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# GILL $NA^+$-$K^+$ ATPASE ACTIVITY AND BODY SILVERING AS INDICES OF SMOLTIFICATION OF HATCHERY-REARED SEA *TROUT (Salmo trutta m.trutta* L.)

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ABSTRACT. Gill $Na^+$-$K^+$ ATPase activity and body silvering were studied in spring in one- and two year old sea trout (*Salmo trutta m.trutta* L.), under hatchery conditions. Two groups were distinguished in each age-class: smolts and non-smolts. Smolts of both age classes showed higher ATPase activity, lower condition coefficient, and more distinct body silvering. One year old smolts were also longer, and their bodies contained more fat compared to non-smolts. In two years old smolts lower fat level was observed. Discriminant functions were proposed, taking into consideration ATPase activity and body silvering, to distinguish smolts from non-smolts. These functions may be used as a smoltification index.

Key words: SALMO TRUTTA, SMOLTIFICATION, $NA^+$-$K^+$ ATPASE ACTIVITY, SILVERING.

## INTRODUCTION

Juveniles of anadromous salmonid fishes pass from parr to smolt stage (smoltification) at the time of migration from fresh to seawater environment. That transformation involves many behavioral, physiological and morphological changes (Hoar 1976). Knowledge of these processes is of key importance for efficient stocking which is often practiced in salmonid management.

The only reliable smoltification estimate is provided by a seawater test (Wedemeyer et al. 1980). The test is, however, difficult to perform under hatchery conditions. Much more often changes of gill $Na^+$-$K^+$ ATPase activity are measured (Zaugg, McLain 1972, Saunders, Henderson 1978, Wedemeyer et al. 1980, Virtanen, Soivio 1985, Tanguy et al. 1994, Siegler et al.1996; Lysfjord, Staurnes, 1998; Stefansson et al.1998; Sundell et al.1998), body silvering is estimated (Vanstone, Markert, 1968; Wedemeyer et al.1980; Kazakov, Kozlov, 1985; Dębowski, Radtke, 1994; Duston, 1995; Haner et al.1995;), or changes of body condition coefficient are calculated (Wedemeyer et al.1980; Dębowski, Radtke, 1994; Tanguy et al.1994; Beeman et al.1995). Using only one parameter may be misleading, especially under artificial con-

ditions. Gill $Na^+$-$K^+$ ATPase activity, considered a good indicator of smoltification, may change due to rearing conditions (Wedemeyer et al.1980; Virtanen, Soivio, 1985), reaching maximum value during migration (Wedemeyer et al.1980; Beeman et al.1995). Fish often still show hypo-osmoregulatory ability, despite decreased ATPase activity (Stefansson et al. 1998). Similarly, also thyroxin concentration is not a reliable index, and its changes poorly correlate with other indices (Winans, Nishioka, 1987; Beeman et al.1995). Body silvering also depends on environmental conditions, especially on light (Vanstone, Markert, 1968; Kazakov, Kozlov, 1985). Silvering is often not accompanied by physiological hypo-osmoregulatory adaptations (Zaugg, McLain, 1972; Wedemeyer et al.1980; Damsgard, 1991; Tanguy et al.1994). Body condition coefficient, however easily measured, is rather risky as a smoltification indicator since it is vulnerable to many environmental factors (Winans, Nishioka, 1987; Beeman et al.1995). Under artificial conditions it strongly depends on feeding, and may not change during smoltification (Sundell et al. 1998).

Smoltification is similar in all anadromous salmonid species (Folmar, Dickhoff, 1980; Wedemeyer et al.1980; Lysfjord, Staurnes, 1998) but in trout (*Salmo trutta m. trutta* L.) is more variable (Okland et al.1993), and changes involved in smoltification process are sometimes less pronounced than in other species (Tanguy et al. 1994).

In the present study usefulness of some indices for evaluation of sea trout smoltification is discussed.

## MATERIAL AND METHODS

### FISH MEASUREMENTS

Juvenile, one- and two years old sea trouts were used in the experiment. The fish were obtained from eggs stripped from the spawners harvested in lower Vistula, and reared in rotation tanks at the Department of Salmonid Fish Breeding in Rutki.

The fish of different smoltification level were chosen basing on different body shape and color. Two years old fish were examined 3 times, and one year old ones – 4 times in 1996, and once in 1997 (Tab. 1). The fish were measured (caudal length, in mm, L variable), weighed (in g, W variable). Light reflection from fish side was also measured (silvering, S variable) according to Kazakov, Kozlov (1985) and Dębowski, Radtke (1994). Activity of gill $Na^+$-$K^+$ ATPase (A) was determined according to modified method developed by Johnston, Saunders (1981). The fish were stunned, and gill

filaments were isolated (without gill archs), blotted, weighed with 1.0 mg accuracy, put into plastic tubes and freezed in liquid nitrogen (-196°C). The samples were thawed in the laboratory at +15°C, transferred to a glass homogenizer and ground in ice, in SEID solution (Zaugg 1982), 1 $cm^3$ of solution per 50 mg of gill tissue. Well ground homogenate was used for immediate enzyme activity measurements. The measurements were performed in 3 replicates for each sample. Quantity of phosphorus released from ATP was expressed in micromoles (μM) of inorganic phosphorus ($P_i$) per mg of protein per hour (μM $P_i$ $mg^{-1}$ $h^{-1}$). Protein content in the homogenate was determined in two replicates, according to Lowry's method (Hatree 1972). Fulton's condition coefficient was also calculated (CF) using the formula: $10^5$*W*$L^{-3}$. In 1997, also total fat content was measured (in % of fresh body weight, F variable) (Dębowski et al. 1999). Fish characteristics and sample size are shown in Table 1.

**TABLE 1**

Sampling dates, sample size (n), and average values of fish body length (L, mm), weight (W, g), condition coefficient (CF), silvering (S), ATPase activity (A), and fat content (F, %). SD in parentheses.

| Age | Sample | n | L | W | CF | S | A | F * |
|---|---|---|---|---|---|---|---|---|
| 1 | 28 Mar 1996 | 10 | 130 (15.4) | 25 (9.4) | 1.07 (0.050) | 9.7 (1.64) | 3.00 (1.450) | |
| | 24 Apr 1996 | 10 | 137 (7.3) | 27 (4.5) | 1.04 (0.037) | 11.1 (2.42) | 3.97 (0.748) | |
| | 6 May 1996 | 10 | 140 (8.1) | 30 (6.2) | 1.08 (0.065) | 10.5 (2.84) | 3.09 (0.758) | |
| | 3 June 1996 | 10 | 155 (12.2) | 42 (10.5) | 1.12 (0.065) | 9.5 (2.07) | 1.58 (0.341) | |
| | 17 May 1997 | 28 | 148 (17.2) | 32 (11.0) | 0.95 (0.066) | 16.4 (5.15) | 4.14 (2.072) | 9.1 (1.09) |
| 2 | 28 Mar 1996 | 10 | 251 (22.4) | 163 (53.9) | 1.04 (0.079) | 14.0 (3.50) | 3.05 (0.965) | |
| | 24 Apr 1996 | 10 | 243 (22.1) | 152 (54.4) | 1.08 (0.112) | 11.2 (3.22) | 2.69 (0.531) | |
| | 6 May 1996 | 10 | 253 (35.8) | 176 (70.0) | 1.02 (0.068) | 13.8 (2.74) | 2.50 (0.965) | |
| | 17 May 1997 | 30 | 248 (25.0) | 169 (57.4) | 1.07 (0.121) | 18.4 (6.83) | 3.98 (2.034) | 9.1 (1.62) |

* *fat level was estimated in 19 1-year and 10 2-year fish.*

## STATISTICAL ANALYSIS

All data were analysed together, separately for each age group of fish.

Relationships among the variables were evaluated using Pearson's correlation coefficients. Then, assuming that silvering and ATPase activity were most the reliable smoltification indices, grouping analysis was done using method of k-averages, for two variables and two groups. Average values of all parameters were calculated, and compared using t-test. The fish showing higher average values of silvering and ATPase activity were considered smolts. From the data set 50% of cases were randomly selected and used for discriminant analysis. The results were tested with the

remaining, independent subset of data. The results of the analysis were used to formulate canonical discriminant function. Values of that function were calculated for each fish and correlated with analysed parameters.

## RESULTS

Ranges of body silvering values and ATPase activity were similar in both age groups of trout – 7-30, and 0.9-9.0, respectively. Similarly, average values of ATPase activity were around 3.4 in both groups. Average silvering, however, was higher in two years old fish (15.7) than in one year old ones (12.8). Silvering, ATPase activity, and other variables were related to one another (Tab. 2). In both age groups of fish silvering positively correlated with ATPase activity, and negatively with condition coefficient. In one year old fish silvering increased also with fish length and fat level. In two years old fish, ATPase activity decreased with fish length and fat content increased (Tab. 2).

**TABLE 2**

Correlation coefficients for comparisons of some parameters, significance level (in the parentheses, NS for p>0.05). Variables as in Tab. 1.

| Age | | CF | S | A | F |
|---|---|---|---|---|---|
| 1 | L | NS | 0.581 (0.000) | NS | 0.717 (0.001) |
| | CF | | -0.468 (0.000) | NS | 0.507 (0.032) |
| | S | | | 0.492 (0.000) | 0.628 (0.005) |
| | A | | | | NS |
| 2 | L | NS | NS | -0.333 (0.011) | NS |
| | CF | | -0.590 (0.000) | -0.605 (0.000) | 0.814 (0.004) |
| | S | | | 0.804 (0.000) | NS |
| | A | | | | -0.735 (0.016) |

According to present knowledge, it was assumed that increase of ATPase activity and change of body coloration (silvering) indicate smoltification. Group analysis resulted in division of each age class of fish into two groups, basing on silvering and ATPase activity values (Fig. 1). In one year old fish the first group contained 48, and in two years old ones – 20 individuals. Fish of the first group were shorter, less silvery, showed better condition but lower fat level and lower ATPase activity comparing to the second group (Tab. 3). Among two years old fish, the first group contained 44 and the second – 14 individuals. The fish of first group showed, similarly as one year old ones,

better condition and lower ATPase activity, and were less silvery. They had, however, higher fat level compared to second group, and average body length did not differ between the groups (Tab. 3). According to the assumption, the fish falling into the first groups were considered non-smolts, and those of the second groups – smolts.

**TABLE 3**

Characteristics of fish groups obtained by grouping for S and A variables. Average values of the variables (SD in the parentheses), and significance level for differences (t test) are shown. Variables as in Tab. 1.

| Age | 1 | | | 2 | | |
|---|---|---|---|---|---|---|
| Group | 1 | 2 | p | 1 | 2 | p |
| n | 48 | 20 | | 44 | 14 | |
| L | 138 (14.4) | 156 (11.5) | 0.000 | 251 (26.3) | 243 (24.3) | 0.310 |
| CF | 1.05 (0.086) | 0.97 (0.064) | 0.000 | 1.09 (0.104) | 0.97 (0.038) | 0.000 |
| S | 10.1 (1.87) | 19.2 (3.54) | 0.000 | 12.9 (2.97) | 24.9 (3.50) | 0.000 |
| A | 3.04 (1.445) | 4.32 (2.042) | 0.005 | 2.68 (0.862) | 5.63 (1.674) | 0.000 |
| F | 8.3 (1.00) | 9.8 (0.58) | 0.001 | 10.3 (0.47) | 8.2 (1.54) | 0.027 |

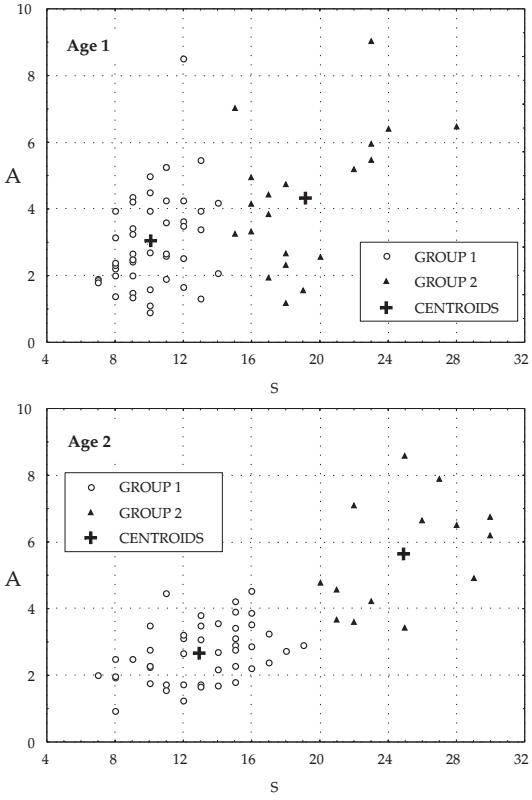


Fig.1. Division of fish into two groups, based on silvering (S) and ATPase activity (A).

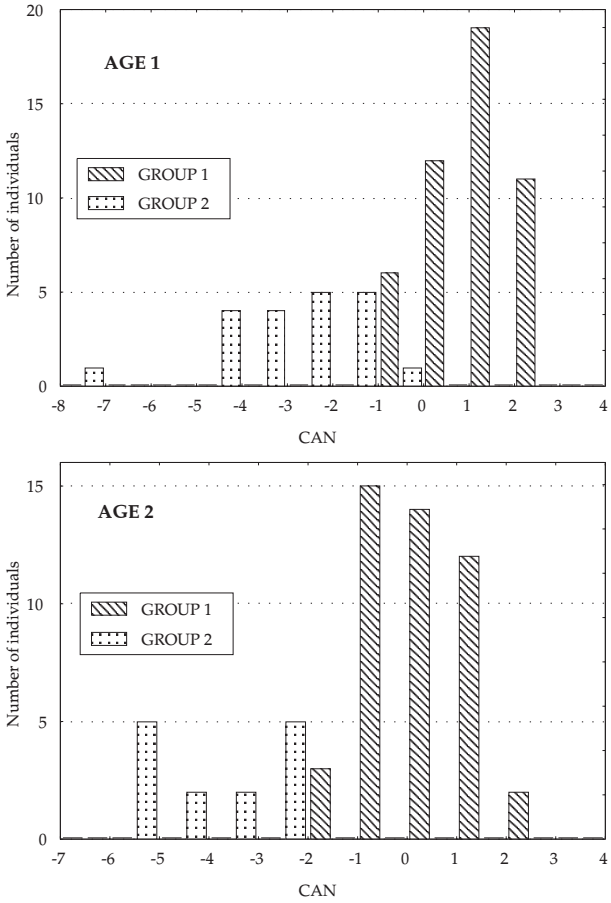


Fig.2. Distribution of canonical function (CAN) values.

Taking into consideration smolts and non-smolts, discriminant analysis was done. Each age group was randomly divided into two subsets. The first subsets were used for development of classification functions, and the remaining data – for their testing. Accuracy of division was high *post hoc* and *a priori* (Tab. 4). Canonical discriminant functions were as follows:

for one year old fish CAN = - 0.5055 * S + 0.2346 * A – 5.6353

for two years old fish CAN = - 0.2710 * S – 0.3705 * A + 4.8857

Canonical variable CAN may be used as smoltification index of trout. The higher the value, the lower smoltification level. Distribution of values of the function for individual fish are shown in Fig. 2. It shows that maximum value for one year old smolts is equal to –1, and for two years old ones to –2.

**TABLE 4**

The results of discriminant analysis: classification of fish into one of the two groups

| Age | Group | Number of fish classified to group: | | % correct |
|---|---|---|---|---|
| | | 1 | 2 | |
| 1 | Training set | | | |
| | 1 | 24 | 0 | 100 |
| | 2 | 0 | 10 | 100 |
| | Independent set | | | |
| | 1 | 24 | 0 | 100 |
| | 2 | 1 | 9 | 90 |
| 2 | Training set | | | |
| | 1 | 26 | 0 | 100 |
| | 2 | 0 | 4 | 100 |
| | Independent set | | | |
| | 1 | 20 | 0 | 100 |
| | 2 | 0 | 10 | 100 |

**TABLE 5**

Correlation coefficients for comparisons between the values of canonical discriminant function and measured parameters (significant level in parentheses, NS for $p>0.05$). Variables as Tab. 1.

| Age | 1 | 2 |
|---|---|---|
| L | -0.601 (0.000) | NS |
| CF | 0.466 (0.000) | 0.619 (0.000) |
| S | -0.988 (0.000) | -0.985 (0.000) |
| A | -0.350 (0.003) | -0.894 (0.000) |
| F | -0.618 (0.006) | 0.658 (0.039) |

Relationships between canonical function and measured parameters are shown in Tab. 5. In one year old trouts smoltification level, beside correlation with silvering and ATPase activity, was higher in larger fish showing higher level of fat, and lower condition coefficient. Minimum body length for smolts was 130 mm (Fig. 3). In two years old fish no effect of body length on smoltification level was observed, the effect of condition coefficient was similar, and of fat level – opposite.

Based on CAN values, smoltification level can be also analysed in particular fish samples (Fig.4). In fish analysed in 1996 (in samples 1-4) no smolts were observed, except several one year old individuals.

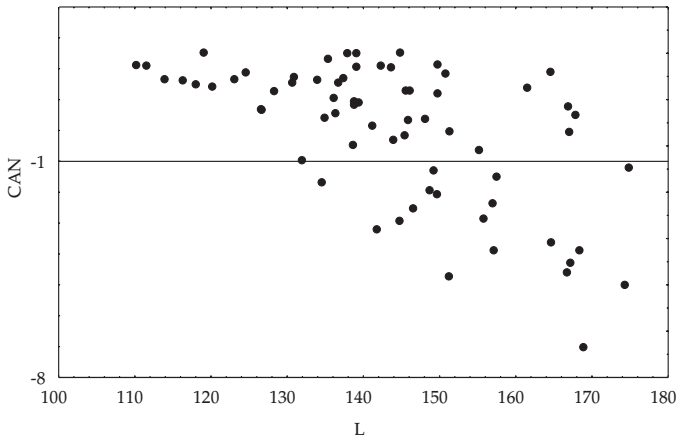


Fig.3. Relationship between canonical function (CAN) values and one year old fish body length. CAN value = -1 assumed maximum for one year old smolts.

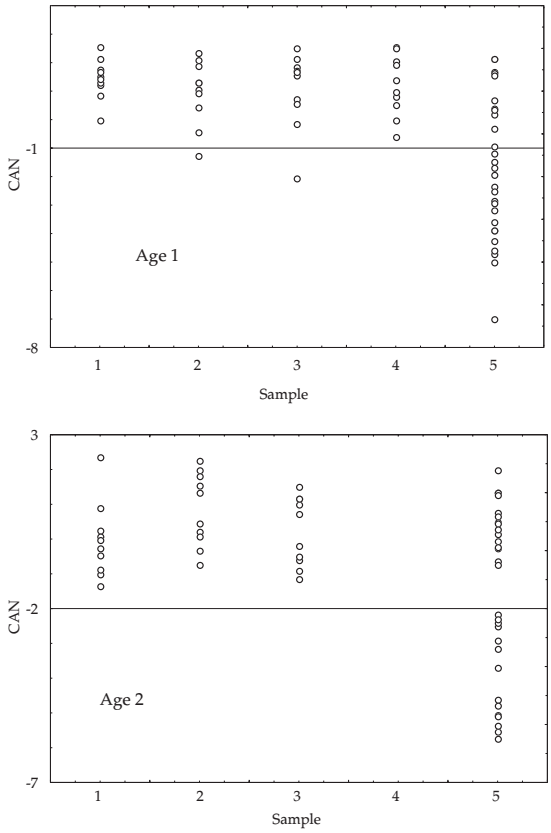


Fig. 4. Values of canonical variable (CAN) for fish in particular samples. Values of CAN = -1 for one year old fish, and CAN = -2 for two years old ones assumed maximum for smolts.

## DISCUSSION

Average ATPase activity in fish considered to be smolts was equal to 4.3 in first year, and 5.6 in the second. The differences between smolts and non-smolts were significant and lower among one year old fish. The values are consistent with those reported by Tanguy et al. (1994) – 4.0-5.0 during migration, or by Sundell et al. (1998) in fish culture. Higher values were often observed before (4.0-6.0) and during migration (8.0-9.0, even to 13.0) (Soivio et al.1989; Muona, Soivio, 1992; Lysfjord, Staurnes, 1998). Higher ATPase activity was usually noted in Atlantic salmon smolts (Saunders, Henderson, 1978; Muona, Soivio, 1992; Siegler et al.1996). ATPase activity did not allow to distinguish smolts from non-smolts.

Body silvering appeared to be a much better smoltification indicator. It correlated with all other indices, and considerably contributed to the developed of discriminant function. Two years old fish showed more pronounced silvering. Silvering values are difficult to compare with the data of other authors due to different measurement devices applied. The same equipment as used in present study was also applied for trout smolts in one of Pomeranian rivers (northern Poland), where both – smolts and non-smolts were more silvery: 14 and 23 (Dębowski, Radtke 1994), and in the study of salmon in Kola Peninsula waters (values up to 65) (Kazakov, Kozlov 1985).

Also condition coefficient considerably differed between the groups, and the values were similar to those noted in other studies (Dębowski, Radtke 1994, Tanguy et al. 1994, Sundell et al. 1998). Analysis of this easily measured parameter seems helpful in smoltification assessment.

Among one year old fish, the largest individuals of the highest fat level became smolts, and in two years old ones – smaller and less fatty fish (but the difference was not significant). In both age groups smolts were thinner than non-smolts. These results are consistent with the general model of salmonid development (Thorpe 1987, 1989). In the first year of life, fast growing fish become smolts, and later on – largest fish did not smoltify and mature in fresh water. Minimum body length for sea trout smolts is 130 mm. Among wild smolts migrating down Pomeranian rivers smaller fish were found, but were scarce (Chełkowski 1995, Chełkowski, Chełkowska 1995, Dębowski et al. 1992, Dębowski, Radtke 1994).

Also Tanguy et al. (1994) observed that trout under 130 mm did not show any smoltification symptoms in Bretagn.

The present study showed that smoltification of hatchery-reared trouts is hard to

evaluate using individual indices, and using them together, as dicriminant function improves accuracy of classification.

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## STRESZCZENIE

AKTYWNOŚĆ SKRZELOWEJ $Na^+$-$K^+$ ATPazy I WYSREBRZENIE CIAŁA JAKO WSKAŹNIKI SMOLTYFIKACJI POCHODZĄCYCH Z WYLĘGARNI TROCI (*Salmo trutta m. trutta* L.)

Badano aktywność skrzelowej $Na^+$-$K^+$ ATPazy i wysrebrzenie ciała wiosną u jedno- i dwuletnich troci wiślanych (*Salmo trutta m. trutta*) z wylęgarni. Na ich podstawie wyodrębniono w każdej grupie wiekowej dwie grupy ryb: smolty i niesmolty. Smolty abu roczników miały wyższą aktywność ATPazy, niższy współczynnik kondycji i były bardziej wysrebrzone od niesmoltów. Smolty jednoroczne były ponadto dłuższe i miały wyższą zawartość tłuszczu w ciele, a smolty dwuletnie – niższy poziom tłuszczu od niesmoltów. Zaproponowano funkcje dyskryminacyjne oparte na aktywności ATPazy i wysrebrzeniu pozwalające na odróżnienie smoltów od niesmoltów. Funkcje te można uznać za indeks smoltyfikacji.

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