

# Classifying Vistula and Pomeranian sea trout populations using discriminant functions based on selected scale characters

Received – 13 March 2009/Accepted – 24 May 2010. Published online: 30 June 2010; ©Inland Fisheries Institute in Olsztyn, Poland

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**Abstract.** This paper presents the results of studies of the effectiveness of a discriminant function system based on selected scale characters of sea trout populations from the Vistula River and Pomerania. Selected parameters of sea trout scales from comparable groups from the period prior to the mixing of the two populations were compiled and function effectiveness was tested. The reference discriminant functions obtained were used to classify the individuals from the mixed stock into corresponding groups. The best results (89.7% conformity) were obtained by applying the discriminant function to classify Vistula sea trout from the winter spawning run from Pomeranian sea trout. The lowest conformity at 64.6% was noted for the function segregating the sea trout from the two Vistula spawning runs. Discriminant analysis of the scale parameters and body length can be used in selection procedures which are used in the program to restore migratory fish species to Poland.

**Keywords:** discriminant function analysis, fish scale characters, Vistula River sea trout, Pomeranian sea trout

## Introduction

The construction of barriers on the Vistula River and its tributaries, overfishing, and pollution were the main factors that limited the population numbers of

Vistula sea trout, *Salmo trutta* L. In an effort to conserve this dwindling population, the Vistula was stocked in the 1970s with material from Pomeranian sea trout originating from the Koszalin-Słupsk region (Wiśniewolski 1987, Bartel 1993). The sea trout from short Pomeranian rivers do not undertake as long spawning migrations as do the Vistula sea trout, nor do they have two annual spawning runs. The fish from the Vistula have gonads in varying states of maturity and ascend rivers in different seasons, and they are larger in size than their Pomeranian relatives (Chełkowski 1969, Sych 1981). The introduction into the Vistula of the different Pomeranian population of sea trout and annual stocking with this material from hatcheries has caused the disappearance of original Vistula sea trout characters. One of the important issues in restoring the natural biodiversity of the Vistula basin ichthyofauna at the species, habitat, and ecosystem levels (Hillbricht-Ilkowska 1997) is to develop a method for identifying fish from within the mixed.

The issue addressed by the current study was whether the differences in biology of Vistula and Pomerania sea trout that are reflected in the characters of the scales, for instance fish age and growth rate (Borzęcka 2001), are distinct enough to permit using them as parameters for discriminant function analysis to effectively classify sea trout individuals correctly. This would create the possibility of gradually eliminating the alien strain from the mixed Vistula stock by avoiding breeding individuals with

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characters of Pomeranian trout that have not been classified with discriminate function analysis as belonging to the Vistula group.

Linear discriminate analysis, which is used to classify comparable groups through measurable, countable, or qualitative characters that describe non-homogeneous groups, evaluates which of these variables is best at predicting group membership. In subsequent steps, the discriminant model evaluates all of the variables tested and indicates the statistical significance of each variable's discriminant power. The discriminant analysis method has been applied for many years in the identification of American and European salmon, *Salmo salar* L., stocks in the fishing grounds of western Greenland (Reddin et al. 1978, Lear and Sandeman 1979, Redin 1981, Potter et al. 1991), as well as in the classification of salmon stocks originating from the northern Baltic basin and those from the Vistula, Drawa, and Grabowa rivers (Tuszyńska and Sych 1983). Functions built based on selected morphological characters of fry, smolts, or adult salmon are used to segregate fish from various rearing facilities and rivers in Ireland (Von Cramon et al. 2005), and differences in the phenotypes of salmon smolts reared in oceanic ponds and originating from three American rivers were used in discriminant analysis to classify individuals to the correct watershed (Sheehan et al. 2005). Morphometric characters of fish have also been used to build discriminant functions that evaluate the smoltification process of farmed juvenile sea trout (Dębowski 1999). Discriminant analysis has been used repeatedly to classify salmon and sea trout from both natural spawning and farming. The accuracy of the classification of 'wild' and farmed fish was evaluated using discriminant analysis built on scale characters in comparison to visual methods to determine the origin of the fish that were assessed by researchers experienced in scale readings (Antere and Ikonen 1983, Borzęcka et al. 1990, Borzęcka 1991, Hiilivirta et al. 1991).

Discriminant functions for classifying stocks of sea trout and salmon were built using meristic and measurable scale characters, including the size of scale growth in subsequent years, which is visible in

the scale images, and biometric characters such as fish body length, fish weight, etc. (Tuszyńska and Sych 1983, Borzęcka et al. 1990, Ikonen and Torvi 1990). In turn, the discriminant function built to segregate a stock of rainbow trout, *Oncorhynchus mykiss* (Walbaum), from natural spawning and farming applied the distance between circuli that were laid down during the river phase of life and the thickness of the circuli (Marcogliese and Casselman 1998, Lacroix and Stokesbury 2004). The discriminant method for classifying fish can also be based on qualitative characters such as the degree to which scales are resorbed or fish age.

The aim of the study was to designate a reference discriminant function for winter and summer Vistula sea trout and Pomeranian sea trout. The results of the analysis will be available to rearing facilities that perform controlled spawning to allow them to classify a group of spawners with characters that are typical of the original Vistula sea trout stock.

## Materials and methods

The scales used for the discriminant analysis presented in this paper came from fish caught in 1970. This year class of fish had not yet been affected by the genetic impact of population mixing in the Vistula that resulted from stocking this river with alien strains of sea trout. Scales from 259 sea trout caught in the Grabowa River in Pomerania and scales from 221 winter and summer sea trout from the same year class that were caught with nets in the Vistula River were used to select the parameters for the reference discriminant functions analysis. The effectiveness of the functions obtained was tested using scales from 34 sea trout of unknown origin caught in the Vistula River mouth and held in a spawning facility for artificial reproduction.

The scales used in the study were collected from between the dorsal and adipose fins from the first row above the lateral line. The scale material occurring in this body location from fish of the genus *Salmo* is homogeneous structurally and with regard

to size (Tuszyńska and Sych 1983). The interpretation of images and measurements of selected growths were performed on plastic imprints of the scales using a projector with magnification of four to seven times (Sych 1964). Scale growth was measured and circuli were counted on the oral scale radius; only circuli that bisected the radius within the field of vision of the apparatus were counted (Tuszyńska and Sych 1983). Functions were created using a program that was especially written for the current study (Sych and Górczyński, personal communication).

Discriminant functions were based on reference scale samples from fish of known origin. They were created based on variations of selected characters from within and between the populations. The general character of the discriminant function was as follows:

$$Y = a_0 + a_1x_1 + a_2x_2 \dots + a_ix_i,$$

where  $x_1, x_2, \dots, x_i$  are the size of the selected characters used to classify the fish groups, while coefficients  $a_1, a_2, \dots, a_i$  describe the discriminant power of a given character, which its ability to identify different groups from within a given set. The function applied is based on establishing the size of characters  $x_1, x_2, \dots, x_k$  for each subsequent fish, and on calculating the corresponding value of  $Y$ . The classification of the fish to one or other of the comparable groups depends on whether  $Y$  is positive or negative. The opposing mean values of the discriminant values of  $+Y$  and  $-Y$  results from the symmetry of the  $Y$  distribution at the point where the fish are segregated into groups (stocks) and is equal to zero.

The characters compiled each time for the pairs of stocks were as follows: 1) winter Vistula sea trout (WVT) and summer Vistula sea trout (SVT); 2) winter Vistula sea trout (WVT) and Pomeranian sea trout (PT); 3) summer Vistula sea trout (SVT) and Pomeranian sea trout (PT); 4) winter and summer Vistula sea trout (VT) and Pomeranian sea trout (PT).

The first step was to verify the discriminant strength of the characters selected to build the function. The discriminant function was created by compiling various configurations of the scale parameters and the fork length and weight of the fish. Then its effectiveness in classifying the fish to the

corresponding stocks was tested. The functions built based on scale growth during the first year the fish inhabited the sea ( $WS_1$ ) and the number of circuli in this annual zone ( $CS_1$ ) did not produce satisfactory results. The discriminant function built for comparable samples regardless of fish age segregated Vistula sea trout from Pomeranian sea trout with total error that exceeded 32%. The same function based on the same scale parameters that was tested on groups of fish aged A.2+ segregated the Vistula sea trout from the Pomeranian sea trout with greater accuracy, but erroneous classification was still high at 23.3%. When attempts were made to segregate the two Vistula stocks, barely 57.5% of the classifications were correct. When fork length ( $L$ ) was added to the parameters used to build the function, correct classifications increased to 64.4%; however, in excess of 30% of the fish were classified incorrectly which indicated that the parameters selected for the function were not effective.

The widths of subsequent annual zones on the scales of older fish were used to increase the effectiveness of the discriminant function since these fish had at least two annual zones on their scales for the period in which they fed in the sea. Such fish in the pure Vistula population accounted for a mean share of 44% of the winter stock and over 60% of the summer stock (Borzęcka 1999). Ultimately, the function that best classified the sea trout to the corresponding stocks was based on the width in mm of the first annual zone on fish scales from the sea period ( $WS_1$ ), the number of circuli in the first annual zone on fish scales from the sea period ( $CS_1$ ), the width in mm of the second annual zone on fish scales from the sea period ( $WS_2$ ), the number of circuli in the second annual zone on fish scales from the sea period ( $CS_2$ ), and fork length in cm ( $L$ ) (Table 1).

## Results

The reference discriminant function built based on the characters of scales of fish that had spent at least two growth periods in the sea was the most effective

**Table 1**

Mean values of scale parameters and mean fork length of sea trout used to build the discriminant functions. Width in mm of the first annual zone on fish scales from the sea period ( $WS_1$ ), number of circuli in the first annual zone on fish scales from the sea period ( $CS_1$ ), width in mm of the second annual zone on fish scales from the sea period ( $WS_2$ ), fork length in cm (L)

	Winter sea trout	Summer sea trout	Pomeranian trout
$WS_1$	1.728±0.276	1.711±0.359	1.152±0.273
$CS_1$	40.1±6.0	41.6±7.2	39.6±5.2
$WS_2$	1.269±0.182	1.311±0.174	1.002±0.332
$CS_2$	28.6±4.6	31.1±3.6	24.4±8.0
L	78.5±5.4	77.4±8.8	67.9±4.2

at classifying fish to the corresponding groups. The components of the linear discriminant function equation are presented in Table 2. The least satisfactory result was obtained when function F(A) was used for the reference group. The variant for

segregating individuals from summer or winter Vistula sea trout from the mixed stocks with the five parameters used was correct in almost 65% of the classifications (Table 3). Function F(B) produced the greatest percentage of correct classifications, and the

**Table 2**

Components of the discriminant function obtained during analysis. Winter Vistula sea trout (WVT), summer Vistula sea trout (SVT), Vistula sea trout (VT), Pomeranian sea trout (PT), width of the first annual zone on fish scales from the sea period ( $WS_1$ ), width of the second annual zone on fish scales from the sea period ( $WS_2$ ), number of circuli in the first annual zone on fish scales from the sea period ( $CS_1$ ), number of circuli in the second annual zone on fish scales from the sea period ( $CS_2$ ), fork length (L)

Function code	Fish origin 1	Fish origin 1	Discriminant functions components					
			$a_0$	$WS_1$	$CS_1$	$WS_2$	$CS_2$	L
F(A)	WVT	SVT	0.342	-0.008	-0.008	0.478	-0.044	0.009
F(B)	WVT	PT	-3.333	0.053	-0.015	0.493	-0.009	0.048
F(C)	SVT	PT	-2.767	0.649	-0.024	0.031	0.028	0.025
F(D)	VT	PT	-2.643	0.518	-0.026	-0.416	0.010	0.029

**Table 3**

Results of applying discriminant function F(A) to classify winter Vistula sea trout (WVT) and summer sea trout (SVT) based on width of the first annual zone on fish scales from the sea period ( $WS_1$ ), the number of circuli in this zone ( $CS_1$ ), the width of the second annual zone on fish scales from the sea period ( $WS_2$ ), the number of circuli in this zone ( $CS_2$ ), and fork length (L)

Fish origin	Classification by discriminant function F(A)		
	WVT	SVT	Total
Winter Vistula River sea trout (WVT)			
N	34	16	50
%	68.0	32.0	100.0
Summer Vistula River sea trout (SVT)			
N	13	19	32
%	40.6	59.4	100.0
N misclassified fish			29
% misclassified fish			35.4

**Table 4**

Results of applying discriminant function F(B) to classify winter Vistula sea trout (WVT) and Pomeranian sea trout (PT) based on the width of the first annual zone on fish scales from the sea period ( $WS_1$ ), the number of circuli in this zone ( $CS_1$ ), the width of the second annual zone on fish scales from the sea period ( $WS_2$ ), the number of circuli in this zone ( $CS_2$ ), fork length (L)

Fish origin	Classification by discriminant function F(B)		
	WVT	PT	Total
Winter Vistula River sea trout (WVT)			
N	45	5	50
%	90.0	10.0	100.0
Pomeranian sea trout (PT)			
N	5	40	45
%	11.1	88.9	100.0
N misclassified fish			10
% misclassified fish			10.5

**Table 5**

Results of applying discriminant function F(C) to classify summer Vistula sea trout (SVT) and Pomeranian sea trout (PT) based on the width of the first annual zone on fish scales from the sea period ( $WS_1$ ), the number of circuli in this zone ( $CS_1$ ), the width of the second annual zone on fish scales from the sea period ( $WS_2$ ), the number of circuli in this zone, ( $CS_2$ ), fork length (L)

Fish origin	Classification by discriminant function F(C)		
	SVT	PT	Total
Summer Vistula River sea trout (SVT)			
N	25	7	32
%	78.1	21.9	100.0
Pomeranian sea trout (PT)			
N	6	39	45
%	13.3	86.7	100.0
N misclassified fish			13
% misclassified fish			16.9

**Table 6**

Results of applying discriminant function F(D) to classify Vistula (VT) from Pomeranian (PT) sea trout based on the width of the first annual zone on fish scales from the sea period ( $WS_1$ ), the number of circuli in this zone ( $CS_1$ ), the width of the second annual zone on fish scales from the sea period ( $WS_2$ ), the number of circuli in this zone ( $CS_2$ ), fork length (L)

Fish origin	Classification by discriminant function F(D)		
	VT	PT	Total
Summer Vistula River sea trout (VT)			
N	72	10	82
%	87.8	12.2	100.0
Pomeranian sea trout (PT)			
N	6	39	45
%	13.3	86.7	100.0
N misclassified fish			16
% misclassified fish			12.6

accuracy of segregating winter sea trout individuals from Pomeranian sea trout into the corresponding groups was nearly 90% (Table 4). Function F(C) produced accurate classifications of 83% of the fish (Table 5). The last of the functions tested, F(D), permitted classifying the Pomeranian sea trout from the mixed Vistula stock with an accuracy in excess of 87% (Table 6).

The most effective function was that used to evaluate sea trout specimens to be used in artificial spawning at the spawning facility in Świbno. Growth achieved during the sea period was measured and the circuli in these zones were counted on the scales of 34 spawners. The origin of the fish to be used in artificial spawning was verified based on scale characters and fork length and the reference function that was determined to be the most accurate at segregating the fish (Table 7). Four of the functions classified all of the fish as originating from the Vistula stock, while the function that analyzed the fork length and individuals age A.1+ and A.2+ classified 97% of the fish as coming from this stock.

## Discussion

The sea trout reproducing in the Vistula system begin their spawning migrations in two distinctly separate periods. After spending one or sometimes many feeding seasons in the sea, the sea trout from the winter spawning run leave saline waters in winter or early spring many months before spawning. Individuals with well-developed gonads from the summer spawning run ascend the rivers in summer immediately before spawning, which usually happens in November (Dixon 1931, Piątek 1961, Żarnecki 1963, Chrzan 1971, Bartel 1988, 1993). Żarnecki (1963) also postulated that there was reproductive isolation between the two groups. Winter sea trout supposedly spawned in the Carpathian tributaries of the Vistula, while summer trout supposedly migrated to the vicinity of Nieszawa, which is in the lower reaches of the Vistula River. Pomeranian sea trout spawns in short rivers, which they usually ascend in September and October, and this strain never attains as a large size as do the Vistula sea trout (Chełkowski 1969, 1974, Sych 1981). Behaviorally and morphologically, these sea trout are more similar to the summer Vistula sea trout.

**Table 7**

Reference discriminant function coefficients used to classify sea trout of unknown origin from the spawning facility in Świbno. Vistula sea trout (VT), summer Vistula sea trout (SVT), Pomeranian sea trout (PT), width of first annual zone on fish scales from the sea period ( $WS_1$ ), number of circuli in the first annual zone on fish scales from the sea period ( $CS_1$ ), width of the second annual zone on fish scales from the sea period ( $WS_2$ ), number of circuli in the second annual zone on fish scales from the sea period ( $CS_2$ ), fork length (L)

Age class, N=34	Sea trout stock	Reference discriminant function coefficients						Separation
		$a_0$	$WS_1$	$CS_1$	$WS_2$	$CS_2$	L	
A.2+ N=10	VT/PT	-2.643	0.518	-0.026	0.416	0.097	0.029	100% to the VT group
A.2+ and A.3+ N=13	VT/PT	-1.228	1.187	-0.046	0.460	0.022		100% to the VT group
A.1+ and A.2+ N=30	VT/PT	-1.759	0.872	-0.035			0.025	97% to the VT group
A.2+ N=15	VT/PT	0.525	1.528	-0.052				100% to the VT group
A.2+ N=15	SVT/PT	-0.78	1.300	-0.036				100% to the VST group

It was interesting to study whether these behavioral, morphological, and physiological differences between winter and summer Vistula sea trout were distinct enough to serve to identify individuals that belonged to different stocks. In ichthyology DNA mitochondrial analysis and searching for genetic markers are some of the methods used to identify genetically different populations. Phenotype differences reflect genotype differences which are the genetic parameters of either individuals or populations that permit identifying individuals or groups (Włodarczyk and Wenne 1996, Łuczyński et al. 1997, Tiffan et al. 2000). However, attempts to find these genetic markers in individuals from close populations do not always succeed, and they require specialized laboratories and experts. However, differences in various population characters such as growth rate, the age distribution of individuals comprising stocks, and the abundance of annual smolt recruitment are all visible in scale images in the width and number of annual zones in subsequent years, the length of time the fish remained in fresh waters prior to smoltification, or in the number and length of feeding periods in the sea. Scales are a readily available study material and selected qualitative or quantitative scale characters are appropriate discriminant function parameters (Reddin 1981, Borzęcka et al. 1990, Borzęcka 1991, DiCenzo and Sellers 1998). Tuszyńska and Sych (1983) attempted to use discriminant function analysis to segregate individuals from different sea trout stocks inhabiting the Vistula by designating a discriminant function based on a single, selected scale character, which was the width of the first annual zone of fish scales from the sea period (WS1) or the number of circuli in this zone (CS1). The results obtained with the function were unsatisfactory. Despite their high correlation (Buras 1999), however, using both characters increased the effectiveness of the function and permitted successfully segregating salmon stocks originating from Finland and Sweden from fish of Polish origin. Attempts to segregate the mixed stock of Vistula and Pomeranian sea trout using this function was loaded with significant error.

The current paper describes the efficiency of discriminant functions based on scale annual zones from the first and second growth periods in the sea, the numbers of circuli in these zones, and fish fork length. These functions accurately segregated the sea trout, but the discriminant power of the scale annual zone from the second feeding period in the sea was ambiguous. Fish fork length was a strong discriminant character. When using these parameters, the final function obtained segregated the Vistula sea trout from the Pomeranian sea trout with more than 87% accuracy. If the winter sea trout are compared to the other groups of sea trout, then the annual zone from the second feeding period in the sea is a stronger discriminant. The precision of segregating the two stocks of Vistula sea trout was not, however, satisfactory at just under 65%. This attests to the closeness of the two stocks and does not support the hypothesis regarding the natural isolation of sea trout that spawn in winter or summer in the Vistula system (Żarnecki 1963). Comparative evidence of this was provided by functions F(B) and F(C). In this instance, there is no doubt regarding geographic isolation, or even regarding the reproduction of sea trout ascending the Grabowa and Vistula rivers. This was noted in the superior segregation of fish with error of 10.5 or 16.9%. Simultaneously, the more precise segregation of fish obtained with function F(B) than with F(C) is understandable since Pomeranian sea trout, because of the season in which they ascend rivers, are of the summer type.

However, it was impossible to evaluate unequivocally the results of using discriminant analysis to choose parental groups of sea trout for breeding at spawning facilities. Most or nearly most of the sea trout spawners tested were classified as Vistula trout regardless of the choice of individual characters and parameters by the reference function. This might indicate that the sea trout inhabiting the Vistula in the 1990s had largely retained their Vistula characters, or that in stocks that are mixed though the introduction of alien stocking material the selected characters do not differentiate strongly enough, or possibly that the scale material used in the tests was not representative. Thus, studies to verify the discriminant

function method using scales from sea trout individuals currently inhabiting the Vistula River should be continued.

The attempt in the current study to apply discriminant analysis can be used in programs to restore migratory fish species to Poland (Sych 1997, Wiśniewolski 2003, Wiśniewolski and Engel 2006). This program proposes to introduce stocking material from anadromous fish populations into corresponding spawning rivers, with the goal of maintaining the natural instinct to return to the river of origin so that the phenomenon of erroneous ascension of rivers is avoided and to fully exploit the volume of continually shrinking area of appropriate spawning grounds.

**Acknowledgments.** The author would like to express her sincere thanks to Prof. Roman Sych for encouraging her to undertake the study of applying discriminant analysis for identifying anadromous fish stocks in Poland and for his kind assistance during work on this topic.

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## Streszczenie

### Separacja troci wiślanej i pomorskiej przy pomocy funkcji dyskryminacyjnych opartych na wybranych cechach łuskowych

W pracy przedstawiono wyniki badania skuteczności funkcji dyskryminacyjnych opartych o cechy łuskowe troci *Salmo trutta* L. pochodzących ze stada wiślanego i pomorskiego. Zestawiano wybrane parametry łusek troci porównywanych grup z okresu przed zmieszaniem populacji i testowano efektywność funkcji. Otrzymane wzorcowe funkcje dyskryminacyjne posłużyły następnie do separacji osobników ze zmieszanych stad do odpowiednich grup. Najlepsze wyniki, wynoszące 89,7% zgodności, uzyskano przy zastosowaniu

funkcji dyskryminacyjnej segregującej trocie wiślane zimowego ciągu tarłowego od troci pomorskiej. Najniższą zgodność, wynoszącą 64,6%, dawała funkcja separująca trocie z obu wiślanych sezonowych ciągów tarłowych. Analiza dyskryminacyjna przeprowadzona na podstawie parametrów łuskowych i długości ciała może być wykorzystana w pracach selekcyjnych prowadzonych w programie restytucji ryb wodnych w Polsce.