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SMOLTIFICATION OF HATCHERY-REARED ATLANTIC SALMON (*Salmo salar* L.) – INDICES AND METHODS OF ESTIMATION

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ABSTRACT. Gill Na^+ - K^+ ATPase activity and body silvering were measured in one- and two-year old salmon. The data allowed to distinguish two fish groups in each age class: smolts and non-smolts. Discriminant functions were developed to qualify the fish into the groups. The values of these functions were assumed as the index of smoltification. Relationship between this index and detailed morphometric data indicates that smoltification involved head size decrease and body height increase in one-year old fish. In two-year old ones – lengthening and thickening of caudal peduncle was noted. These changes, however, were too little pronounced to enable accurate assessment of smoltification level, and silvering measurements were necessary.

Key words: *Salmo salar*, SMOLTIFICATION, Na^+ - K^+ -ATPASE ACTIVITY, SILVERING, MORPHOMETRY, MODEL, METHOD

INTRODUCTION

Beginning from the '50, Atlantic salmon (*Salmo salar* L.) gradually disappeared from Polish rivers, and at the end of '80 it was already extinct (Bartel 1997). In 1994 salmon restoration project started, in which fish from Latvian Daugava River were used. The project involved stocking the rivers with hatchery-reared salmon smolts (Bartel 1997). Stocking efficiency usually depends on fish smoltification level (Isaksson, Bergman 1978, Bartel, Dębowski 1996). Accurate evaluation of smoltification is difficult. Sea water test is considered the most reliable; biochemical (gill Na^+ - K^+ ATPase activity, thyroxin concentration) and morphologic (body silvering, condition factor, body shape) indices are also applied (Folmar, Dickhoff 1980, 1981, Wedemeyer et al. 1980, Kazakov, Kozlov 1985, Virtanen, Soivio 1985, Beeman et al. 1994, 1995, Dębowski et al. 1999b, 1999c).

Most of these indices, however, require the use of special equipment, are laborious, and destructive to the fish. Additionally, smoltification assessment is more difficult in hatchery-reared fish, in which smoltification may be incomplete or disturbed due to environmental conditions (Wedemeyer et al. 1980, Wedemeyer 1982, Virtanen, Soivio 1985, Winans, Nishioka 1987, Stefansson et al. 1998, Sundell et al. 1998).

In the present study some smoltification indices of hatchery-reared salmon were measured, and quantitative model of evaluating smoltification level was developed, as well as non-destructive methods of smoltification assessment.

MATERIAL AND METHODS

FISH

Juvenile, one- and two-year old salmon were studied. The fish were obtained from the eggs stripped from the spawners reared in cages in Puck Bay. Young salmon were kept in tanks in Osowo Hatchery, Aquamar.

Fish of different smoltification degree were chosen, showing different body coloration and shape. Measurements were performed 4 times in spring 1996, and once in spring 1997 (Tab. 1). The following measurements were taken: caudal body length (L) in mm, body weight (W) in g, body silvering (S) (Kazakov, Kozlov 1985, Dębowski et al. 1999b), gill Na^+ - K^+ ATPase activity (A) in $\mu\text{M Pi} \cdot \text{mg of protein}^{-1} \cdot \text{h}^{-1}$ (Dębowski et al. 1999b), and in 1997 also body fat content in % of fresh weight (F) (Dębowski et al. 1999a). All the fish were recorded using S-VHS camera, and then measured using a computer image analysis system (Dębowski et al. 1998). Four body heights, and 28 distances between fish body landmarks (truss network – Fig. 1) were measured. Condition factor ($\text{CF} = 10^5 \cdot \text{W} \cdot \text{L}^{-3}$) was also calculated. Number of measured fish and their characteristics are shown in Tab. 1.

TABLE 1

Dates of sampling, number of fish (n), mean: length (in mm)(L), weight (in g)(W), condition factor (CF), body silvering (S), ATPase activity (A) and fat level (in %)(F). SD in parentheses.

Age	Sample	n	L	W	CF	S	A	F*
1	28 Mar 1996	10	123 (11.4)	25 (9.4)	1.15 (0.106)	10.9 (1.79)	1.82 (0.680)	
	24 Apr 1996	9	117 (4.3)	27 (4.5)	1.18 (0.050)	6.3 (2.06)	1.92 (0.365)	
	6 May 1996	11	122 (7.5)	30 (6.2)	1.21 (0.075)	7.2 (1.25)	3.49 (1.725)	
	3 June 1996	10	121 (4.2)	42 (10.5)	1.11 (0.037)	8.6 (1.96)	2.36 (1.454)	
	17 May 1997	30	144 (12.7)	32 (11.0)	1.21 (0.058)	16.3 (6.35)	6.23 (3.960)	11.2 (1.84)
2	28 Mar 1996	10	213 (27.3)	127 (45.9)	1.27 (0.063)	12.9 (1.60)	1.73 (0.987)	
	24 Apr 1996	6	223 (11.3)	138 (27.5)	1.23 (0.093)	9.7 (2.07)	1.67 (0.443)	
	6 May 1996	10	229 (28.3)	150 (54.0)	1.20 (0.089)	10.8 (3.19)	1.61 (0.760)	
	3 June 1996	10	251 (23.2)	198 (52.9)	1.22 (0.061)	11.3 (2.50)	3.49 (1.520)	
	17 May 1997	29	192 (23.3)	88 (32.9)	1.19 (0.064)	14.8 (3.26)	3.98 (2.034)	8.9 (2.31)

* fat level was estimated in 15 1-year and 14 2-years old fish.

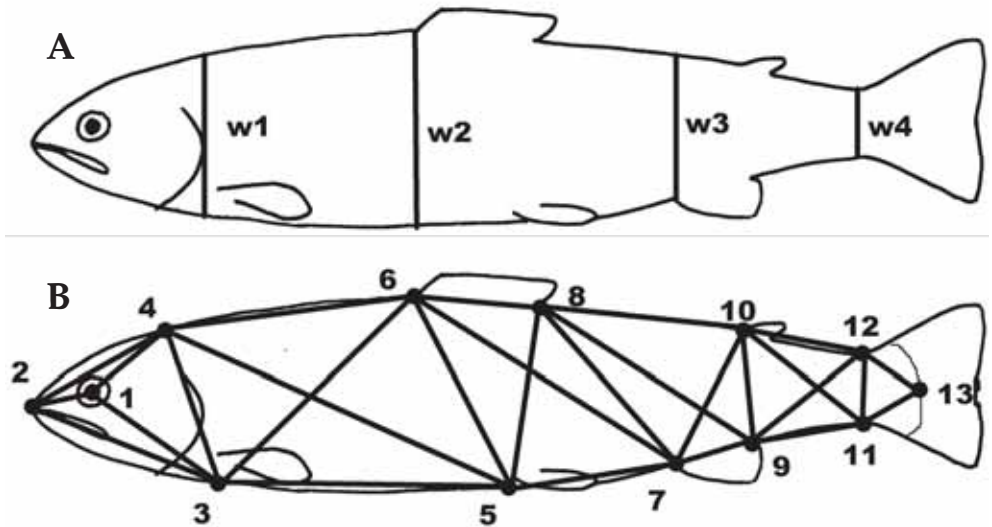


Fig. 1. Body heights (A) and truss network characters (B)

STATISTICAL ANALYSIS

The analysis was carried out for all measurements together, separately for each age group. Relationships between the parameters were calculated using Pearson's coefficients of correlation. Then, grouping of the fish in variable S (silvering) and A (gill $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ activity) ranges was performed and analysed. Fish group of higher values of these parameters were considered to be smolts. From all the data, 50% of the fish were randomly selected for discriminant analysis. The data were tested using the remaining 50% of the data. Canonical discriminant function was formulated to evaluate the level of smoltification (IS).

Morphometric data were standardised: body height with the formula $W_i = w_i \cdot L^{-1}$ (where W_i – relative height, w_i – measured height, $i = 1, 2, 3, 4$), and truss network measurements according to the formula $X = \ln(x) - \beta (\ln(L) - \ln(L_m))$ (where X – standardised value, x – measured value, β – regression coefficient of $\ln(x)$ versus $\ln(L)$, L_m – mean fish length in a sample).

In order to reduce the number of truss network variables, factorial analysis was performed. Principal components (PC_j , where j – number of components) were used in further calculations, and stepwise multivariate regression was used for IS values versus the measured variables. Several regression models for various data sets were tested (Dębowski et al. 1999c).

RESULTS

Silvering values ranged from 4 to 30. Average silvering value was equal to 11.7 for one-year old fish, and 12.9 for two-year old ones. The difference between these values was insignificant. Maximum gill $\text{Na}^+\text{-K}^+$ ATPase activity in one-year old fish was 14.86, and average 4.06 being significantly ($p < 0.001$) higher compared to that observed in two-year old ones (2.45). One-year old salmon showed significantly lower condition factor (1.19 versus 1.21) but higher fat content (11.2 v. 8.9).

Several relationships were found between the parameters (Tab. 2). Correlations between silvering (S) and gill $\text{Na}^+\text{-K}^+$ ATPase activity (A) were particularly high. Relationship between enzyme activity and body length (L) was positive in one-year old fish, and negative in two-year old ones.

TABELA 2

Correlation coefficients for comparison between some variables, p-level (in parentheses; NS for $p > 0.05$). Descriptions as in Table 1.

Age		CF	S	A	F
1	L	NS	0.734 (0.000)	0.494 (0.000)	0.626 (0.013)
	CF		NS	NS	NS
	S			0.684 (0.000)	NS
	A				NS
2	L	NS	NS	-0.332 (0.007)	NS
	CF		NS	-0.423 (0.000)	0.793 (0.001)
	S			0.558 (0.000)	NS
	A				NS

Analysis allowed to distinguish two groups of fish, according to A and S variables (Fig. 2). Group I contained 52 one-year old fish, significantly shorter, less silvery, and of lower gill $\text{Na}^+\text{-K}^+$ ATPase activity, compared to 18 fish of group II (Tab. 3). In two-year old salmon only body silvering and ATPase activity differed between the groups (average value were higher in group II – Tab. 3). Fish of group I were considered to be non-smolts, and group II – smolts.

Based on this division, discriminant analysis was carried out. Functions developed for a sub-sample were tested on the remaining data set. *Post hoc* and *a priori* accuracy were very high, except the qualification of two-year old smolts and the testing data (Tab. 4). Thus, the values of canonical discriminant functions may be used as smoltification indices (IS).

TABLE 3

Characteristics of the fish groups obtained from grouping in variable S and A ranges. Average values (SD in parentheses), and significance level for t-test are shown. Descriptions as in Tab. 1.

Age	1					2				
Group	1		2		p	1		2		p
N	52		18			49		16		
L	125	(10.7)	149	(12.3)	0.000	214	(32.1)	210	(32.4)	0.707
CF	1.18	(0.078)	1.21	(0.058)	0.123	1.22	(0.078)	1.18	(0.053)	0.081
S	8.6	(2.39)	20.6	(4.03)	0.000	11.4	(2.15)	17.4	(2.33)	0.000
A	2.64	(1.474)	8.17	(3.859)	0.000	2.01	(1.019)	3.80	(1.836)	0.000
F	10.1	(1.80)	12.0	(1.49)	0.041	9.8	(2.67)	8.1	(1.67)	0.183

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	0	7	100
	2	26	0	100
	Independent set			
	1	1	10	91
	2	26	0	100
2	Training set			
	1	0	6	100
	2	27	0	100
	Independent set			
	1	4	6	60
	2	22	0	100

TABLE 5

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

Age	1	2
L	-0.688 (0.000)	NS
CF	NS	NS
S	-0.943 (0.000)	-0.993 (0.000)
A	-0.888 (0.000)	-0.654 (0.000)
F	NS	NS

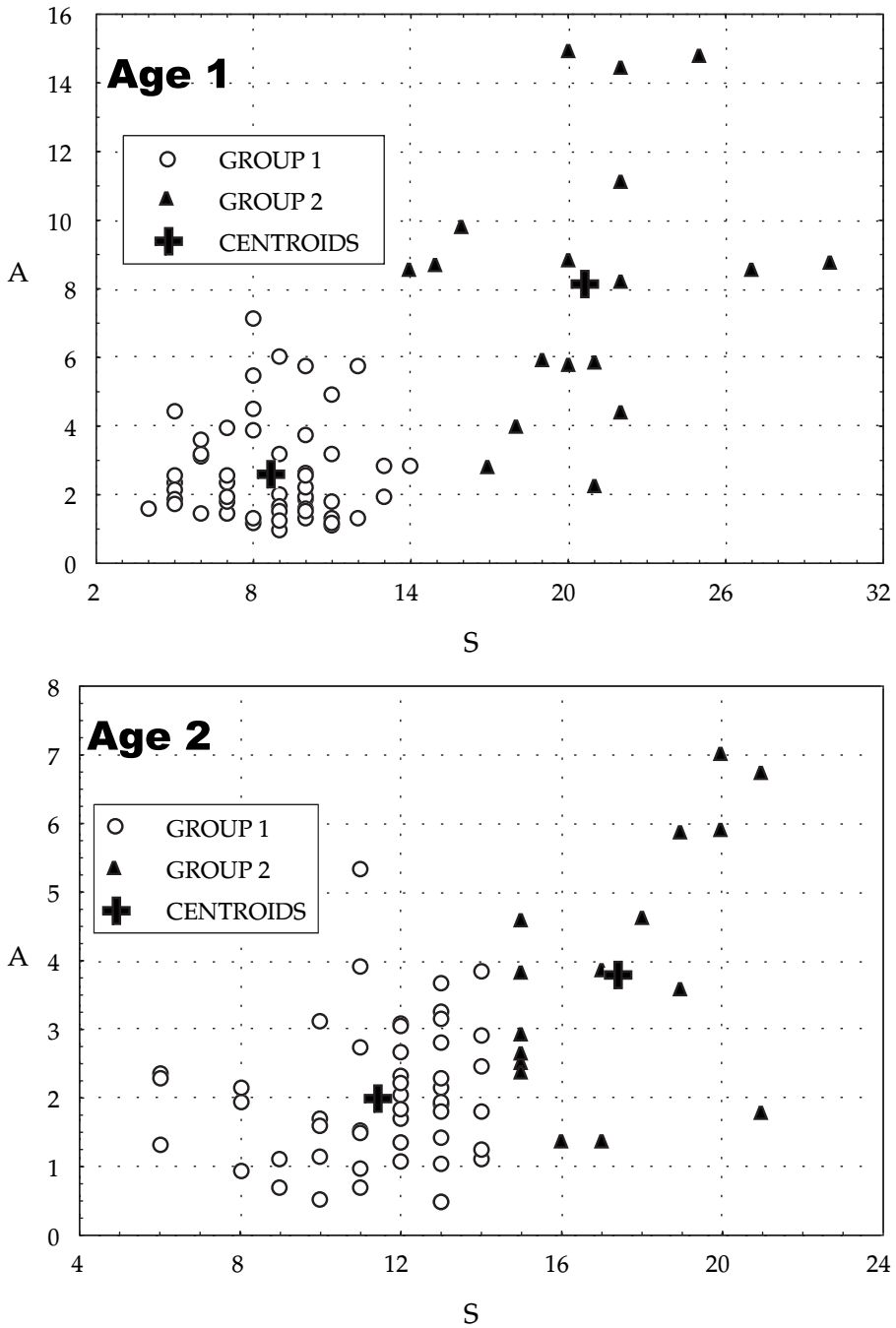


Fig. 2. Division of fish into the two groups based on silvering factor (S), and gill Na⁺-K⁺ATPase activity (A)

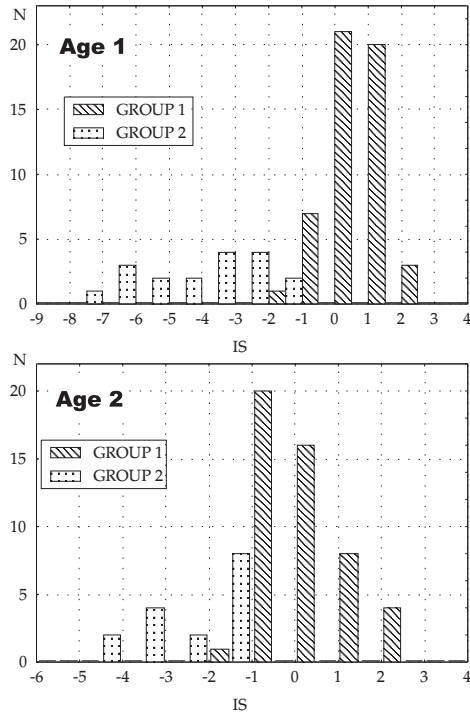


Fig. 3. Distribution of smoltification index (IS)

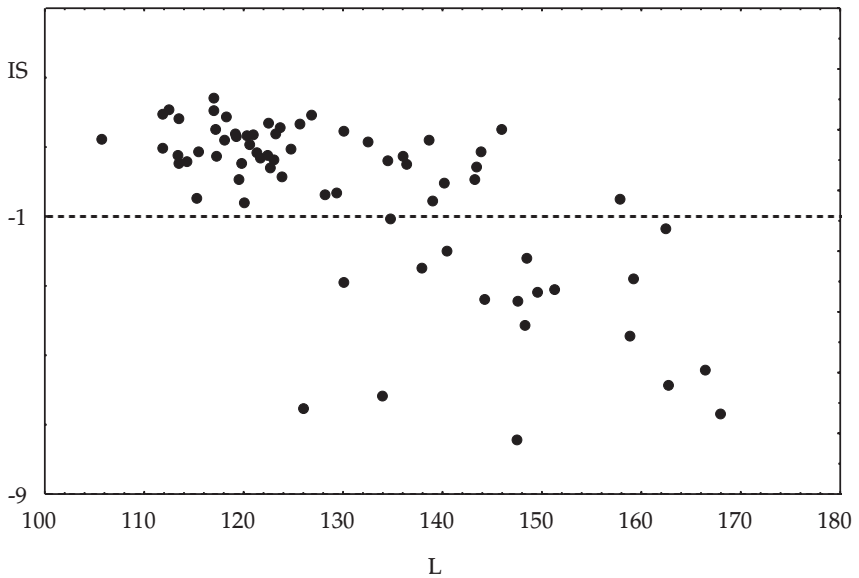


Fig. 4. Relationship between smoltification index (IS), and body length in one-year old fish. IS = -1 was assumed as a maximum for smolts

The functions were:

for one-year old fish $IS_1 = -0.2576 * S - 0.3353 * A + 3.9756$

for two-year old fish $IS_2 = -0.4411 * S - 0.1611 * A + 5.7757$

The lower the IS value, the higher the smoltification level. Maximum IS value for smolts was found using distribution analysis. This value was $IS = -1$ for both groups (Fig. 3). Minimum body length at which smoltification occurred was about 130 mm (Fig. 4).

Factor analysis of truss network morphometric data resulted in 6 principal components for each age group (Tabs. 6, 7) which explained 71.7% of variation in one-year old fish, and 78.7% in two-year old ones. None of the components was correlated with fish body length ($p < 0.001$), indicating that they described fish shape and not size (Humphries et al. 1981).

Multivariate regression models (Tab. 8) revealed correlation between smoltification index and various sets of measurements. Equations 1.2, 4.1, and 4.2 describe changes of fish body shape during smoltification. They show that the rear body part becomes higher in both age groups, and in two-year old salmon also caudal peduncle is larger (Fig. 5). The height of the central part of the body decreases in two years old fish, and increases in one year old ones. In the latter also head becomes lower, and in two years old ones – shorter.

Accuracy of estimates for morphometric data alone is low. Determination coefficient was under 0.50 for all models, and in case of truss network no significant relationship was found for one-year old fish. Incorporation of the fish length and condition factor into the models increased determination coefficients, especially in younger fish (Tab. 8, equations 2.1, 2.2, 5.1, 5.2). In the models incorporating silvering factor, IS for one-year old fish depended only on this factor (determination factor 0.89, Tab. 8,

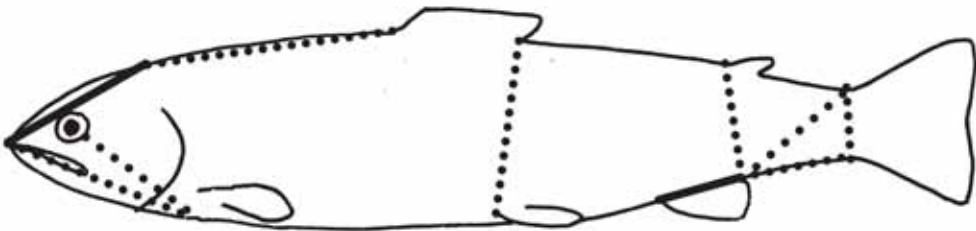


Fig. 5. Morphometric characters positively (solid lines) and negatively (broken lines) correlated with index of smoltification (IS) for two years old salmon

TABLE 6

Loadings from principal component analysis of morphometric characters of 70 one-year old salmon. Characters are distances between landmarks (Fig.2). Loadings $>|0.7|$ are distinguished.

Character	PC1	PC2	PC3	PC4	PC5	PC6
1-2	.002921	-.061412	.422012	-.092143	-.357174	.032482
1-3	.218234	-.082502	-.016832	.066437	.887678	-.116607
1-4	.536657	.165219	-.012068	-.447767	.284128	.156814
2-3	.327077	-.035149	.075088	.025814	.791473	-.061609
2-4	.570266	.073343	.293969	-.485577	.087502	.113563
3-4	-.069181	.222972	.073075	.044651	.779155	.249338
3-5	-.746687	.013906	.132634	-.241534	-.235036	.202080
3-6	-.802371	-.045065	.049046	-.007729	-.334362	.229266
4-5	-.864074	-.025879	.019727	.042715	.127421	.145570
4-6	-.809817	-.179039	-.123768	.291496	-.140354	-.105985
5-6	-.514484	.263405	.145592	.098899	.257737	.575528
5-7	.535583	-.277492	-.304711	.507636	-.303645	.092769
5-8	-.053306	.235049	.107595	.494325	.058916	.446384
6-7	.462776	-.149827	-.186363	.181229	-.051885	.583877
6-8	.257930	-.152759	.784487	.112289	.188172	.073037
7-8	.174707	.076923	-.811781	-.010651	-.154043	.422528
7-9	-.247163	-.448813	.133605	-.168254	.371957	-.303922
7-10	-.243594	.644381	.017636	.122309	.390069	.054012
8-9	.041882	-.282533	-.860714	-.193060	.005781	.206428
8-10	.179138	.390641	-.829702	-.059401	.052563	-.138069
9-10	-.273625	.238739	-.086555	.042050	-.003268	.717308
9-11	.190834	.909571	-.065639	-.188664	-.050667	.081871
9-12	.075160	.920515	-.105107	-.045208	.010105	.232914
10-11	-.039017	.248357	-.073919	-.527114	-.106098	.657677
10-12	.031954	-.035611	.006015	-.564424	-.046958	.484160
11-12	-.224375	.526173	-.201448	.077753	.059788	.562998
11-13	-.030253	-.097811	.057608	.708642	.104330	.076071
12-13	-.038484	.078239	.150503	.691619	.049781	.069818
% of total variance explained	17.8	16.8	12.5	10.1	8.0	6.5

equations 3.1, 6.1). In older fish, models of determination factor 0.99 contain silvering, body length, and condition factor (3.2), or silvering and two body heights (6.2).

DISCUSSION

Average gill $\text{Na}^+ \text{-K}^+$ ATPase activity for one-year old smolts was equal to 8.17, and for two-year old ones – 3.80. The first value was similar (or slightly lower) than

TABLE 7

Loadings from principal component analysis of morphometric characters of 65 two-years old salmon. Characters are distances between landmarks (Fig.2). Loadings >|0.7| are distinguished.

Character	PC1	PC2	PC3	PC4	PC5	PC6
1-2	.016681	-.439890	-.231182	-.063798	.026589	-.521576
1-3	.164326	.134203	.150828	-.123694	.039375	.908957
1-4	.099188	-.661790	.197816	.050296	-.086076	.447704
2-3	-.001773	.128048	.180483	-.135231	-.032366	.860688
2-4	.105668	-.897821	.061237	.008300	-.047714	.068147
3-4	.593116	-.260319	-.168755	.065660	.043339	.658847
3-5	.163307	-.213538	-.752373	.168460	-.044408	-.402111
3-6	.544589	-.073304	-.367799	.322487	.116624	-.047088
4-5	.261107	.292010	-.856674	.056092	-.016526	-.064037
4-6	-.004766	.845203	-.118175	-.012389	.120936	.168438
5-6	.661214	-.353438	-.532890	.209097	-.017914	-.058754
5-7	.216929	.205063	.856959	.046967	.131685	.117360
5-8	.797051	-.254623	-.022573	.192779	.140012	.086984
6-7	.689661	-.258205	.134339	.114675	.022468	-.235417
6-8	.010470	-.217037	.047405	-.901232	.048757	-.093109
7-8	.443833	-.003037	-.013588	.847141	-.007570	-.082537
7-9	-.074395	-.734050	-.423598	-.051855	.004622	-.159711
7-10	.661816	-.026312	-.222641	.321770	.105761	.203298
8-9	.264028	-.293104	-.143183	.833048	-.044971	-.189686
8-10	.080325	.089293	.018861	.942998	.049823	-.097318
9-10	.839126	.236301	.035966	.081137	-.107074	.240646
9-11	-.137192	.726140	.225598	.045345	-.394799	.261379
9-12	.055242	.775751	.260239	.099851	-.306537	.249913
10-11	.415515	.161112	-.056820	-.141367	-.807784	.080449
10-12	-.106621	.180725	-.004971	-.082438	-.874543	-.070600
11-12	.775025	.185407	.020728	-.027860	.030345	.078497
11-13	.389728	.243336	.080458	-.206997	.683518	-.016496
12-13	.358032	.345801	.200870	-.264051	.491349	.032032
% of total variance explained	23.3	19.1	12.6	9.3	8.8	5.6

those usually reported for salmon smolts (Saunders, Henderson 1978, Virtanen, Soivio 1985, Muona, Soivio 1992, Tanguy et al 1994), and similar or higher than the values observed in trout smolts (Soivio et al. 1989, Tanguy et al. 1994, Dębowski et al. 1999b). Gill $\text{Na}^+ - \text{K}^+$ ATPase activity in two-year old smolts was, compared to the data of other authors, very low. Younger smolts were also more silvery than older ones – 20.6, and 17.4, respectively, contrary to trout studied at the same time in a nearby

TABLE 8

Regression models for some sets of variables on indices of smoltification for one-year (IS_1) and two-years (IS_2) old salmon. Variables: principal components (PCj), body heights (Wi), length (L), condition factor (CF) and body silvering (S). „X” denotes variable taken into account. For all models $p < 0.001$.

Variables					R ²	Model
PCj	Wi	L	CF	S		
X					0.45	regression non significant $IS_2 = -0.282*PC1 - 0.646*PC2 - 0.643*PC6 - 0.129$ (1.2)
X		X	X		0.56	$IS_1 = -0.106*L + 13.721$ (2.1)
					0.48	$IS_2 = 7.504*CF - 0.689*PC1 - 0.604*PC2 - 0.616*PC6 - 9.285$ (2.2)
X		X	X	X	0.89	$IS_1 = -0.364*S + 3.931$ (3.1)
					0.99	$IS_2 = -0.479*S + 0.001*L + 0.880*CF + 4.486$ (3.2)
	X				0.49	$IS_1 = 148.41*W1 - 105.76*W2 - 94.40*W3 + 11.69$ (4.1)
					0.31	$IS_2 = 74.52*W2 - 103.14*W3 - 130.05*W4 - 9.36$ (4.2)
	X	X	X		0.56	$IS_1 = -0.106*L + 13.721$ (5.1)
					0.31	$IS_2 = 74.52*W2 - 103.14*W3 - 130.05*W4 - 9.36$ (5.2)
	X	X	X	X	0.89	$IS_1 = -0.364*S + 3.931$ (6.1)
					0.99	$IS_2 = -0.467*S - 7.339*W1 + 11.427*W2 + 4.521$ (6.2)

hatchery (19.2, and 24.9, respectively) (Dębowski et al. 1999b). Thus, it may be concluded that in one-year old smolts, body characteristics typical for this developmental stage were more pronounced than in older fish. Between these two characteristics, body silvering appeared to be a more accurate index of smoltification, similarly as in trout. The third indicator – condition factor – was completely useless, which confirmed conclusions of Winans, Nishioka (1987), Beeman et al. (1995), and Sundell et al. (1998).

Among one-year old fish, smolts were larger (over 130 mm), fatter, with smaller heads and higher bodies. In two-year old salmon, smolts showed longer and thicker caudal peduncles. Some changes of fish body shape occurred during smoltification, but not distinct enough to indicate smoltification level. Such evaluation may be done using body silvering factor, and in case of two-year old fish – incorporating also body length, and condition factor, or else body heights.

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STRESZCZENIE

SMOLTYFIKACJA ŁOSOSIA ATLANTYCKIEGO (*Salmo salar* L.) POCHODZĄCEGO Z WYLĘGARNI – WSKAŹNIKI I METODY OCENY

Zbadano aktywność skrzelowej $\text{Na}^+\text{-K}^+$ ATPazy i wysrebrzenie u jedno i dwuletnich łososi. Na podstawie wyników wyodrębniono w każdej grupie wiekowej dwie grupy ryb: smolty i niesmolty. Zaproponowano funkcje dyskryminacyjne pozwalające na kwalifikowanie ryb do jednej z tych grup. Ich wartość przyjęto za wskaźnik stopnia smoltyfikacji. Badając zależności pomiędzy tym wskaźnikiem a szczegółowymi danymi morfometrycznymi stwierdzono, że w czasie smoltyfikacji u ryb jednorocznych maleje głowa i wzrasta wysokość ciała, a u ryb dwuletnich wydłuża się i pogrubia trzon ogonowy. Zmiany te są jednak zbyt mało wyraźne, aby można było na ich podstawie oszacować stopień smoltyfikacji. Konieczne jest do tego dokonanie pomiarów wysrebrzenia.

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