Osmoregulation and smolt physiology of sea-run brown trout (*Salmo trutta*)

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Introduction:

Most fish can only survive in a small range of water salinities, and are classified as stenohaline. However, some fish can acclimate to a wide range of salinities, and are referred to as euryhaline. Of these euryhaline fish, some are also anadromous, living in freshwater early in life as "parr", migrating to the ocean as "smolts", and then returning to rivers to spawn. Before they leave freshwater, the fish undergo a complex suite of transformations in order to survive in the ocean (McCormick 2001). This is referred to as the parr-smolt transformation.

Figure 1: Two year-old freshwater *S. trutta* at the CT DEEP hatchery in February 2016, showing pre-smolt coloration.

Physiological changes are necessary to maintain the appropriate balance of salt and water in the fish's body, a process referred to as osmoregulation. The most important site of osmoregulation in fish are the gills. Cells in the gills called ionocytes have the specific function of regulating ion concentrations (Pierre and Perry 1991). Transporter proteins within the ionocytes move ions across membranes. Of these, the most important is the enzyme Na^+/K^+-ATPase (NKA) that uses cellular energy to move sodium ions. As salinity increases, gill osmoregulation by way of NKA activity increases as well (McCormick et.al. 1989). Thus, as anadromous fish change into their saltwater phase, changes in gill physiology are critical for survival.

The parr-smolt transformation for many species of fish, especially Atlantic salmon (*Salmo salar*), has been well documented; however, there are other species of anadromous fish such as brown trout (*Salmo trutta*) whose smoltification process has not been studied in as great a depth. Unlike salmon which are entirely migratory, only some populations of brown trout migrate to the ocean. Anadromous brown trout transform from parr to smolt somewhere between 1 and 2 years old (Thomsen et.al. 2007). Once physiologically ready, they go out to sea, and grow substantially larger than their freshwater relatives. Sea-run brown trout are desirable to fisheries and recreational fishermen. Additionally, these differences in migratory behavior within the same species make brown trout an excellent subject for the study of the evolution and maintenance of osmoregulatory physiology in fishes.

Research Objectives:

- Find baselines in physiology for the smolting process of *Salmo trutta* at one and two years of age.
- Determine the ability of both one and two year-old to survive and acclimate to marine water.

Methods and Materials:

- 24 hour challenge: One and two year-old fish were transferred from freshwater (FW), to saltwater (SW). After transfer the fish were sacrificed by means of rapid euthanasia using MS-222 anesthetic. Blood was drawn by caudal vessel puncture and the gill arches were extracted. Blood was spun down in a centrifuge in order to separate the plasma, which was held at -80°C to be analyzed later. The gill arches were used to find the activity of NKA. For control, freshwater fish of each age were sampled in the same manner.
- 21 day acclimation: A third group was allowed to acclimate to SW for 21 days. Due to the fish not accepting food while in captivity, there were only 6 left to sample from at the end of the 21 day period.
- Analysis: Plasma chloride was determined by a salt analyzer (SAT-500). NKA activity was determined by a kinetic enzyme spectrophotometric assay.
- Statistics: One-Way ANOVA tests were performed on both the one year-old and two year-old age groups. Tukey's HSD post-hoc analysis was performed to determine differences between the 2 year-old, freshwater, SW and 21 day acclimation fish.

Table 1: Summary of treatment groups (±1 SD).

Treatment	N	Total Length (cm)	Mass (g)	Temperature (°C)	Salinity (ppt)
1 Year-Old Freshwater	24	13.8 (±2.3)	29.0 (±8.2)	8.5 (±1.0)	0
1 Year-Old Saltwater	23	14.1 (±1.1)	26.1 (±6.5)	8.5 (±1.0)	35
2 Year-Old Freshwater	12	23.8 (±4.0)	119.6 (±39.4)	8.5 (±1.0)	0
2 Year-Old Saltwater	12	23.1 (±1.7)	111.8 (±23.0)	8.5 (±1.0)	35
2 Year-Old 21 Day Acclimation	6	24.9 (±2.4)	131.2 (±32.7)	8.5 (±1.0)	35

Results:

The one year-old fish showed a significant difference within each parameter measured between the SW and FW. There were significant chloride concentration increases between the FW and SW ($p<0.001$, Fig. 2). The NKA activity between the FW and SW groups significantly increased ($p< 0.05$, Fig. 3). The correlation between length and chloride concentration was not a strongly correlated in either the FW or SW groups (Fig. 4). In the two year- old fish, there is an increase of chloride concentration between the FW and SW fish ($p<0.001$, Fig. 2). The chloride concentration decreased in the 21-day acclimation group ($p<0.001$, Fig. 2). However, there was no significant difference between the FW two year-olds and the fish that were allowed to acclimate ($p=0.159$, Fig. 2). The NKA activity however, is not significantly different between any of the three groups (Fig. 3). The two year-old SW fish show a slight negative correlation between the length and chloride concentrations however, these chloride concentrations are still higher than the FW group. There is no correlation between the length and chloride concentrations in the FW group. The strongest correlation is between the length and chloride concentration in the acclimated fish (Fig. 4).

Results (continued):

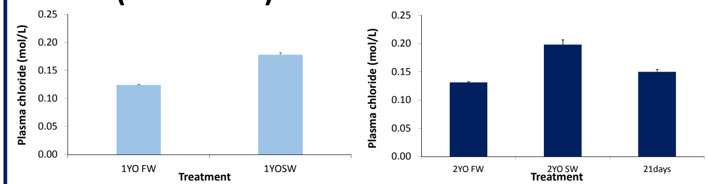

Figure 2: Chloride concentrations for all treatments. 1 year-old groups are on the left (light blue) and 2 year-old groups are on the right (dark blue). Error bars represent ±1 SEM.

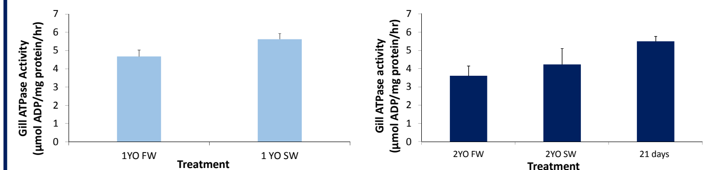

Figure 3: ATPase activity for all treatments. 1 year-old groups are on the left (light blue) and 2 year-old groups are on the right (dark blue). Error bars represent ±1 SEM.

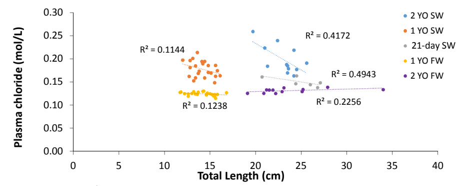

Figure 4: Correlation between fish total length and plasma chloride concentration in all treatments.

Discussion:

Contrary to our original expectations, neither the one year-old nor two year-old fish appear to be smolts. Fish that are already smolts would have no difference in plasma chloride concentration when transferred into SW, however; none of the fish tested had the same concentrations of plasma chloride in the SW challenge as they did in FW. The 21-day acclimation group did show that plasma chlorides return to the FW levels after time passes. There is also no clear length threshold at which the fish are able to osmoregulate like smolts. This suggests that this population of *S. trutta* do not have a defined smolting period but rather are similar to other euryhaline fish that can tolerate a wide range of salinity without a distinct smoltification event.

The fish used in the experiment are native to Finland and would naturally migrate into the Baltic Sea. The Baltic Sea is only 2-7 ppt near the Finnish coast. The natural environment for these sea-run fish is not that much saltier than FW (Forman et. al 1998). In other species of euryhaline fish, it has been shown that pre-exposure to a wide range of salinity has increased ability to osmoregulate (Haney 1999). This population could have a lower affinity for osmoregulation at a higher salinity since they were never pre-exposed before this experiment. Nonetheless, some populations of *S.trutta* are known for their ability to osmoregulate in hyperosmotic environments (Forman et al. 1998). This is likely why there was high survival during the experiment and why plasma chloride levels remained largely unaffected.

Populations of *S. trutta* have huge variations in individual migratory behavior as well as osmoregulatory abilities (Forman et. al. 1998). Johnsson (1989) believed that some of the variation in migration could be caused by the growth rates of the animals. The slow growing individuals are small and tend to stay in the protected areas of the streams and rivers. Fast-growing individuals need to eat a lot more food and are quickly by limited by small FW habitats. Therefore, the fast growing, young fish will migrate out to sea where they can satisfy their need for more food, leaving the slow growing fish of the same age behind. The main concern of CT DEEP was that their trout were small for their age. In the wild they would be considered to be slow-growing and may require additional time before they are ready to tolerate SW. In conclusion, although these trout will be able to survive in SW after an acclimation period, none of either the one or two year-old *S. trutta* that the state reared can be considered to be smolts.

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