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COLOURATION OF RECIPROCAL HYBRIDS OF SALMON (*SALMO SALAR* L., 1758) AND SEA TROUT (*SALMO TRUTTA* M. *TRUTTA* L., 1758) AGED 0+ AND 1+ GROWN IN THE NATURAL CONDITIONS

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ABSTRACT

The study was performed on the reciprocal hybrids of *Salmo salar* and *Salmo trutta* aged from 4 to 24 months, grown in natural conditions. As far as colouration is concerned, the ♀ trout x ♂ salmon hybrids aged 0+ and 1+ were found to resemble salmon of the same age (similar number of spots above and below the lateral line and on the opercular bones, and the spots below the lateral line reached the distance marked by the end of the dorsal fin). The salmon x ♂ trout hybrids resembled trout, but the ranges of variation of individual features were greater than in trout. The hybrids of the two types differed from the parent species in the greater ranges of variation of particular characters, irregular distribution of spots, brighter colours and irregular shapes of larval spots and other spots. Some hybrids representing the two types had untypical lighter colouration with larval spots only or with additional few little spots.

Key words: colouration; hybrids; *Salmo salar* x *Salmo trutta*; *Salmo trutta* x *Salmo salar*

INTRODUCTION

In natural conditions hybridisation between salmon (*Salmo salar*) and trout (*Salmo trutta* m. *trutta*) is relatively often at the localities of their common occurrence. The level of hybridisation between these two species varies from 0.15% in Finland and Norway [9], to 4.7% in Canada [24] up to 18.8-31.4% in the Baltic Sea area [29]. The factors favouring hybridisation comprise anthropopressure [15, 26, 30, 6, 33, 4, 32, 12, 13, 23] excessive fish catchment [22], restitution of salmon over limited areas [17]. Hybridisation between these species is undesirable because of introgression and leading to the loss of spawning sites of salmon and trout. For this reason early identification of hybrids and their elimination from the environment of pure species are of importance. The hitherto analyses of the look and external features of the natural hybrids of salmon and trout have been based on investigation of individuals

of unknown direction of hybridisation, on a small sample or on the only one cross [1, 18, 27, 30, 5, 16, 34, 2]. In this study a detail analysis of colouration of the reciprocal hybrids of salmon and trout, aged 0+ and 1+ grown in natural watercourses, was made.

MATERIAL AND METHODS

The study was made on 416 hybrids of ♀ trout x ♂ salmon and 158 hybrids of ♀ salmon x ♂ trout aged from 4 to 24 months (Tab. 1). Reciprocal crossing of salmon and trout was made during artificial spawning. The salmon spawn and milt were obtained from individuals grown in the Fish Farm in Miastko and in the River Wieprza, originating from the school imported from the River Dougava, while the trout spawn and milt were taken from the school brought from the River Rega. It spawning every during one from sexual products have were collected from 3-5 individuals. Fertilisation was performed at the PZW Hatchery in Goleniów, where the spawn was also incubated. The hatch was introduced into the watercourses near Szczecin, ending in the municipal sewage system, which ensures that the hybrids would not get into the natural environment to have contact with pure species. The growing fish were regularly caught with the help of electric current producing aggregate JUP-23 (Approved by the Local Commission for Ethical Research no. 24/02 of 3.06.2002). The hybrids were caught and transported to laboratory for analyses. At first the photographic documentation was made and then the fish were put to sleep. The fish were weighted, their caudal length and total length of the body were measured and their look was described in details, taking into account the colour of the body, fins, colour, size, shape and distribution of spots. The number of spots was counted on the dorsal fin, on the left and right side of the body including the larvae spots (large, grey spots along the lateral line), spots above and below the lateral line, behind the eye and on the opercular bones; moreover their distribution and shapes were described in salmon, trout [20] and their hybrids [14]. Significance of differences was calculated using the Kruskal-Wallis test (examined indicators of spots hybrids and pure species) and Spearman correlation (correlation between the length of the fish and the and the number of spots) were used. Performed a factor analysis using the indicators of spots. Statistical analysis was made using the program STATISTICA (data analysis software system), version 9.1. www.statsoft.com., StatSoft, Inc. (2010). Henceforth, as it is generally accepted, in the names of hybrids the maternal species is given first and paternal second [5].

Tab. 1. Summary of materials used in the study.

Hybrids	Number (n)		Total length (cm) mean ± S.D. (range).		Fish weight (g) mean ± S.D. (range).	
	0+	1+	0+	1+	0+	1+
♀ trout x ♂ salmon	307	109	8.44±1.84 4.90-13.60	13.75±2.40 8.50-18.90	8.82±5.99 1.5-27.50	35.01±19.08 6.40-73.3
♀ salmon x ♂ trout	101	57	8.79±1.96 5.5-15.5	12.33±2.76 7.76-19.2	9.4±6.73 1.7-39.4	25.46±15.19 5.20-71.00

RESULTS

Trout x salmon hybrids

At the age of 4 months, the trout x salmon hybrids had grey dorsal area, light-grey and sandy sides and white abdomen area (Fig. 1). With age the colouration of their body was getting darker. At the age of 15-22 months the individuals with fewer spots had darker body colours. Together with body colouration also the colours of the fins changed. In all hybrids aged 4-6 months, the edges of the adipose and caudal fin were orange or dark grey (Fig. 2), while the other fins were milky white. In some 6-month old individuals the anal fin had black and white edge, which was orange in some older individuals.



Fig. 1. 4-month-old hybrid ♀ *Salmo trutta* x ♂ *Salmo salar*, scale of 1 cm

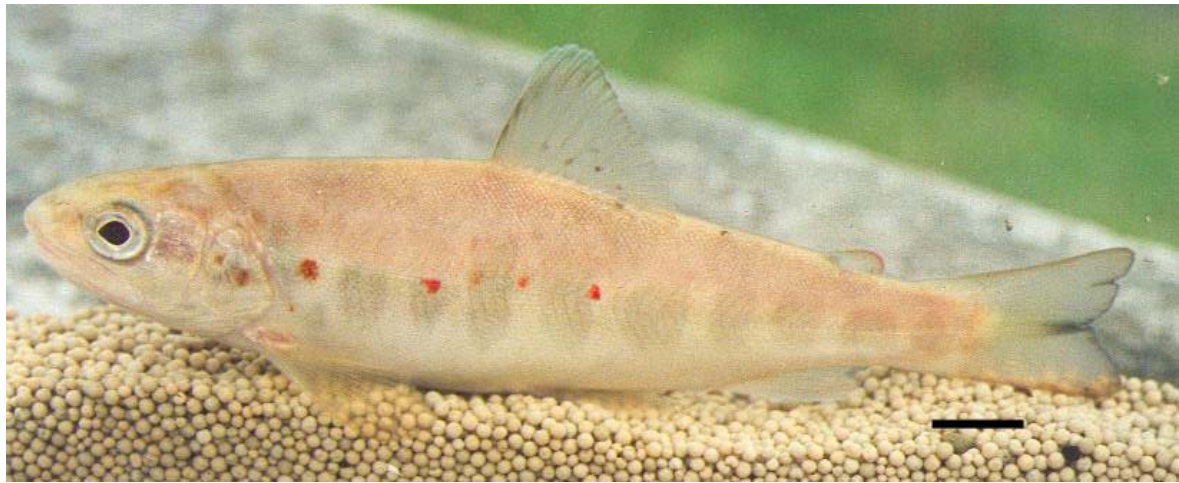


Fig. 2. 6-month-old hybrid ♀ *Salmo trutta* x ♂ *Salmo salar*, scale of 1 cm

The trout x salmon hybrids in the first year of age had from 3 to 11 larvae spots on the left side of the body, while in the age 1+ the number of these spots varied from 7 to 10 (Fig. 3a). In 4-month old individuals the spots were surrounded by light-grey aureoles. The smallest number of the larvae spots (3-10) was found in the group of 6-month old fish. In all samples of the hybrid of this type there were some individuals in which the larvae spots were of irregular shape, were broadened, joined or divided at the height of the lateral line, shifted towards dorsal area or with additional grey spots in the space among the larvae spots from top, (Fig. 4). In the following the results presented concern the left side of the body.

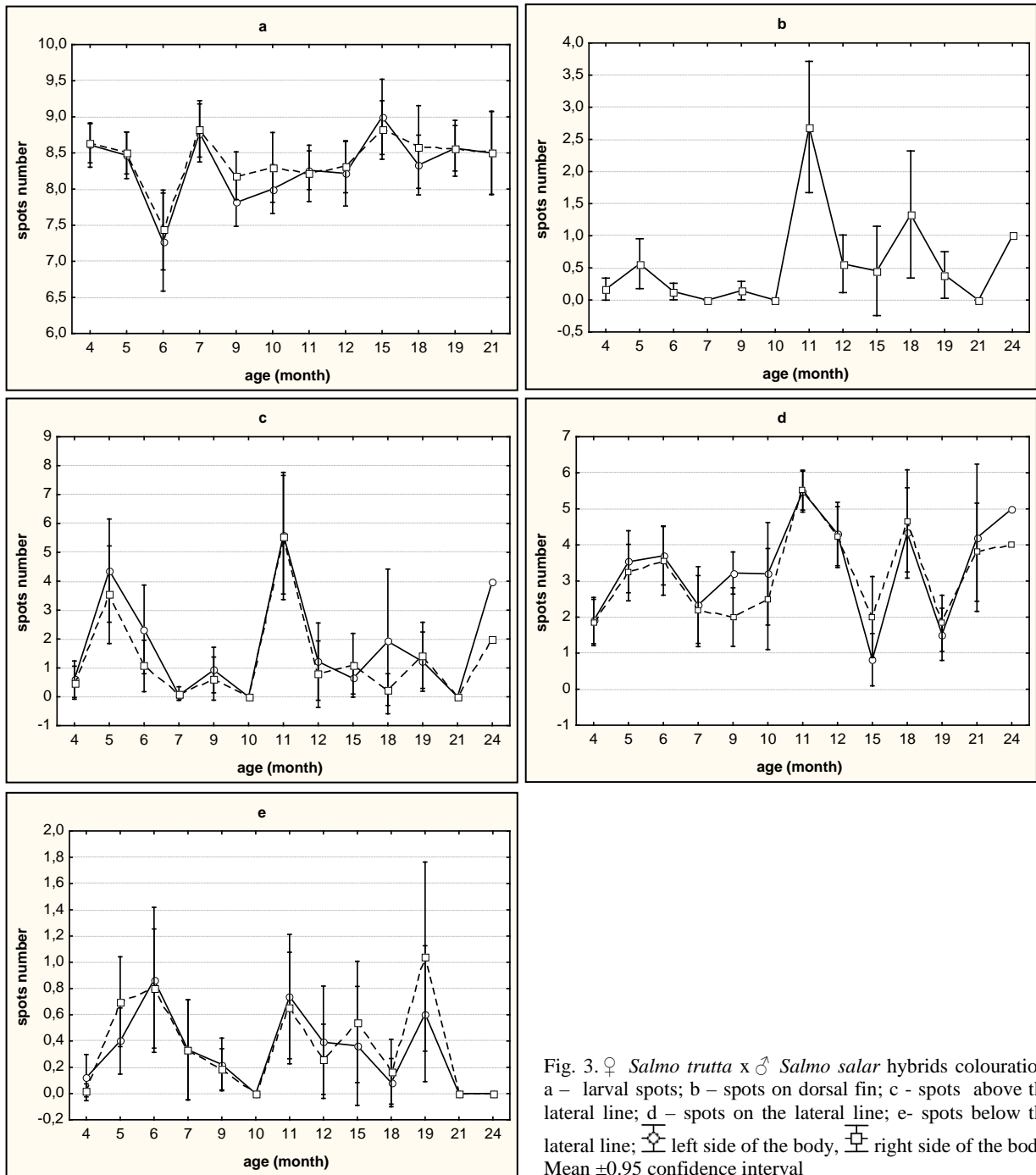


Fig. 3. ♀ *Salmo trutta* x ♂ *Salmo salar* hybrids colouration: a – larval spots; b – spots on dorsal fin; c - spots above the lateral line; d – spots on the lateral line; e- spots below the lateral line; \circ left side of the body, \square right side of the body. Mean \pm 0.95 confidence interval



Fig. 4. 6-month-old hybrid ♀ *Salmo trutta* x ♂ *Salmo salar*, scale of 1 cm

In the hybrids of the trout x salmon type aged 0+ and 1+, the majority of individuals had no spots on the dorsal fin; no individual aged 7, 10 and 21 months had spots on the dorsal fin, while in the others the number of spots on the dorsal fin varied from 1 to 9, (Fig. 3b). On the dorsal fin single spots were placed at the bases of the rays, and if present in a greater number they were irregularly distributed in the majority of individuals and only in a few fish they were arranged in two rows. The number of spots above the lateral line in the hybrids aged 0+ varied from 0 to 17, while in those aged 1+ from 0 to 14, (Fig. 3c). At the age of 10 months all the individuals did not have any spots over this area. At the age of 4 months the spots above the lateral line were red or orange, while in older individuals grey spots were prevalent.

On the lateral line in the trout x salmon hybrids aged 0+ and 1+ (on the left side of the body) the number of spots was 0-10 and 0-8, respectively (Fig. 3d). The spots were orange or red and were on the lateral line, below or above but close to it. The spots below the lateral line in the majority of individuals were in the front side of the body till the end of the dorsal fin. The number of these spots in the hybrids of this type at the age 0+ and 1+ was 0-6 and 0-4, respectively (Fig. 3e). All the individuals at the age of 10, 21 and 24 months had no spots below the lateral line on both sides of the body.

At the age of 0+ the hybrids had 0-3 spots on the opercular bones, while at the age 1+ the number of these spots was 0-2, the spots could be small dots or larger size spots. In all 4- month old and almost all 5-month old individuals no spots occurred on the opercular bones. The spots in individuals aged 6-15 months were red or red-black, while in older fish the opercular spots were grey or black. In the hybrids of trout x salmon type usually there were no spots behind the eye, but in some individuals one black spot was found. Usually in all areas in which the spots were counted they were irregularly distributed, only in a few individuals they were arranged in one row above the lateral line. Below the lateral line the spots were usually distributed to the distance marked by the end of the dorsal fin. In all samples representing the trout x salmon hybrids, there were a few individuals that had very light colouring and had only larvae spots or only a few spots besides them.

Salmon x trout hybrids

The hybrids of the salmon x trout type at the age of 4-month had dorsal area from dark grey to steel grey colour, sides grey to the distance marked by the end of the pectoral fin, abdomen band white, the dorsal fin with black-white or orange-black edge, the edge of the adipose fin in orange and that of the caudal fin in orange-grey colour. Older hybrids were a bit darker, their fins were more colourful (the dorsal fin had grey-white or orange-black edge, the adipose fin and caudal fin had orange edge) and the spots were brighter (Fig. 5).



Fig. 5. 5-month-old hybrid ♀ *Salmo salar* x ♂ *Salmo trutta*, scale of 1 cm

The number of larvae spots varied in a greater range (4-10) in the hybrids aged 0+ than in the hybrids aged 1+ (7-10), (Fig. 6a). Similarly as in the reverse hybrid, in all samples of the salmon x trout hybrids there were individuals with untypical divided spots. At the age of 5 months the hybrids has lighter aureoles. The salmon x trout hybrids aged 0+ had from 0 to 15 spots on the dorsal fin, while those aged 1+ had from 1 to 13 spots. The hybrids aged 4 months had the lowest number of spots, while those aged 22 months had their highest number (Fig. 6b). The spots were grey, grey-orange or orange, usually they were unevenly distributed and in a few individuals they were arranged in rows. Above the lateral line the hybrids aged 0+ had 0-42 spots, while at the age of 1+ they had 9-52 spots (Fig. 6c). On the lateral line the number of spots was 0-12 and 3-11 for the hybrids aged 0+ and 1+, respectively (Fig. 6d). In the majority of salmon x trout hybrids at least some spots were directly above or below the lateral line. At the age of 0+ the hybrids of salmon x trout type had from 0 to 16 spots below the lateral line, while at the age of 1+ this number varied from 1 to 16 (Fig. 6e). The lowest number of spots was found in the hybrids of lighter colouring. In a few hybrids aged 4, 6 and 9 months no spots were found below the lateral line. In individuals with a greater number of spots below the lateral line they were distributed over the whole length of the fish. The salmon x trout hybrids aged 0+ and 1+ had from 0 to 3 black or black-orange spots on the opercular bones. Only in a few of the youngest hybrids, aged from 4 to 6 month, no spots were noted on one or two opercular bones. Behind

the eye on the left side of the body the hybrids aged 4 -14 months had 0-2 spots, in older individuals – 1 spot. The spots behind the eye and on the operculum bones were round or of kidney-like shape, dark grey or black. The spots above and below the lateral line in the salmon x trout hybrids aged 4 and 5 months were grey, while those on the lateral line were red or grey. In older individuals the number of orange or orange-grey spots below and above the lateral line increased, and in some individuals the orange spots on the lateral line had light aureoles. In the oldest hybrids of this type studied the grey spots were dominant below and above the lateral line. In the majority of samples of this hybrid there were single fish of very light colouring and with larvae spots only or with larvae spots and a few additional ones (Fig. 7).

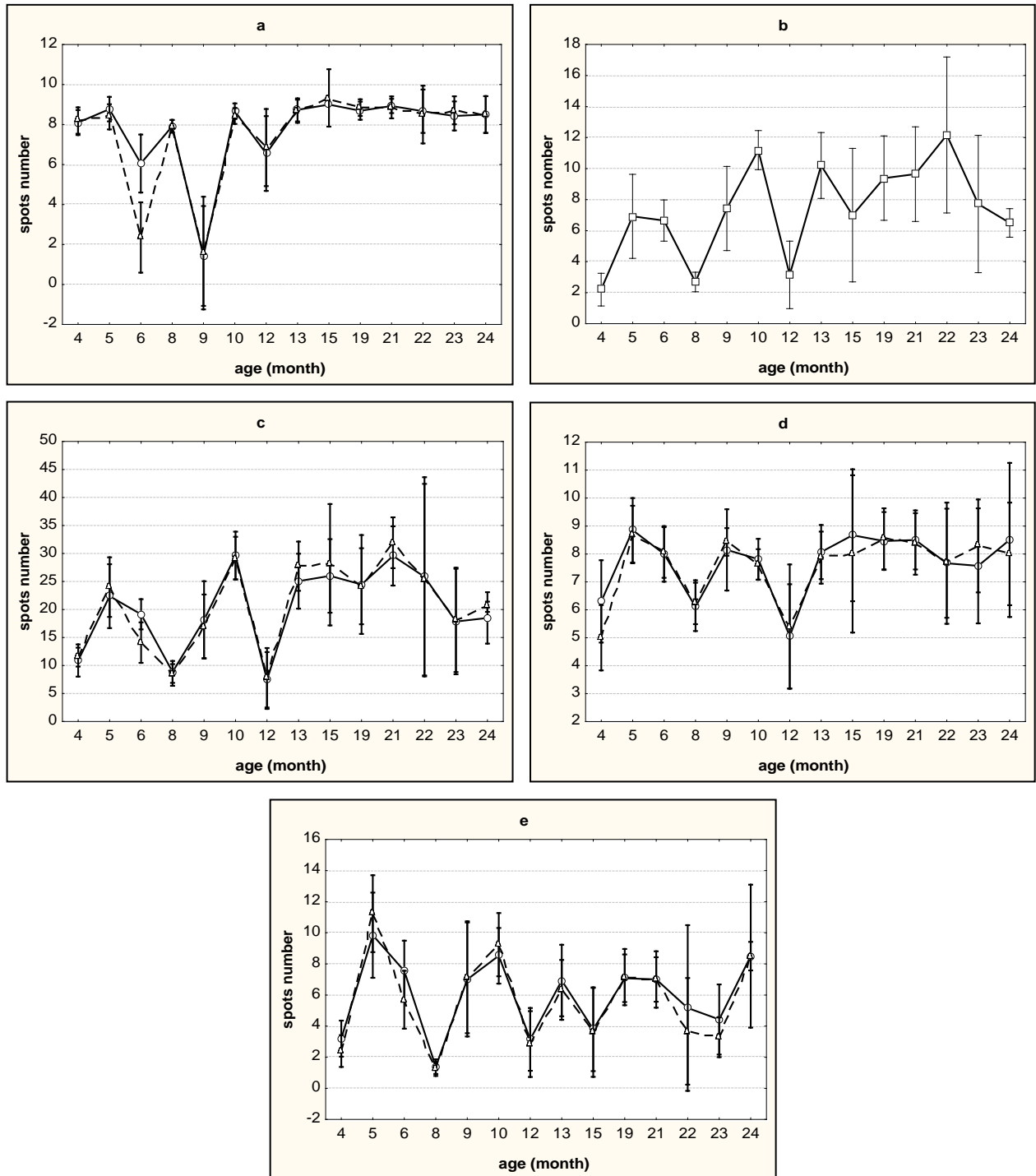


Fig. 6. ♀ *Salmo salar* x ♂ *Salmo trutta* hybrids colouration: a - larval spots; b – spots on dorsal fin; c - spots above the lateral line; d – spots on the lateral line; e- spots below the lateral line; ○ left side of the body, □ right side of the body. Mean ±0.95 confidence interval



Fig. 7. Hybrid ♀ *Salmo trutta* x ♂ *Salmo salar* at the age of 11 months showing untypical light body colouration, scale of 1 cm

STATISTICAL ANALYSIS

For the hybrids of the two types aged 0+ and 1+ , the relations between the fork length and the number of spots and dots were analysed by calculating the Spearman correlation coefficient. For the trout x salmon hybrids aged 0+ or 1+ , statistically significant correlations were found between the fork length of the fish and all the characters analysed except for the number of spots above the lateral line. For the salmon x trout hybrids aged 0+ , a statistically significant correlation was noted between the fork length and the number of larvae spots, while in those aged 1+ correlations were observed between the fork length and all the characters analysed except the number of spots above the lateral line.

The Kruskal-Wallis test has shown in the trout x salmon hybrids aged 0+, the differences in the number of spots and dots between the left and right sides of the body were statistically significant in the majority of areas considered ($p < 0.05$), while at the age of 1+ these differences were nonexistent. In the salmon x trout hybrids aged 0+ the left and right sides of the body statistically significantly differed in the number of larvae spots and the spots behind the eye, while at the age of 1+ the differences disappeared. Statistical analysis of the number of spots and dots on the left and right sides of the body between the hybrids of the two types, has shown no statistically significant differences in the number of larvae spots both at the age of 0+ and 1+ ($p < 0.05$) but the differences in the number of spots on the other areas considered were statistically significant.

DISCUSSION

The first characters used for identification of pure species and their hybrids are the easiest noticeable colouration and shape of the body. The hitherto data on the colouration of hybrids gave general description without a detail analysis of the colour, number, shape and distribution of larval spots and other spots. In literature on the salmon and trout hybrids these features were usually considered together by describing the hybrids as salmon-like [1, 31] or trout-like [18, 1, 27, 30, 5, 2] or as having looks intermediate between the parent species [35, 25, 14]. It should be mentioned that the studies reported in hitherto literature were based on analysis of hybrids of unknown direction of hybridisation, on a small number of samples or on only one cross [26, 19, 34]. In this study an attempt was made to perform a detail comparative analysis of the reciprocal hybrids and the pure parent species. The comparison was made between the hybrids and the trout [20] and salmon [8] growing in forest watercourses in similar conditions, as according to Kuzyshchyn *et. al.*, [22], who analysed the river trout individuals coming from different watercourses, the pattern and intensity of coloration can be affected by the geological structure of the watercourses of origin. Representatives of the hybrid ♀ trout x ♂ salmon aged 0+ and 1+ in body colouration resembled salmon at the same age [8], as they had a similar number of spots on and above the lateral line and on the opercular bones. In the majority of trout x salmon hybrids the spots below the lateral line appeared to the distance determined by the end of the dorsal fin – as in salmon [8]. The differences between the hybrids and salmon were the smaller number of spots, in particular in the hybrids aged 0+ , the distribution, shape and colour of larvae spots and other spots.

Analysis of the ♀ salmon x ♂ trout hybrids, aged 0+ and 1+ revealed similarity to trout of the same age [8], the differences were found in the ranges of variation in the number of spots, which were larger in the hybrids. The exception was the number of spots on the opercular bones, which in the hybrids aged 1+ was similar to that in salmon [8]. The features differentiating the two types of hybrids from the parent species were irregular distribution of spots, the shape of spots and – observed in some individuals- untypical light colour of the body with a small number of spots. Similar results were published by Dellinger *et al.* [7], who analysed the hybrids of *Salmo*

marmoratus x *S. trutta* and distinguished 4 types of colouration; two similar to the parent species and two others of poorer colouration than the parent species. The ranges of variation in the number of larvae spots were always greater in the hybrids than in the young fish of salmon [31, 28, 3] and trout [31, 3]. Hedenskog et. al. [14] studied hybrids of salmon and trout of unknown direction of hybridisation and found that the number of spots on the dorsal fin, above and below the lateral line in the hybrids aged 1+ were similar to those in the parent species or intermediate between them. The hybrids of salmon and trout from the watercourses of southern England, (in which reciprocal hybridisation took place, Hartley, [16]) had colouration similar to that of the parent species and of intermediate features [16]. According to Hammar et al. [12] the hybrids of *Salvelinus alpinus* x *S. fontinalis* were similar to one of the parent species. According to Solomon and Child [30], the salmon and trout hybrids at the stage of parr resembled trout, while in the stage of smolt they resembled salmon in the majority of characters but had orange adipose fin, similar although less intense as in trout. Garcia de Léaniz and Verspoor [10] reported that smaller wild hybrids from the river Pas were similar to salmon, while the greater ones to trout or had features intermediate between those of the parent species. Form among the fish analysed in our experiment, the hybrids salmon x trout in the stage of parr and the two types of hybrids in the stage of smolt were similar to trout as they had bright orange adipose fin as in trout, while in salmon smolts the adipose fin is colourless [30, 8].

The factor analysis of the number of larvae spots and other spots in the two types of hybrids analysed aged 0+ and 1+ indicated that factor 1 was mainly affected by the number of spots on the dorsal fin, below, on and above the lateral line and on the opercular bones. The analysis confirmed the similarity of trout x salmon hybrids to salmon and those of salmon x trout to trout at the age 0+, (Fig. 8a). The analysis of these characters for the fish aged 1+ revealed that the trout x salmon hybrids begin to differ in the colouration from the parent species and from the salmon x trout hybrids (Fig. 8b).

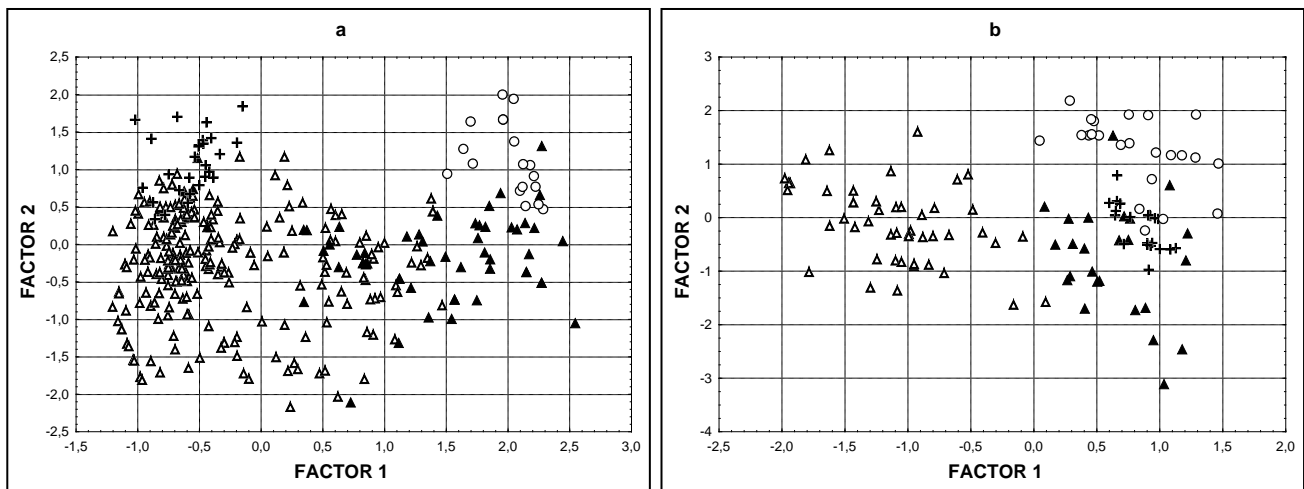


Fig. 8. Values of the factors constructed in the Factor Analysis of the colouration of 0+ old (a) and 1+ old (b) Δ - trout x salmon; \blacktriangle - salmon x trout; + - salmon (Domagała and Kirczuk, 2004); \circ - trout (Kirczuk and Domagała, 2003)

In general, the detailed analysis of the coloration of the reciprocal hybrids of salmon and trout has shown that the majority of hybrids resembled the father species, but their correct identification is possible when analysed together with other features [16], e.g. metric characters [7] or deformations [21]. The other representatives of the two types of hybrids had untypical light colouration. Identification of the hybrids on the basis of colouration is of great importance as the salmon and trout hybrids pose threat to the parent species because of the possibility of introgression and phasing out the pure species from their spawning localities [11].

SUMMARY

In natural conditions, hybridisation between *Salmo salar* and *Salmo trutta* m. *trutta* is most often a result of anthropopressure. The study was made on 416 hybrids of ♀ trout x ♂ salmon and 158 hybrids of ♀ salmon x ♂ trout aged from 4 to 24 months. Reciprocal crossing of salmon and trout was made during artificial spawning. The hatch was introduced into the watercourses near Szczecin (Poland), ending in the municipal sewage system, which ensures that the hybrids would not get into the natural environment to have contact with pure species. In the watercourses in which the hybrids were grown there were no representatives of pure parent species or other predators. The growing fish were regularly caught each month. The fish were weighted, their caudal length and total length of the body were measured and their look was described in details, taking into account the colour of the body, fins, colour, size, shape and distribution of spots. The number of spots was counted on the dorsal fin, on the left and right side of the body including the larvae spots, spots above and below the lateral line, behind the eye and on the

opercular bones. As far as colouration is concerned, the ♀ trout x ♂ salmon hybrids aged 0+ and 1+ were found to resemble salmon of the same age (similar number of spots above and below the lateral line and on the opercular bones, and the spots below the lateral line reached the distance marked by the end of the dorsal fin). The salmon x ♂ trout hybrids resembled trout, but the ranges of variation of individual features were greater than in trout. The hybrids of the two types differed from the parent species in the greater ranges of variation of particular characters, irregular distribution of spots, brighter colours and irregular shapes of larval spots and other spots. Some hybrids representing the two types had untypical lighter colouration with larval spots only or with additional few little spots.

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