microscopy

To evaluate the amount of mitochondria, triads and lipid droplets in the muscles of wild and reared salmon (II) a transmission electron microscopy method was used. Muscle blocks were fixed in glutaraldehyde and formaldehyde solution according to Proctor et al. (1980) and embedded in Epon as described in (II). Leica Ultracut UCT ultramicrotome (Leica Microsystems, Austria) was used to cut 80 nm thin sections. The sections were examined with a Philips CM100 transmission electron microscope. CCD camera was used to capture the images and the analysis was done with TCL-EM-Menu version 3 from Tietz Video and Image Processing Systems GmbH (Gaunting, Germany). The density of mitochondria and lipid droplets was analyzed by counting the number of organelles from an area of 220 µm² (magnification used 1600×). For triad density the measuring area was 70 µm² (magnification used 2850×). The size of mitochondria and lipid droplets was analyzed by taking an average from cross-section area of ten transversely cut organelles from different sections.

4.8 SDS-PAGE and Western blotting

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting were used to analyze the relative level of DHP and Ry receptors in the white muscles of fish (I, III). Blocks of muscle were homogenized in homogenization buffer and denatured in sample buffer (Laemmli 1970) as described in (I and III). The total protein concentration in the samples was assayed according to Bradford (1976). Samples containing 24 µg of protein were separated electrophoretically at 150 V for 40 minutes. The method of Towbin et al.
(1979) was used to electroblot the proteins to nitrocellulose membrane. Western immunodetection of proteins was performed by incubating the membranes for two hours in primary antibody solution containing DHP receptor α1S antibody (1:250, Santa Cruz Biotechnology Inc., USA) and Monoclonal Anti-Ryanodine receptor Clone 34 C antibody (1:4000, Sigma-Aldrich Inc., USA). After the first antibody incubation the membranes were incubated with secondary antibody solution containing Blotting Grade Affinity Purified Goat Anti-Mouse IgG H+L Alkaline Phosphatase Conjugate antibody (1:3000, Bio-Rad, USA), also for two hours. The proteins were visualized using BCIP/NBT substrate. The optical densities of the detected bands were analyzed with the FluorS MultiImager program (Bio-Rad, USA).

The average coefficient of variation (CV) for Western blotting method was 0.4 in (I) and 0.3 in (III). The CV values of the fluorescence labeling method, also used for determining the relative level of receptors in muscles, were 0.4 (I) and 0.4 (III), respectively.

4.9 Recapture rates

To evaluate the downstream migration speed and the recapture rates of control, trained and wild salmon (V), a smolt trap was placed close to the estuary of the River Simojoki, 44 km downstream from the Iso-Tainikoski rapids, where the salmon were released (Fig. 5). The trap net closed about one-third of the river. Wild salmon smolts caught with a rotary smolt screw trap were tagged with plastic streamer tags (PST) 2 km upstream from the smolt trap. The number of control, trained and wild salmon caught per day with the smolt trap was calculated and compared between different groups (2007). In 2008 the study was repeated to ensure that environmental variables did not influence the results. The migration speed and recapture rates of trained and control fish were analyzed in 2008.

4.10 Statistics

The differences in the physiological parameters between reared and wild juvenile salmon were analyzed with independent samples t-test (I-II). Spearman’s correlation test was used to evaluate the correlation between DHP and Ry receptor levels with the activity of SDH in muscle section (I). For the training studies (III-V) the differences in muscular parameters of trained and control fish were
analyzed with independent samples t-test. A Mann-Whitney U-test was used to analyze the differences in swimming performance between control and trained groups (IV-V). Independent samples t-test was used to analyze the difference in migration velocity between trained and control salmon (V). The differences between changes in training groups were evaluated by two-way ANOVA using training velocity, and duration as the factors (III-IV). One-way ANOVA was used to compare the effects of training between fish species (IV), sex (V) and condition factors (III-IV). All the statistical analyses were made with R for Windows software (R-2.2.1 or R-2.6.0 version).
5 Results

In general, the relative level of DHP and Ry receptors as well as the oxidative capacity was higher in wild salmon as compared to reared fish. Training of reared fish brought the levels of parameters closer to those of wild fish. The detailed results are described in the original papers.

5.1 Differences in muscle contraction parameters between wild and reared fish (I, II)

In the first and second paper of the thesis the functional parameters of muscles of wild and reared juvenile salmon were compared. Shortly, the results show that the muscles of wild salmon have both higher level of receptors involved in the muscle contraction mechanism and higher capacity for oxidative metabolism.

5.1.1 Water flow velocity

In order to evaluate the differences between water flow habitats for reared and wild salmon, the water flow velocities in the river and tanks were measured (I–II). In 2005, the flow velocities for the wild and reared fingerlings were 5.36±0.22 BL s⁻¹ and 0.82±0.03 BL s⁻¹, respectively. For the yearlings, the same values were 3.16±0.06 BL s⁻¹ and 0.66±0.03 BL s⁻¹. There was a 554 and 379% difference between the wild and reared salmon (p<0.001) (I). In 2006, the flow velocities for wild and reared yearlings were 4.86±0.06 BL s⁻¹ and 1.21±0.04 BL s⁻¹. The percentual difference was 301.7% (p<0.001) (II). There was a significant difference in water flow velocities between the years since in 2006 both wild (p<0.001) and reared (p<0.001) yearlings had to swim in higher water flow velocity compared to the yearlings from 2005.

5.1.2 Size of fish

In both 2005 and 2006, the condition factor (p<0.05) as well as the size (p<0.01) of the wild fish was significantly lower compared to reared fish. However, in 2006 the difference in the size between wild and reared salmon was not as great as in 2005 (p<0.001). The reared fish in 2006 were significantly smaller than the reared fish in 2005 (p<0.001).
5.1.3 *DHP and Ry receptors in swimming muscles*

Fluorescence labeling and Western blotting methods were used to evaluate the relative level of dihydropyridine and ryanodine receptors in the swimming muscles. The results showed a significant difference between the wild and reared juvenile salmon. In the first study done in 2005 (I), the relative level of DHP and Ry receptors were higher (p<0.001) in both red and white muscle types of fingerlings as analyzed with the fluorescence labeling method. Similar differences were also noted in the yearlings in the DHP receptor levels (p<0.001) and even higher in the Ry receptor levels (p<0.001). The percentual differences in the relative level of DHP and Ry receptors between wild and reared fish are presented in Tables 3 and 4. Interestingly, with the Western blotting method the differences were not that significant when analyzed from the white muscles of fingerlings (p<0.05). In the yearlings there was no significant difference between wild and reared salmon, or the difference was reversed (p<0.01). According to the results of the fluorescence labeling method, the relative level of Ry receptor decreases as a function of age in the reared fish, since in the fingerlings the level of receptors was higher compared to the yearlings (p<0.001). However, the Western blotting method showed opposite results, since the level of receptors was higher in the older fish (p<0.01). In wild salmon the fluorescence labeling method showed a decreasing trend in the level of DHP receptors as a function of age (p<0.05). With the Western blotting method, no significant differences were observed between the age groups of wild salmon.

In the second study (2006, II), where the muscles of wild and reared salmon were compared, only the fluorescence labeling method was used. The results showed that the levels of receptors were also significantly higher in the muscles of wild yearlings as compared to the reared ones (p<0.001, Tables 3 and 4). When comparing the first (I) and second (II) study together in 2006, some of the values were higher when compared to 2005 as analyzed from fluorescence labeling results. In the wild 2006 yearlings, the level of both receptors was higher in the white muscle when compared to the white muscle of wild yearlings from 2005 (p<0.01). In the reared 2006 yearlings the levels of Ry receptors were higher in both muscle types as compared to the muscles of reared yearlings from 2005 (p<0.001).
Table 3. Percentual difference in the relative level of dihydropyridine receptor (DHPR) and ryanodine receptor (RyR) as well as in the activity of succinic dehydrogenase (SDH) in red muscle of wild salmon as compared to reared salmon.

<table>
<thead>
<tr>
<th>Fish group</th>
<th>DHPR</th>
<th>RyR</th>
<th>SDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fingerling -05</td>
<td>109.8% ***</td>
<td>123.3% ***</td>
<td>570.2% ***</td>
</tr>
<tr>
<td>Yearling -05</td>
<td>153.5% ***</td>
<td>459.1% ***</td>
<td>889.8% ***</td>
</tr>
<tr>
<td>Yearling -06</td>
<td>131.4% ***</td>
<td>89.8% ***</td>
<td>50.0% **</td>
</tr>
</tbody>
</table>

Significance of difference between wild and reared salmon *** p < 0.001, ** p<0.01.

Table 4. Percentual difference in the relative level of DHPR and RyR as well as in the activity of SDH in white muscle of wild salmon as compared to reared salmon. The methods used for analyzing the level of DHPR and RyR were fluorescence labeling (Fluor) and Western blotting (WB).

<table>
<thead>
<tr>
<th>Fish group</th>
<th>DHPR Fluor</th>
<th>RyR Fluor</th>
<th>SDH Fluor</th>
<th>DHPR WB</th>
<th>RyR WB</th>
<th>SDH WB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fingerling -05</td>
<td>128.6% ***</td>
<td>75.0% *</td>
<td>186.0% **</td>
<td>92.9% **</td>
<td>72.1% **</td>
<td></td>
</tr>
<tr>
<td>Yearling -05</td>
<td>131.2% ***</td>
<td>-24.1%</td>
<td>858.4% ***</td>
<td>-55.5% **</td>
<td>87.4% ***</td>
<td></td>
</tr>
<tr>
<td>Yearling -06</td>
<td>169.9% ***</td>
<td>NA</td>
<td>150.9% ***</td>
<td>NA</td>
<td>16.1%</td>
<td></td>
</tr>
</tbody>
</table>

Significance of difference between wild and reared salmon *** p < 0.001, ** p<0.01, * p<0.05. NA not analyzed.

5.1.4 Enzyme activities and capillary density in muscles

The activities of aerobic and anaerobic enzymes as well as capillary densities were measured in order to analyze the energy metabolism of the swimming muscles. According to the results, in the muscles of wild salmon the activities of aerobic enzymes were significantly higher compared to reared salmon (I-II). Both in the fingerlings and yearlings, the activity of SDH was higher in the wild group (p<0.01, Tables 3 and 4) (I-II). Moreover, the activity of SDH correlated positively with the level of both receptors (p<0.01) (I). According to the results, the activity of NADH reductase, LDH and cytochrome c oxidase was higher in the red muscles of wild as compared to reared salmon (76%, 63% and 37%, respectively, p<0.01). A similar result was found concerning capillary densities of both red and white muscle types (23 and 28%, respectively, p<0.01). However, the activity of the anaerobic enzyme phosphorylase was considerably lower in the muscles of wild salmon as compared to reared salmon (49 and 47%, p<0.001) (II).
5.1.5 Mitochondria, triad and lipid content of muscles (II)

The density and size of mitochondria were significantly higher in the muscles of wild as compared to reared salmon (II). In red muscle, the density was 39\% (p<0.01) higher in wild fish, and in white muscle the same difference was 68\% (p<0.05). The diameter of the mitochondria in the red muscle of wild salmon was 46\% (p<0.001) and in the white muscle 57\% (p<0.001) higher than in the muscles of reared salmon. The density of triads followed the same pattern (69 and 73\%, p<0.001).

The density and diameter of lipid droplets were, on the other hand, opposite to those of mitochondria. In the red muscle of wild salmon, the density of lipid droplets was 62\% lower than in the red muscle of reared salmon (p<0.01). A similar difference was observed in the diameter of droplets (66\%, p<0.001).

5.2 Effects of training on swimming musculature of reared fish (III-V)

In the third, forth and fifth paper of the thesis the effects of training on the muscular parameters of different fish species and on migration were evaluated. In general, the swimming capacity, level of DHP and Ry receptors as well as oxidative capacity increased after training. Moreover, the training affected the migration speed of salmon.

5.2.1 Swimming capacity and condition factor

To analyze the swimming capacity of the fish, different kinds of tests were used. In paper IV, the \( U_{crit} \) of the brown trout and whitefish was measured before the onset of training. The values were 1.6±0.07 BL s\(^{-1}\) for trout and 2.0±0.06 BL s\(^{-1}\) for whitefish. The time to 50\% of fish in the tank to fatigue by swimming against velocity of 2.5 BL s\(^{-1}\) was measured after each training duration (i.e. 2, 4, and 6 weeks). Compared to control fish, the time to fatigue was significantly longer in trained fish. In brown trout, the group training with the velocity of 2 BL s\(^{-1}\) achieved the best results (p<0.01). In whitefish, the best group was the one training with velocity of 1.5 BL s\(^{-1}\) (p<0.05) (Fig. 1 in IV).

In paper V, the fish were allowed to swim against a water velocity of 4.1 BL s\(^{-1}\) in a flow tunnel, and the time to fatigue was measured. After 2 weeks of
training the trained salmon had at least 280% longer time to fatigue when compared to the control salmon (p<0.05).

The condition factors of fish were slightly dissimilar between the groups (p<0.01). In the whitefish (IV) the CF of the control fish seem to be slightly higher when compared to the training groups. In the migration study the fish were bigger in 2007 than in 2008 (length: p<0.001 and weight: p<0.001).

5.2.2 DHP and Ry receptors in trained fish

Training enhanced the level of receptors in both muscle types and in all fish species tested (III-V). The positive changes were seen with all water flow velocities and training durations used. However, the training programs differed from each other both according to training duration (p<0.001) and velocity (p<0.05, although in brown trout and whitefish the training velocity was not that significant a factor) (Figs 1, 2 in III and Figs 2-5 in IV). In Tables 5 and 6 the training programs enhancing the level of DHP and Ry receptors the most in different species are gathered together. The responses to training varied between all the fish species tested (DHP receptor in red muscle F=3.8, p<0.05; DHP receptor in white muscle F=11.0, p<0.001; Ry receptor in red muscle F=8.0, p<0.001; Ry receptor in white muscle F=121.8, p<0.001).

| Table 5. Training programs increasing the level of dihydropyridine receptor (DHPR), ryanodine receptor (RyR) and activity of succinic dehydrogenase (SDH) the most and reducing the activity of phosphorylase the most in red muscle of different species. |
|---|---|---|---|---|
| Species | DHPR | RyR | SDH | Phosphorylase |
| Salmon | 1.5 BL/s for 2 wk *** | 1.5 BL/s for 2 wk *** | 1.5 BL/s for 2 wk *** | 1.5 BL/s for 2 wk *** |
| Brown trout | 1.0 BL/s for 2 wk *** | 1.0 BL/s for 2 wk *** | 1.5 BL/s for 2 wk *** | 1.5 BL/s for 2 wk *** |
| Whitefish | 1.5 BL/s for 2 wk *** | 1.0 BL/s for 2 wk *** | 1.5 BL/s for 2 wk *** | 1.5 BL/s for 2 wk *** |

Significance of difference between training group and control group *** p < 0.001

| Table 6. Training programs increasing the level of DHPR, RyR and activity of SDH the most and reducing the activity of phosphorylase the most in white muscle of different species. |
|---|---|---|---|---|
| Species | DHPR | RyR | SDH | Phosphorylase |
| Salmon | 1.5 BL/s for 6 wk *** | 1.5 BL/s for 6 wk *** | 1.5 BL/s for 2 wk *** | 1.5 BL/s for 2 wk *** |
| Brown trout | 2.0 BL/s for 6 wk *** | 2.0 BL/s for 6 wk *** | 2.0 BL/s for 2 wk *** | 2.0 BL/s for 2 wk *** |
| Whitefish | 2.0 BL/s for 6 wk *** | 1.5 BL/s for 6 wk *** | 1.0 BL/s for 4 wk *** | 1.5 BL/s for 2 wk *** |

Significance of difference between training group and control group *** p < 0.001, ** p<0.01
In the migration behavior study (V), training with 1.5 BL s\(^{-1}\) also increased the level of receptors (p<0.01) in both muscle types of salmon. After training, both trained and control salmon were released to the wild. Swimming for three days in the river against the current and at the same time migrating downstream increased the level of receptors in the control salmon in 2007 (p<0.01). In the trained salmon the level of receptors was still higher when compared to the controls (p<0.05). The highest level of receptors was, however, observed in the muscles of wild salmon after the migration in 2007 and 2008 (p<0.05). The fish in 2007 and 2008 differed from each other. In 2008 the level of receptors was significantly higher than in 2007 (p<0.05).

5.2.3 Oxidative capacity in the muscles of trained fish

Training generally increased the oxidative capacity of the swimming muscles in all fish species (III-V). There were again significant differences between training protocols, with training duration being a factor (p<0.05) (Fig. 3 in III and Figs 2-5 in IV). Tables 5 and 6 show which training programs altered the most the activity of SDH in the swimming muscles of salmon, brown trout and whitefish. The response to training of SDH activity in the muscles of different fish species differed from each other (red muscle F=157.2, p<0.001; white muscle F=83.4, p<0.001). In paper V there was also significantly higher activity of SDH in the muscle of trained salmon as compared to the reared ones (p<0.001), although after migration the activity of SDH increased in the control salmon in 2007 (p<0.05). In wild salmon, the activity of SDH was, however, the highest measured in 2007 and 2008 (p<0.01). Moreover, the proportion of red muscle was significantly higher in the trained salmon as compared to the controls (p<0.01). A similar difference remained after migration (p<0.05). The proportion of red muscle in the lateral musculature was, however, the highest in wild salmon (p<0.01).

While the oxidative capacity of the muscles was enhanced as a result of training, the activity of anaerobic enzyme phosphorylase decreased in the swimming muscles. In a similar manner as the responses in SDH, the fish had differential responses to different training durations at least in white muscle (p<0.05). Tables 5 and 6 present the most effective training programs decreasing the activity of phosphorylase. In the migration study (V), training also reduced the activity of phosphorylase (p<0.001), and a similar difference between trained and control salmon remained also after migration (p<0.05). In wild salmon, the
activity of phosphorylase was the lowest (p<0.001). The activity of both phosphorylase and SDH differed between the years in the study (V). In 2008, the activity of SDH was higher and phosphorylase lower when compared to most of the groups in 2007 (p<0.05).

5.3 Migration speed and recapture rates (V)

To investigate the migration speed of salmon, a smolt trap was placed downstream from the releasing site. The temperature in the river varied between 13.0–18.2°C in 2007 and between 12.5–16.1°C in 2008 during the experiment. In 2007, the control salmon migrated downstream in the river in 1.77±0.08 days, while in trained salmon the migration took 2.43±0.12 days. The groups differed from each other significantly (p<0.001). In the wild salmon a migration of two kilometers took 1.33±0.079 days. The catching rates of control salmon per day were not equally divided since 67.9% of all caught control salmon were caught the first day after the release. The corresponding number for trained salmon was 49.8%. The total recapture rates of all released salmon were 19.3% for control salmon, 12.9% for trained salmon and 14.2% for wild salmon. In 2008, the downstream migration time of control fish was 6.55±0.25 days and that of trained salmon 7.26±0.31 days (p<0.05). Total recapture rates were 13.6% for control salmon and 12.0% for trained ones.
6 Discussion

In the past few decades more and more attention has been given to the inconclusiveness of fish stocking programs. Only a minor fraction of the released reared fish survive until maturity. Among the wild fish the probability to survive is substantially higher, although their survival probability has also decreased since the 1990s (ICES 2008). Previously, the reasons for the lower survival rate of reared fish have been analyzed by comparing e.g. the behavior and swimming capacity of wild and reared fish. In the current study, the comparison goes a degree further. The differences in the muscular parameters are evaluated in order to clarify which cellular factors could explain the previously measured differences in e.g. swimming capacity between wild and reared fish. As it is, the whole performance of the fish is eventually based on the cellular mechanisms.

In order to evaluate the role of cellular parameters in the observed differences in swimming capacity and, thus, in the survival rate of wild and reared salmon, the muscles of wild and reared fish were first compared at cellular level. Moreover, the habitats of reared and wild fish were compared in order to analyze which factors led to the muscular differences.

In addition to comparing wild and reared fish, one of the main objectives of the thesis was to shift the level of cellular, functional parameters of the muscles of reared fish closer to those of wild ones. By influencing the cellular mechanisms, the overall fitness of the fish could be impacted, thus affecting the survival of the fish. In order to achieve changes in the muscles, the habitat of reared fish has to resemble wild conditions more closely. According to the analysis, one of the factors varying between habitats is water flow velocity. Therefore, increasing the water flow velocity in rearing tanks, i.e. inducing training, was chosen as the way to influence the functional parameters of muscles.

In general, it was observed that the level of dihydropyridine and ryanodine receptors, the two main receptors involved in excitation contraction coupling, is significantly higher in the muscles of wild salmon than in the muscles of reared ones. A similar result is seen in the amount of membrane structures expressing these receptors. In the swimming muscles of wild fish, the activity of enzymes involved in the aerobic energy metabolism is also considerably higher as compared to the muscles of reared ones. On the other hand, the activity of anaerobic enzymes is lower. These results are related to differences in mitochondrial composition. The number of mitochondria is significantly higher in the muscles of wild salmon. The habitats of wild and reared salmon also differ
since wild fish have to swim in water flow velocity that is more than four times higher. This is one of the factors affecting the muscular fitness of fish.

In this study it was also observed that by training it is possible to enhance the level of DHP and Ry receptors in swimming muscles of reared fish. After exercise the muscular Ca$^{2+}$-regulating mechanism begins to resemble more that of wild fish. A similar result is seen in the oxidative capacity of muscles. Moreover, the swimming performance of fish increases. According to the results, training improves the measured muscular parameters in all fish species studied. However, there are variations in responses to training between species. The differential swimming pattern is most likely one of the factors affecting the results. Moreover, the intensity of training is also a major factor influencing this variation.

The changes in muscular as well as swimming capacity of trained salmon also affect migration speed, which begins to resemble more that of wild fish. It is thus concluded that by modifying the cellular mechanism of the muscles, the whole behavior and performance of reared fish could be modified to bear closer resemblance to wild fish.

6.1 DHP and Ry receptors in the muscles of wild, trained and reared fish

One of the functional parameters of muscles analyzed in the current study is the level of DHP and Ry receptors. The amount of the receptors in the muscles of wild fish formed a reference level at which the training of the reared fish aimed.

6.1.1 The relative level of DHP and Ry receptors in swimming muscles of wild and reared salmon

The results clearly show for the first time that the level of DHP and Ry receptors is significantly higher in both red and white muscle of wild juvenile salmon compared to reared salmon.

These two receptors are a relevant functional part of the muscles. They are involved in the initiation of muscle contraction by regulating the release of calcium from SR to cytoplasm (Lamb 2000; Fill & Copello 2002). Moreover, the Ca$^{2+}$ concentration in the cytoplasm which these receptors regulate is one of the parameters defining muscle force (Hellam & Podolsky 1969). Ca$^{2+}$ is involved in changing the conformation of the troponin-tropomyosin complex, which thereafter allows cross-bridge binding between myosin and actin. Since Ca$^{2+}$
regulates the binding of myosin and actin (Gordon et al. 2000) and the force of the muscle is dependent on the amount of actin-myosin overlap (Herzog et al. 2008) and the amount of Ca\(^{2+}\) activated binding site for myosin (Gordon et al. 2000), it can be said that force development is at least partly dependent on Ca\(^{2+}\) concentration (Westerblad & Allen 1996). On the other hand, a high level of receptors involved in the release of Ca\(^{2+}\) from SR can both accelerate the rise of Ca\(^{2+}\) concentration in cytoplasm and probably increase the Ca\(^{2+}\) concentration during muscle contraction. This explains the observed positive correlation between the level of receptors and velocity and force of the contraction in mammals (e.g. rodents) (Kandarian et al. 1992; Zhong et al. 2001; Golden et al. 2003; Määttäri & Järvelä 2005). In fish, the connection is only shown in different muscle types; white muscle has both a higher level of receptors and faster contraction velocity compared to red muscle (Akster et al. 1985). Thus, based on the receptor level results of the current study and on the studies of the level of receptors and the contraction efficiency of the muscles, it is concluded that the muscle contraction ability of wild salmon is superior compared to reared ones.

Moreover, according to the results, the density of membranes expressing the DHP and Ry receptors, i.e. triads, is higher in the muscles of wild fish. Since the action potential moves along T-tubules into the muscle fibers (Lamb 2000; Fill & Copello 2002), the higher level of triads could lead to more efficient conduction of depolarization wave into the muscles and thus possibly faster contraction velocity. The activation of the contraction machinery of muscles still occurs through the receptors, not through the membranes themselves. Since both the amount of receptors and the density of triads is significantly higher in the swimming muscles of wild salmon, the EC coupling as a whole is more efficient in the muscles of wild fish as compared to reared ones.

### 6.1.2 Effect of training on the relative level of DHP and Ry receptors

Since there were significant differences in the relative level of both DHP and Ry receptors between wild and reared fish, one of the purposes of the current study was to increase the level of muscular variables of reared fish closer to that of wild fish. This was done by means of training. Interestingly, there are no previous studies available concerning the effects of training on DHP and Ry levels in fish swimming muscles. It was thus observed for the first time that training in general increases significantly the level of receptors in the muscles of all of the fish
species studied. As a result of training, the muscle structure of reared fish actually begins to resemble that in wild fish.

The increase in water flow velocity in the rearing tanks for training leads to more active swimming and thus requires more active use of muscles. Since the level of receptors has a positive effect on the contraction capacity of muscles as discussed above (Kandarian et al. 1992; Zhong et al. 2001; Golden et al. 2003; Mänttäri & Järvilehto 2005), the increment in receptor density is one way to respond to the demands of increased muscle activity. Moreover, the results indicate that the efficiency of muscle contraction is higher in trained fish as compared to normally reared, control ones. According to the current results there actually seems to be a connection between the level of receptors and the swimming capacity of fish. In both brown trout and whitefish, the anaerobic swimming capacity, measured with fixed-velocity test, and the level of receptors in white muscle measured with both fluorescence intensity levels and with relative difference to the control group are convergent in most of the training groups. According to McDonald et al. (1998a) the fixed-velocity test is optimal for comparing the swimming capacity with other physiological capacities. In the swimming capacity test the fish are forced to swim actively. This means that the contraction-relaxation cycle of muscle fibers, including release of $\text{Ca}^{2+}$ from SR and pumping it back to SR, must act fast and continuously. High levels of both DHP and Ry receptors enable fast and high-volume release of $\text{Ca}^{2+}$ from SR to force production, which results in fast contraction velocity (Kandarian et al. 1992; Zhong et al. 2001; Golden et al. 2003). The high level of receptors on its part accelerates the contraction-relaxation cycle of muscle fiber, which enables high activity level of muscles for the swimming test. Moreover, a high level of receptors may affect the fatigue time. It has been shown that one factor causing fatigue is inhibition of receptors and $\text{Ca}^{2+}$ release rate from SR by e.g. low ATP concentration, raised concentration of Mg$^{2+}$ or metabolites of ATP (Lamb 2002). The high level of receptors in the triad junction may enable longer time until fatigue since it takes a longer time to inhibit the receptors if receptor density is high. This is also seen in the current study since there seems to be a connection between a higher level of receptors and longer time to fatigue in trained fish as compared to control ones. According to our results, the cellular function thus really does have an effect on the performance of the body as a whole.

The present results also support the observations of previous studies in mammals. Training has been observed to increase the level of DHP and Ry receptors in the skeletal muscles of mice, rats, and humans (Saborido et al. 1995;
Moreover, Ørtenblad et al. (2000; Mänttäri et al. 2006). Moreover, Ørtenblad et al. (2000) showed that besides an increase in the level of receptors after training, muscle performance also becomes more effective.

Although training increased the level of both DHP and Ry receptors in the swimming muscles of fish, the increment seems to be more evident in the level of Ry receptors. One of the factors explaining the result could be the differential myosin heavy chain (MHC) profile of trained and reared fish. At least in mammals, the muscles containing different MHC composition have a different ratio of DHP and Ry receptors (Fill & Copello 2002) and the changes in MHC type composition at muscular level thus have a differential effect on Ry receptor level as compared to DHP receptor level. It is noted that active swimming alters the MHC type profile of fish muscles (Mänttäri et al. 2005), and thus also in the current study, one parameter that changes after training could be myosin heavy chain composition. Moreover, besides the differences in DHP and Ry level, the possible differences in MHC composition between trained and reared fish could also influence the muscle performance since the different MHC types are connected to the contraction velocity of muscles (Pette & Staron 2001; Weaver et al. 2001). However, the level of DHP and Ry receptors, regardless of the changes in MHC composition, affects the contraction efficiency of muscle. In the study of Ørtenblad et al. (2000) there is a significant increase both in the level of receptors and in muscle performance, but no changes in the MHC composition. In conclusion, the results of this study show that in trained fish both the level of receptors and swimming capacity is higher as compared to non-trained ones. Moreover, one explaining factor for the higher swimming capacity, i.e. longer time to fatigue in swimming performance test in trained fish is their higher level of both DHP and Ry receptors.

Interestingly, the white muscle responded to training at all water flow velocities used. It has been shown that the activity pattern of muscles follows the swimming velocity. Red muscle is active in slow swimming events and the activity level of white muscle increases as the swimming velocity increases (Altringham & Ellerby 1999). However, it has been shown that white muscle is also active at slower swimming velocities (Wilson & Egginton 1994; Day & Butler 1996), and the characteristics of white muscle change as well after training with sustainable swimming velocities (Gruber & Dickson 1997; Sänger & Pötscher 2000). This could explain the result that white muscle responded to training also at the velocity of 1 BL s⁻¹. On the other hand, the changes in red muscle at slow swimming velocities could also activate genes in white muscle via
paracrine factors, since it is well known that muscle activity releases paracrine factors from muscle cells, which may alter the expression of genes in neighboring cells (e.g. Hawley 2002; Bugeon et al. 2003).

### 6.1.3 Methods for determining the level of DHP and Ry receptors

In the current study the measurement of the relative level of DHP and Ry receptors was done with both Western blotting and fluorescence labeling methods. Both of the methods indicated that the levels of DHP and Ry receptors were higher in wild and trained fish. However, there were some discrepancies in the results. According to fluorescence labeling results, the relative levels of DHP and Ry receptors were higher in younger fish. On the other hand, the Western blotting method showed quite the opposite result. Moreover, there were differences in the increment of the level of receptors in salmon after training when analyzed with different methods. When analyzing the coefficient of variation values of different methods they were approximately the same. Thus, the dissimilar results of the methods do not seem to result from the inaccuracy of either of the methods. Moreover, quite low CV values of both of the methods indicate that the methods are quite reliable. Furthermore, since the CV values of the methods were approximately the same the variations between samples are the main cause for CV values, not the variations of methods. Thus, the reason for the dissimilar results of the methods must be more physiological rather than an error in the measurements. One reason for the dissimilar results of Western blotting and fluorescence labeling could, thus, be the several isoforms of the receptors. In fish muscles, there are at least three different Ry receptor isoforms (Franck et al. 1998; Morrissette et al. 2000). The dihydropyridine receptors have only been studied in white fibers of fish (Grabner et al. 1991) and there are thus no data about different DHP receptor isoforms in fish muscles. In mammals there are two different DHP receptor isoforms in striated muscles (Fill & Copello 2002). In the Western blotting method, the receptors are identified with antibodies that bind to the specific sequence of receptors. Since the sequences of receptor isoforms may differ from each other, the antibodies might not react with all isoforms in an equal manner. This is the reason why in mammalian studies different antibodies are used when analyzing different receptor isoforms in muscles (Froemming et al. 2000; Jeftinija et al. 2007). The fluorescence labeling method is not based on antibodies, but on fluorescence-labeled dihydropyridine and ryanodine molecules. Since every isoform of the receptors is dihydropyridine or ryanodine sensitive
(Knaus et al. 1992; Fill & Copello 2002; Jeftinija et al. 2007) all of them are fluorescing. The differences between Western blotting and fluorescence labeling results could arise from the observation that increased muscle contraction frequency e.g. after training might change the isoforms of receptors, as is seen in rabbits (Froemming et al. 2000). There might thus be dissimilar isoforms in the muscles of wild, trained and reared salmon. Moreover, there could be changes in the isoforms of receptors during the development, as has previously been observed in rabbits (Froemming et al. 2000). This could explain the differential results from fingerlings and yearlings when analyzed with different methods.

Interestingly, the fluorescence labeling results indicate that the fluorescence is more intense in the red as compared to the white muscle. Previously with Western blotting, fluorescence labeling and electron microscopic methods, it has been shown in both fish and mammals that the amount of receptors is higher in white fibers (Akster 1985; Froemming et al. 2000; Mänttäri et al. 2001; Anttila et al. 2007). One reason for the opposite result in the current study could be the localization of receptors. According to the results of this study, the receptors are localized near the sarcolemma in the white fibers, whereas in the red fibers there are also receptors in the medial parts. In mammals (e.g. guinea pigs) it has been shown that the SR is more complex in the red muscle as compared to the white muscle (Fanzini-Armstrong et al. 1988). In fish, it has also been shown that the T-tubular system of red muscle is particularly well developed (Johnston 1982). Since the fluorescence was measured from the area of fixed size there was more fluorescence in the red fibers as compared to the white ones, probably because of the different localization of receptors.

### 6.2 Oxidative capacity and structure of swimming muscles; relation to swimming capacity

In addition to differences in EC coupling machinery, the differences in energy metabolism were measured from the swimming muscles of wild, trained and reared fish in order to evaluate the efficiency of energy production. Among others, the activity of aerobic and anaerobic enzymes was first compared between the muscles of wild and reared fish. After the comparison, the effects of training on energy production machinery were analyzed in order to evaluate whether training changes the energy metabolism of muscles of reared fish closer to that of wild fish.
6.2.1 Aerobic and anaerobic capacity of muscles of wild and reared salmon

The present study is the first one to evaluate the size and density of mitochondria in wild and reared salmon. Electron microscopy showed that the red muscle of wild salmon contains more and substantially larger mitochondria when compared to the muscles of reared salmon. Since the aerobic metabolism of muscles occurs inside the mitochondria, the results indicate that the oxidative capacity of muscles of wild salmon is superior to reared fish. According to the enzyme activity analysis this is the case since the activity of SDH, one of the citric acid cycle enzymes, is significantly higher in the muscles of wild salmon. Moreover, the activities of NADH reductase and cytochrome c oxidase are higher in the muscles of wild salmon as compared to reared salmon. These enzymes are involved in forming the electrochemical gradient between the intermembrane space and the matrix of mitochondria. ATP synthase uses this gradient when forming ATP (Saraste 1999). The high activities of the mitochondrial enzymes in the muscles of wild salmon concurrently indicate a significantly greater ATP synthesis capacity.

Besides differences in the oxidative enzyme activities, there are also differences in the activity of the anaerobic enzyme phosphorylase between the muscles of wild and reared salmon. Phosphorylase is an enzyme involved in breaking glycogen into glucose molecules (glycogenolysis). Especially white muscle uses anaerobic glycogenolysis for energy supply, and the glucose is mainly transformed into lactate in white muscles during burst swimming (Johnston 1980). According to the results, the density of glycogen granules and the activity of phosphorylase is significantly higher in the white and also in the red muscle of reared fish compared to wild fish. The result indicates indirectly that lactate production is more intense in the muscles of reared salmon. Moreover, comparison of the activity of H-LDH shows that lactate is transformed into pyruvate more actively in wild as compared to reared salmon. The considerably denser capillary network in the muscles of wild salmon further enables more efficient waste removal. On the other hand, the substrates, i.e. glucose and fatty acids for ATP synthesis and O₂ for aerobic metabolism reach the fibers more easily. Thus it is confirmed that wild salmon have a greater ability to both avoid and recover from fatiguing swimming events due to more oxidative energy metabolism and efficient waste removal. The current results, moreover, support
the results of McDonald et al. (1998b), which showed that reared fish became fatigued more quickly than wild ones in a swimming performance test. Interestingly, the differences in the oxidative capacity between wild and reared salmon were more pronounced in red than in white muscle. Moreover, while the density and diameter of mitochondria was higher in white muscle of wild salmon as compared to the white muscle of reared salmon, there were no differences in the activity of aerobic enzymes (year 2006), or the differences were not as significant as those seen in red muscle (year 2005). One explanation for the dissimilarities of red and white muscle could be the differential use of muscles in swimming events and, thus, differential energy metabolism of muscle types. Red muscle is used in cruising swimming events, while white muscle is active in burst type swimming, e.g. escape responses (Gibb & Dickson 2002). Since red muscle is used in long-lasting swimming, the activity of aerobic enzymes is significantly higher as compared to white muscle (III; Love 1980). In the white fibers the activity of glycolytic enzymes is, on the other hand, higher (III; Johnston 1980). The low initial activity of aerobic enzymes in white muscle could be the reason for the observation that there is no significant difference between wild and reared salmon in the oxidative capacities in white muscle.

In addition to enzymes involved in energy metabolism, the source of energy was also determined in the muscles of wild and reared salmon. According to the results, there are more and larger lipid droplets in the muscles of reared salmon as compared to wild ones. The droplets are in large clusters taking space from myofilaments. Moreover, the whole composition of muscles in reared fish is more loose, with significantly greater amounts of cytoplasm and membrane structures between the myofibrils. The structure of muscles with large lipid droplets may also affect the contraction efficiency of muscles. McDonald et al. (1998b) speculated that the high lipid level in the muscles of reared fish could disturb glycolysis. Moreover, the high level of lipids in muscle is connected to low swimming performance.

### 6.2.2 Effects of training on energy metabolism

The results of the current study show that training has a positive effect on the aerobic capacity of the muscles of reared fish. The activity of SDH increases, indicating a higher ATP production capacity of trained fish as compared to those reared normally. Moreover, the proportion of red muscle is higher in trained fish. On the other hand, the activity of phosphorylase declines after training. The
The aerobic pathway to produce ATP is about 15 times more efficient when compared to anaerobic production. This indicates that both the capacity to produce energy and the ability to resist fatigue are also significantly higher in the muscles of trained fish as compared to normally reared ones. Training thus increases the capacity to produce ATP in the muscles of reared fish closer to that of wild fish. The results of the study are supportive to previous investigations where it has been shown that in trained fish, lipids become a more important energy source and the activities of enzymes involved in $\beta$-oxidation (Davison 1997) and aerobic metabolism increase (Davison 1997; McClelland et al. 2006). However, in some studies the activity of anaerobic enzymes has, on the contrary, improved (Johnston & Moon 1980a).

Although the responses in the aerobic capacity were positive in both muscle types after training, there were, however, also dissimilarities between red and white muscles. In red muscle the greatest responses are seen with lower swimming velocity and duration as compared to white muscle. The effect declines with higher intensity of training, whereas the opposite is seen with white muscle. On the other hand, at least in salmon the SDH activity in red muscle did not increase as much as other parameters after two weeks of training, but the activity increased more significantly until four weeks of training and decreased after six weeks. One explanation for this could again be the differential use of muscle types in different swimming events and, thus, differential initial energy metabolism of muscles (Altringham & Ellerby 1999; Gibb & Dickson 2002). In low-frequency training the red muscle mostly powers the swimming, and the properties of red muscle are thus enhanced especially at these velocities. Increasing the intensity of training leads to more active use of white muscle and the activity of red muscle may decline, which is seen in the changes of muscle properties as the training intensity increases. On the other hand, the initial aerobic capacity of red muscle is high and the activity of enzymes may thus be adequate during the initial phases of training. This could explain the result that red muscle SDH activity did not increase significantly until four weeks of training.

### 6.2.3 Connection between the aerobic capacity of muscles and swimming capacity of fish

In general the results showed that the activity of enzymes involved in the aerobic energy metabolism is significantly higher in the muscles of wild and trained fish than in the muscles of reared fish. Previously, it has been noted that the muscles
of wild fish contain more protein and myosin heavy chains (Carpene’ et al. 1998; McDonald et al. 1998b). Moreover, the muscles of reared fish contain more lipids and glycogen when compared to wild fish (McDonald et al. 1998b; Grigorakis et al. 2002). Overall, the fitness of reared fish seems to be more deficient (Grigorakis et al. 2002). According to Martinez et al. (2003) the lower fitness of muscles affects swimming capacity and it has been noted that both endurance (Rimmer et al. 1985; Pedersen et al. 2008) and sprint performance (McDonald et al. 1998b; Basaran et al. 2007) of wild fish are superior.

In the current study it was noted that training increased swimming performance. Control fish had an over three times shorter time to fatigue as compared to trained fish. The activity of SDH was also higher in the trained fish. These results support the previous observations that training increases both the oxidative activity (Davie et al. 1986; Davison 1997; Gruber & Dickson 1997; Sänger 1997; McClelland et al. 2006; van der Meulen et al. 2006) and swimming performance (Holk & Lykkeboe 1998; McDonald et al. 1998b; McFarlane & McDonald 2002; McClelland et al. 2006; van der Meulen et al. 2006) of fish. It has also been noted that the size of muscle fibers increases (Hinterleitner et al. 1992; Gruber & Dickson 1997) via cell proliferation (Martin & Johnston 2006; van der Meulen et al. 2006) after training. Moreover, the proportion of red muscle in the lateral musculature of fish increases (V; Davison 1997; van der Meulen et al. 2006). The better efficiency to convert food to energy in trained fish may enable the hypertrophy of muscles (Davison & Goldspink 1977; Davison 1997; van der Meulen et al. 2006). Furthermore, at least in mammals (including humans) the hypertrophy of muscles seems to be connected to high muscle performance (Bell et al. 2000), and this also seems to be the case in fish. The increment in the proportion of red muscle and the enhanced activity of aerobic enzymes seems to be connected to the increased endurance capacity (Houlihan & Laurent 1987; van der Meulen et al. 2006). Critical swimming velocity ($U_{crit}$) has also been noted to increase (Young & Cech 1993a; Holk & Lykkeboe 1998; McClelland et al. 2006), which could be related to the result that LDH activity increases (Pearson et al. 1990; Gruber & Dickson 1997). In trained fish, the muscles are more capable of resisting fatigue as seen in the current study and, moreover, recovery is faster (Young & Cech 1993b). It has been shown that trained fish are more capable of resisting the water current both in swimming tests and in rivers. According to the results of this and previous studies (Kolok 1992) it is thus confirmed that the muscle fitness of fish is related to their swimming capacity.
6.3 Factors influencing measured differences between wild, trained and reared fish

In order to be able 1) to alter the muscular condition of reared fish closer to that of wild fish and 2) to evaluate the effects of training on muscular capacity, the factors influencing muscular condition and differences between wild and reared fish have to be analyzed. Factors having an effect on the measured differences between wild and reared salmon as well as on the changes in trained fish are numerous. Some factors are environmental, e.g. temperature and flow conditions, while others are more physiological, including the size and the age of fish.

6.3.1 Environmental factors

Flow conditions

One of the most significant factors having an effect on the differences in the muscular parameters between wild and reared salmon is probably the difference in their habitat. Mostly for economical reasons fish are reared in the hatcheries in high densities, and the growth and subsistence of the fish is ensured with unlimited food resources and protection from predators (Olla et al. 1998; Einum & Fleming 2001; Jonsson & Jonsson 2006). In rearing tanks the water flow conditions with constant, low-velocity water current hardly represents that in the wild. Actually, in the current study it was observed that the water flow velocity in the river was 4–6.5 times higher than in the rearing tanks. Therefore, wild salmon are forced to swim in higher water flow velocity. In the training studies, flow conditions mimicked the high water flow velocity in the natural habitat. As seen in previous and current studies, the enhanced water flow and, thus, swimming activity achieve changes in the muscles of fish (Table 2). One factor affecting the differences in the DHP and Ry receptor level as well as in the oxidative capacity between wild and reared salmon is thus most likely the natural “training” of wild salmon. Furthermore, when analyzing the differences between trained and non-trained fish the differences are for the most part the same as found between wild and reared fish (Table 7). Moreover, it has been speculated that differences in water flow habitats could be one of the factors explaining the observed differences in e.g. morphology and physical fitness between wild and reared fish (McCormick et al. 1998; Pakkasmaa & Piironen 2001; Jonsson & Jonsson 2006).
Table 7. Comparison of the muscles of trained, non-trained and wild fish. The table is compiled from the results of current (I, II, III and IV) studies (*) and from the studies of Rimmer et al. (1985); Davie et al. (1986); Sänger (1997); McDonald et al. (1998b); McFarlane & McDonald (2002); McClelland et al. (2006) and Basaran et al. (2007)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild</th>
<th>Reared</th>
<th>Trained</th>
<th>Non-trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swimming capacity</td>
<td>High</td>
<td>Low</td>
<td>High*</td>
<td>Low*</td>
</tr>
<tr>
<td>Endurance</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Mitochondria level</td>
<td>High*</td>
<td>Low*</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Oxidative activity</td>
<td>High*</td>
<td>Low*</td>
<td>High*</td>
<td>Low*</td>
</tr>
<tr>
<td>Capillary density</td>
<td>High*</td>
<td>Low*</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>DHPR and RyR level</td>
<td>High*</td>
<td>Low*</td>
<td>High*</td>
<td>Low*</td>
</tr>
</tbody>
</table>

Also in the training studies, it was noted that water flow velocity was a significant factor influencing the muscles of reared fish. Water flow velocity was shown to partly determine the effects of training. Moreover, red and white muscle responded differently at different velocities. The same finding was seen between different fish species.

Since water flow velocity is shown to be such a significant factor for the physiology of fish, water flow velocity is also probably one factor behind the differences found between the measurements made in 2005 and 2006 (I and II). In 2006, the muscles of both wild and reared yearlings contained a higher density of DHP and Ry receptors as compared to the muscles of yearlings in 2005. There was a similar difference in water flow velocities in the different years. The water flow velocity was significantly higher in 2006.

Not only does flow velocity differ between rivers and rearing tanks, but there are also differences in flow kinematics. In the hatcheries, the water flow is constantly slow, thus inducing slow, endurance-type swimming, whereas in the wild the flow pattern is more turbulent. The different flow pattern in the environments could, thus, be one factor affecting the muscles of fish. According to the results of the current study (I and II), more burst-type swimming with high intensity seems to have a greater effect on the muscles of salmon as compared to slow, endurance-type swimming. In other species, the responses to different kinds of flow patterns could be different compared to salmon, depending on the initial living environment and swimming pattern of the fish (Pakkasmaa & Piironen 2001).
Selection pressure and food supply

In addition to water flow kinematics, selection pressure differs between the hatchery and the natural habitat. The treatment of diseases, feeding the fish and a sheltered environment affect the fact that mortality in the hatcheries is significantly lower when compared to the natural habitat (Einum & Fleming 2001; Jonsson & Jonsson 2006). This leads to the result that individuals that would have died in the wild survive in the hatcheries. In the natural habitat, on the other hand, only the strongest individuals survive (Einum & Fleming 2001). In the current study, most of the weakest fish had probably already disappeared from the population in the wild at the time of capture, so that the analyzed wild fish represented the strongest individuals. However, the reared fish from the hatchery represented the average population since weak individuals were probably also still alive. The differences in selection pressure between habitats could thus have a major effect on the results.

There are also differences in the availability and quality of food between habitats. In the hatcheries the amount of food is abundant when compared to the wild, and the reared fish are not forced to catch prey (Olla et al. 1998). This could have an effect on the muscles of reared fish. Furthermore, the composition of food differs between hatcheries and the natural habitat. For example, the lipid level in pellets is high (26% in the current study). This is most probably associated with the high concentration of lipids in the muscles of fish, as also seen in previous studies (Sheikh-Eldin et al. 1996; McDonald et al. 1998b; Grigorakis et al. 2002). This supports the assumption that the composition of food influences the composition of muscles. For example, the fatty acid isoforms of reared and wild fish differ, which is speculated to depend on the differential fatty acid compositions of the food (Carpene’ et al. 1998; Grigorakis et al. 2002). Previously it has been observed that a high lipid level in the muscles disturbs glycolysis. Moreover, the lipid concentration affects the swimming capacity of fish. Fish having a high concentration of lipids in their muscles fatigue fast (McDonald et al. 1998b). Food thus has an effect on both lipid concentration and thereafter on the competence of muscles.

Temperature

Among other environmental factors, temperature has also been shown to affect the muscles of fish. In previous studies, it has been noted that cold acclimatization
increases the functional capacity of muscles. Exposure to cold increases for example myosin ATPase activity (Hwang et al. 1990). Differences in the composition of MHCs have also been noted (Crockford et al. 1991; Watabe 2002). The swimming capacity of fish has, moreover, observed to be unchanged in a broad range of temperatures (Randall & Brauner 1991). There must thus be such changes in fibers that the muscle is able produce an equal size of force although the temperature declines. As a result of cold acclimatization the proportion of red muscle in the swimming musculature increases and the density of mitochondria and oxidative capacity is enhanced (Randall & Brauner 1991; Sylvestre et al. 2007). In a cold environment, the fish are furthermore forced to activate more fibers at the same swimming velocity as compared to a warm environment (Randall & Brauner 1991). In the current study (IV), the temperature increased as the duration of training experiment was prolonged. The level of receptors and the swimming capacity of non-trained, control fish declined, on the other hand (Fig. 1 and Tables 2, 3 in IV). It thus seems that the fish in the cold water had more efficient muscle contraction kinetics, and the efficiency declined as the temperature rose. It has been noted that in polar species the maximum muscle tension capacity declines as the temperature rises (Johnson & Johnston 1991). The changes in water temperature could thus partly explain the result that swimming capacity and level of receptors declined during spring, as seen in control fish. However, in the current study the changes in the water temperature were the same for the control and trained salmon. The effects of training could therefore be differentiated from the effects of temperature by analyzing the percentual changes between trained and control fish.

6.3.2 Functional and physiological factors

Smoltification

Besides environmental factors, physiological and functional factors also have a significant impact on the changes observed between wild, trained and reared fish. For example, the level of DHP and Ry receptors changed over time in the non-trained control salmon in III (Tables 2, 3 in III). Although the change in temperature could be one factor affecting the level of receptors as discussed above, the changes in swimming muscles of salmon could also depend on smoltification. Since the experiment was done at a northern location (65°34’) the
salmon underwent smoltification during the experiment (e.g. the color of scales became more silvery, own unpublished data). Previously, it has been observed that the activity of phosphofructokinase increases in white muscle during smoltification (Leonard & McCormick 2001). There is also a decrease in the lipid concentration and the CF of smolts (McCormick et al. 1998). Martinez et al. (1993) noted that the MHC composition was different in the muscles of parrs as compared to smolts. This could be related to the results that parrs have shorter activation and relaxation times as compared to smolts and that the swimming frequency differs between the fish (Coughlin et al. 2001). It has been speculated that these changes during smoltification prepare the smolts for the downstream and feeding migration (Leonard & McCormick 2001). According to these results smoltification could thus be one factor affecting the muscles of salmon. However, the effects of training could again be separated from the effect of smoltification by analyzing the percentual changes between control and trained salmon because the smoltification occurred at the same time in all of the groups.

Swimming pattern

In addition to smoltification, swimming pattern could be one factor influencing the muscles of fish. In the training studies (III, IV) it was observed that different fish species reacted differently to different training programs. In the red muscle of salmon the highest changes in the DHP and Ry receptor levels were noted with the intermediate training intensity while in the brown trout the highest change was seen in the group training with the lowest training intensity. In whitefish, also the intermediate training program produced the most significant changes in the receptor level of the red muscle. One factor influencing the observed differences between species could be the differential living environment and, thus, morphology and swimming pattern of the species. Brown trout live their entire lives in small streams without major migrations (Heggenes 1996). Juvenile salmon, on the other hand, live in fast-flowing rivers, and migrate to the sea after smoltification (Crisp 1996). Whitefish swim continuously in the sea or rivers (Salama & Nikinmaa 1989). According to Pakkasmäa & Piironen (2001), the morphology of fish changes according to the environment. Streamline shape, with a crescent-shaped tail and narrow peduncle, has been shown to correlate with prolonged swimming capacity, while large body depth along the body length is associated with burst-type activity (Webb 1984). When comparing the living environments and body shapes (Fig. 6) of fish species, the body of whitefish is
the most streamlined, and whitefish uses a continuous swimming pattern. On the other hand, salmon is also accustomed to high water flow (Crisp 1996) and, moreover, smoltified salmon are starting to prepare for migration. In both of these species, the highest changes at the receptor level in the red muscle were induced in faster flowing water velocity as compared to brown trout. In brown trout, even a small increment in the water flow velocity caused a significant change in the muscles. According to these results, the initial swimming pattern could be a significant factor affecting the results after training. The observations of the current study support the result of Pakkasmaa & Piironen (2001), who observed that in salmon and lake trout the changes in the fin size and body shape after fast water flow treatment were opposite, and depended on the initial swimming pattern.

Fig. 6. Graph of whitefish (Coregonus lavaretus), Atlantic salmon (Salmo salar) and brown trout (Salmo trutta m. fario). Modified from Čihař (1991).
Age and size of the fish

The age of the fish could also have an effect on the observed differences between wild and reared fish. The level of receptors was 110–186% higher in the muscles of wild fingerlings as compared to reared ones. On the other hand, in yearlings the difference was 131–858%. It seems that the difference was more evident in yearlings than in fingerlings. The result is in accordance with a previous study of McDonald et al. (1998b) where wild and reared salmon from the age group 1+ had a more significant difference in the anaerobic swimming performance than salmon from the age group 0+. Moreover, in the current study a shift in the isoforms of receptors may have occurred during the development, as discussed on page 57. In previous studies a reduction in the tail beat frequency as a function of age has been noted (review of Goldspink 1998). Moreover, the MHC composition changes (Chan et al. 2003), which is thought to have a connection with the change in the swimming pattern (Goldspink 1998). The change in the MHC composition could, on the other hand, be associated with the isoforms of receptors as seen in mammals when different MHC-type muscles and levels of different receptor isoforms are compared (Fill & Copello 2002).

In addition to smoltification, swimming pattern and age, the size of the fish must also be considered as a factor having an effect on the musculature of fish. In the current study, it was observed that wild fish were significantly smaller than reared ones. Moreover, the CF was lower indicating lower weight-to-length ratio of wild fish. There was also a significant difference in the size of salmon between the different years in the migration study (V). The factors influencing the size of the fish are most probably environmental. In the hatcheries fish are fed and they are not forced to swim against high flow velocities (Jonsson & Jonsson 2006). This enables high growing velocity for reared fish. Moreover, it has been shown that fish have a higher growth rate in stable, high water level as compared to more fluctuating conditions (Flodmark et al. 2004), which could be related to water conditions in the hatcheries and in the wild, respectively. In the muscles of most fish species the number of fibers increases throughout life. Moreover, the diameter of fibers increases for example in the cod until the fish is 80 centimeters long (Johnston 1982). Thus, the high growth rate of reared salmon leads to an increased number and size of fibers, but it is not certain whether the inner structures of the fibers develop simultaneously. In the study of Pelletier et al. (1993) it was noted, for example, that the activities of aerobic enzymes were lower in the muscles of bigger fish. Moreover, the density of muscle fibers is
lower in the muscles of bigger fish (Johnston et al. 2000). Besides the inner structure of fibers, the size of fish also has an effect on the swimming pattern. Smaller fish swim with higher tail-beat amplitude than bigger ones (Webb et al. 1984). In the current study the reared fish were bigger than the wild ones. Moreover, the activities of aerobic enzymes were lower in reared fish and there were more cytoplasm and membrane structures between filaments. The size of fish could thus be one of the factors explaining the observed differences between wild and reared fish.

In the current study it was also observed that the level of receptors and activity of SDH in muscles of reared fish in 2006 resembled more that of wild ones than in 2005, i.e. the percentual difference between wild and reared salmon was smaller in 2006. This is also the case with respect to differences in size between wild and reared fish. The bigger size of reared fish in 2005 could also partly explain their lower level of Ry receptors.

Although there are numerous factors explaining the physiological fitness of fish it does not remove the fact that the fitness of wild fish is superior compared to reared ones. However, physiological fitness is not fixed, and the fitness of reared fish can be enhanced by changing their habitat as also noted in the current study after training the fish. By making the habitat more natural both fitness and behavior (Mirza & Chivers 2000; Braithwaite & Salvanes 2005) can be improved.

6.4 Migration

The migration of smolts is divided into two stages: 1) downstream migration in the river from the rearing tributaries to estuary and 2) feeding migration from the estuary to the feeding areas in the sea (McCormick et al. 1998). In previous studies, it has been noted that by changing the habitat in the hatchery to a more natural one (including e.g. the composition of food, presence of predators and flow velocity) before release to the wild could influence the survival of fish (Cresswell & Williams 1983; Olla et al. 1998; Davison 1997; Jonsson et al. 1999; Mirza & Chivers 2000; Stunz & Minello 2001; Braithwaite & Salvanes 2005). In the current study, the smolts were exposed to sustained training with constant water flow before the release. When comparing this environment to the natural habitat of migrating smolt (e.g. long migration to feeding areas in the sea) they are approximately the same. Thus, the training prepares the fish for the migration. Since migration is usually hundreds of kilometers in length (McCormick et al. 1998) it is important that especially sustained swimming
capacity is improved, i.e. the properties of red muscle and oxidative capacity. In the wild fish, the feeding migration occurs at the velocity of 0.5–1.8 BL s\(^{-1}\) whereas the reared fish migrate at a velocity of 0.5–0.6 BL s\(^{-1}\) (Jonsson & Jonsson 2006), which is the velocity used in normal rearing tanks. In the current study the salmon were trained with the velocity of 1.5 BL s\(^{-1}\) for two weeks before releasing them to the wild since 1) the training regimen enhanced the properties of red muscle the most and 2) this training level has the potential to produce a migration pattern resembling that of wild fish. The fish were thus trained with a proper program in order to improve their survival probability. Previously, fish have been trained or acclimated in the water flow before releasing them to the wild, and the survival estimation results have in general been positive (Cresswell & Williams 1983; Davison 1997; Jonsson et al. 1999). The current study differs from the previous ones in that the training program was tested before releasing the fish. According to the results (III, IV), it is not rational to train the fish at whatever velocity or time; the properties of muscles are enhanced the most only with the proper training program, which varies from species to species, depending on their natural environment and swimming pattern.

In the current study the downstream migration pattern of trained and normally reared salmon was analyzed. According to the results, the non-trained reared fish were recaptured significantly earlier as compared to the trained fish. According to McCormick et al. (1998), spring flood produces the highest water flow velocity to the river and the downstream migration is partly a passive drift within the watercourse. Despite this, the fish are partly swimming actively against the current and move in and out of the main current, for example to avoid being caught in backwaters and for capturing prey. According to the results, lower swimming capacity and aerobic metabolism as well as a quicker fatigue rate of reared fish could explain the fact that reared fish appeared in the trap earlier than the trained ones. The reared fish had a lower capacity to swim against the current as compared to the trained ones (V), which may be the reason why the reared fish did not have as good an ability to move out of the current actively as the wild and trained ones. It has been observed that there is a similar difference in the migration pattern between wild and reared fish. Reared fish migrate to the estuaries significantly faster than wild ones (Einum & Fleming 2001; Jonsson & Jonsson 2006). It has been speculated that one reason for the longer time of wild fish to migrate to sea could be their feeding during the migration (Heinimaa & Erkinaro 1999; Jutila & Jokikokko 2008). In the present study, the migration speed of trained smolts, however, began to resemble that of wild fish.
Besides swimming capacity and muscular differences, water flow velocity and temperature obviously have an effect on migration. The migration speeds differed between the years, although in both years the reared smolts were recaptured with the trap earlier than the trained ones. McCormick et al. (1998) have pointed out that smolts will not migrate until the water temperature is above a specific value. The differences in the water temperature could thus explain the variation in the timing of migration between the years. On the other hand, water flow velocity also affects migration. Migration is faster in years when the water flow velocity in the river is high (McCormick et al. 1998). Environmental factors could thus be factors explaining the observed differences in the migration speeds between 2007 and 2008. However, morphology and muscular capacity could also be factors worth consideration. In 2008, both reared and trained smolts migrated downstream at a lower speed compared to the migration in 2007. Moreover, the muscles of fish in 2008 contained significantly higher numbers of DHP and Ry receptors and their oxidative capacity was also higher. The size of all fish in 2008 was closer to wild fish than the size of fish in 2007. Thus, the fish in 2008 could have a better capacity to resist the current as compared to those in 2007.

It has been noted that the timing and duration of the migration differs in reared and wild fish. This is presumed to be one of the factors explaining the observed differences in the survival rate between reared and wild fish (Einum & Fleming 2001; Leonard & McCormick 2001). Migration at an incorrect time could result in environmental circumstances that are energetically and predationally more costly than the circumstances for wild smolts migrating at an environmentally more suitable time (review of Einum & Fleming 2001). This could reduce the survival probability of reared smolts. According to the results of the current study, the migration pattern of trained fish resembles more that of wild ones. This could mean that the survival probability of reared fish could increase by releasing trained fish in the future.
7 Conclusions and future objectives

Billions of reared fish are released annually to nature although their survival probability is extremely low. This means huge economical, conservational as well as investigational losses. The survival probability of wild fish is, on the other hand, significantly higher. In order to enhance the survival probability of reared fish, the reasons for the difference in the survival probability between wild and reared fish have to be found out. Swimming capacity differs between wild and reared fish. Since swimming forms the basis for the whole lifecycle of fish it is suggested that the lower swimming capacity of reared fish is one of the reasons for their lower survival rate. Since muscular capacity depends on molecular interactions at cellular level, one of the basic aims of the current study was to find out how the cellular parameters of muscles differ between wild and reared fish.

In general, the results show that in the muscles of wild fish the prerequisites for active swimming are better met as compared to reared fish with the same genetic background. The relative level of DHP and Ry receptors is significantly higher in wild salmon. Furthermore, the higher oxidative energy metabolism and more efficient lactate removal machinery in the muscles of wild fish enable high-intensity and long-lasting swimming events. The current rearing practices with e.g. low water flow velocity and a constant supply of food seem to be the primary factors explaining the impaired functional variables of muscles.

Since the muscular capacity of reared fish was significantly lower as compared to that of wild ones, one of the major aims of the study was to increase the muscular performance of reared fish so as to be closer to that of wild fish. This was done by making the habitat of reared fish more natural by increasing the water flow velocity in the rearing tanks. For the first time it was observed that training in general increases the level of DHP and Ry receptors in the swimming muscles of fish so that the relative level approaches that of wild fish. A similar change after training was also observed in the activity of aerobic enzymes and in the swimming capacity of the fish.

However, the changes are dissimilar when using different training protocols and different fish species. Low intensity training does not increase the level of receptors sufficiently. On the other hand, overtraining also reduces the level of receptors. In fish species that are used to swimming in low water flow velocity, larger changes are observed with lower intensity training programs as compared to fish used to swimming at a higher velocity. The optimal training program enhancing muscular fitness the most is species-specific.
Besides the increase in the muscular capacity of reared fish closer to that of wild fish, the migration behavior, one of the factors affecting the survival of fish, also changes to a more natural direction after training. In general, downstream migration is slower in trained fish as compared to reared ones. The difference is similar to that observed between wild and reared fish. According to the physiological and histochemical analyses, the swimming and muscular capacity explain the better ability of trained and wild fish to resist the current. The capacity at the cellular level thus has an effect on the behavior and fitness of the fish. Moreover, since migration is energetically extremely expensive, the higher muscular fitness of trained fish could enhance their survival probability.

In conclusion, according to the results the current rearing practices should be changed so as to be more natural. As seen in the current study, just changing the water flow velocity in the tanks induced a shift in the muscle structure and thereafter swimming performance and behavior of reared fish closer to that of wild ones. According to the results, these changes will most probably increase the survival rate of reared fish, which will be analyzed in the future. Other circumstances could obviously be changed as well so as to resemble more circumstances in the wild, and combining these methods will most probably result in the highest increase in the survival rate of reared fish in the future.

Analysis of the survival difference could also be extended to other age classes since releasing yearlings is also common in stocking programs. The differences between wild and reared fish could also be investigated even more closely in order to reveal the factors affecting the survival rate of fish. For example, the activity of lipid metabolism could be compared between wild and reared fish. The rearing facilities could thereafter be modified by changing the composition of food and analyzing its effects on muscular capacity and survival.

Besides studies on survival differences, the molecular properties of fish muscles should be investigated more thoroughly in order to relate the molecular mechanism more closely to the performance of the whole animal. The analysis of e.g. the connection between the level of receptors and swimming capacity of fish deserves more examination. Although there seems to be a connection between these parameters, the result could also be affected by other muscular factors. These factors should be eliminated in order to find out the real connection. Moreover, the molecular analysis of DHP receptors and their isoforms should be extended to red muscle. This way the effect of training on different receptor isoforms could be studied separately and their function in muscle contraction could be evaluated.
References


Original papers


Original publications are not included in the electronic version of the dissertation.
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