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The analysis of sample size in morphometric measurements of fish

Andrzej Dowgiałło

Sea Fisheries Institute, Kołłątaja 1, 81-332 Gdynia, Poland

Abstract. This paper presents the results of analyses of methods of obtaining morphometric equations which determine the dependencies among fish body parameters. A new method of obtaining these equations is also proposed in which the geometric similarity of fish is used and which depends on the search for invariants of their similarity. The results of analyses confirm that the new method, which is much more effective, is at least as precise as the previously applied estimation of equations to describe relationships between particular linear parameters of fish body and fish length.

Key words: morphometric measurements, sample quantity, accuracy of estimation

INTRODUCTION

During machine fish processing, the measurement of particular fish body parameters is not always technically easy or even possible. In such cases, these parameters are determined by the measurement of another parameter which is easier to determine. This is providing that the latter is related to the former parameter in a well-defined and constant way. Therefore, many researchers (Terentiew 1964, Dutkiewicz 1971, Umiancew 1980, Dutkiewicz and Dowgiałło 1995, Pierowa and Kowaliew 1995) have carried out measurements of the morphometric features of fish. These allow for the estimation of equations of the first order, which describe the relationship between particular fish body parameters and fish length l . In the available literature, these so-called morphometric equations appear in two forms, with or without an intercept. The form of the equation results from the type of the statistical method used in processing the measurement results. The equations with an intercept are characteristic of investigations which were carried out until the mid-1980s. The computation methods and models used then, which were recommended in the literature (e.g. Volk 1958, Draper and Smith 1966), led to equations of the following type: $y = bx + a$ (b – regression coefficient, a – an intercept). It was not until the time when statistical computer programs became commonplace that estimations of morphometric equations without an intercept ($y = bx$) were simplified. Such equations are logically closer to the character of the dependencies which they describe. The data which are available, due to variety of methods through which they are obtained and the laconic form of their presentation, are usually limited to the equation and its determination coefficient R^2 ; however, it is impossible to determine which of the two types of equations approximate the results of morphometric measurements more precisely.

The method of morphometric measurements of fish that was described by Adamkiewicz and Matuszek (1967), among others, and which has been applied so far does not allow for the determination of the number of measurements necessary to estimate the morphometric equation to predict the expected value y with acceptable accuracy. The confidence limits of the mean value and the prediction limits of single observations can be determined a posteriori after the estimation of the morphometric equation has been made. Therefore, the rule was that the more measurements the better, although it is obvious that by increasing the number over a certain level changes the estimation level insignificantly in terms of machine design or steering. On the contrary, such actions increase costs and measurement efforts. Therefore, in order to estimate the mean values of mutual proportions of fish body linear parameters (morphometric equations), it would be advantageous to apply methods which allow the accuracy of estimation to be determined a priori. The aim of this paper is to present such a method and to verify it statistically using the results of morphometric measurements.

MATERIALS AND METHODS

The possibility of a new approach in the method of taking morphometric fish measurements is made possible by the geometric similarity, which has been proven empirically, of the individuals of a given species. This similarity is significant in that particular fish body proportions which describe its geometric shape, e.g. the ratio of maximum fish width to its length, are constant and independent of size. These proportions are the invariants and they are related to the regression coefficients in morphometric equations of the $y = bl_c$ type. The accuracy of invariant estimation, which reflects the closeness of similarity, is concerned with the quantity and accuracy of measurements which are carried out in order to determine them. All deviations from the theoretical similarity can be interpreted as measurement errors resulting from specimen differences, which are characteristic of raw material of biological origin, and measurement errors. However, the ratio of, for example, the total lengths l_c of two different fish is the coefficient of scale change (Johnstone and Thring 1960).

By utilizing the geometric similarity of fish and employing proper statistical techniques, the confidence limits of the mean value and standard deviation of an arbitrary invariant for the whole population based on the n -element sample is easy to describe. By assuming an arbitrary alpha level, it is possible to determine the minimum number of independent measurements which allow for the determination of an arbitrary invariant with accepted error. The variance of a particular invariant in a population, which is necessary in order to determine the sample size, can be determined based on preliminary investigations, the results obtained from earlier investigations, by using the preliminary sample or by using knowledge of the population structure. It must be noted that such procedures are based on the assumption that the sampling distribution is normal.

In order to compare the accuracy of estimations of the morphometric dependencies of fish, analysis of these dependencies was carried out using the three previously mentioned methods. The results of measurements of the morphometric parameters of 300 sprats, which were carried out according to the procedure presented in Figure 1, were used for the analysis.

The analysis was begun by checking that the distributions of measurement results of particular invariants in the 300-element sample were normal. It was confirmed that all mea-

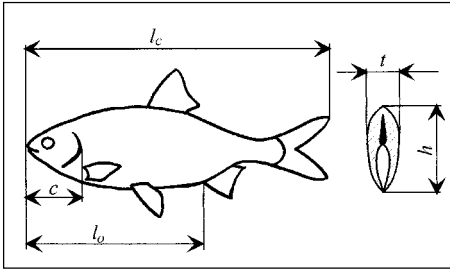
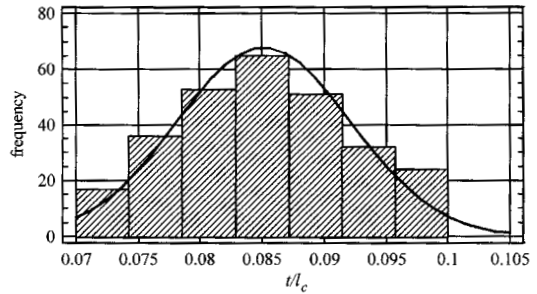


Fig. 1. Diagram of morphometric measurements

Fig. 2. Distribution of invariant t/l_c values obtained from measurements of 300 sprats

measurements had a normal distribution. Figure 2 presents the distribution of measurements of the invariant most vulnerable to distortions $t : l_c$, which depends on the fullness of the stomach, among others. The sample size is insignificant in comparison to the abundance in the general population, which, in this case, includes all the sprats in the Baltic Sea. However, on the basis of the results which were obtained and by keeping the natural character of the phenomena under investigation in mind, it was determined that there is a basis for making assumptions regarding the normal distribution of invariant values in the general population.

The program *STATISTICA* was employed for further analysis with the assumed level $1 - \alpha = 0.95$ and the following parameters were determined:

1) morphometric equations (with and without an intercept) which describe the relationships between maximum thickness t , head length c , maximum height h , the distance from the anus to the beginning of the head l_o and total fish length l_c (based on measurements from the whole set),

2) the confidence and prediction intervals of these equations,

3) probability invariants ($b_t = \frac{t}{l_c}$, $b_c = \frac{c}{l_c}$, $b_h = \frac{h}{l_c}$ and $b_{l_o} = \frac{l_o}{l_c}$) and their standard deviations for the general population (based on the measurements from the whole set),

4. similarity invariants (a_b , a_c , a_h and a_{l_o}) and their standard deviations for the general population (based on measurements of 10 randomly chosen sprats)

5. using the results obtained from the 10-element random sample, disregarding the influential observations (Ekschlager 1974) and accepting the values of estimation error, the smallest number of measurements n_{imin} was derived for each invariant a_i using the Barnett formula (Barnett 1974):

$$n_o = \left(\frac{u(1-0.5\alpha)}{d} \right)^2 \sigma^2 \quad [1]$$

where

$(1 - \alpha) = 0.95$ – confidence level,

$u(1 - 0.5\alpha) = 1.96$ – quantile $(1 - 0.5\alpha)$ in normal distribution table value,

σ^2 – variance of measurement error,

d – half of the confidence limit for the mean measurement value.

RESULTS AND DISCUSSION

The results of analyses which were carried out for the whole sprat sample are presented in Table 1.

The comparison of the resultant morphometric equations (with and without an intercept) with those obtained through transformations of invariant b_i reveals that the differences between the errors of estimation of particular parameters are negligible. The absolute value of differences between the predicted mean values which were derived from particular equations for the investigated fish ranges from 0 to 0.6 mm. Figures 3 to 5 illustrate the graphic presentation of the analyses which were carried out for parameter t , which is the most vulnerable. These figures reveal that the range of confidence limits of the mean values is very narrow for such a numerous sample. The figures also reveal that the prediction limits of 95% of future observations which were derived for each of the methods are almost identically wide.

Before proceeding with further analyses, parameter d from equation [1] was determined. This parameter describes the accuracy of a particular invariant derivation ($d = |\Delta a_i|$). Since the variation of invariants from their theoretical values characterizes perfect similarity, then in the current analyses the random values for parameters t , c , h and l_o are decisive:

$$b_i \pm \Delta b_i = \frac{y_i \pm \Delta y_i}{l_c} \quad [2]$$

where y_i denotes an arbitrary fish body parameter. The transformation of equation [2] yields:

$$\Delta b_i = \frac{\Delta y_i}{l_c} \quad [3]$$

Assuming that, in the case of sprats, it is sufficient to derive an arbitrary parameter y_i with an accuracy of $\Delta y_i = 0.5$ mm, then:

$$\Delta b_i = \frac{0.5}{l_c} = d \quad [4]$$

Dependence [4] reveals that half of the confidence limit for the mean measurement value b_i (error in determining b_i) decreases with the increase of fish length l_c . This means that it is more advantageous to determine d for longer fish, since it increases the accuracy of the

Table 1. Morphometric equations and invariants obtained for a 300-element sample

Equation								Invariant		
$y = bx + a$	R^2 [%]	SEE ^a	MAE ^b	$y = bx$	R^2 [%]	SEE	MAE	b_i	σ	
$t = 0.107l_c - 2.32$	82.3	0.814	0.665	$t = 0.086l_c$	99.1	0.887	0.700	b_t	0.085	0.0066
$c = 0.167l_c + 2.22$	87.3	1.039	0.721	$c = 0.187l_c$	99.7	1.091	0.767	b_c	0.188	0.0100
$h = 0.203l_c - 1.54$	99.2	0.963	0.773	$h = 0.189l_c$	99.7	0.990	0.795	b_h	0.188	0.0095
$l_o = 0.637l_c - 1.74$	99.0	1.017	0.822	$l_o = 0.621l_c$	99.9	1.050	0.851	b_{l_o}	0.621	0.0237

^a Standard error of estimate, ^b Mean absolute error.

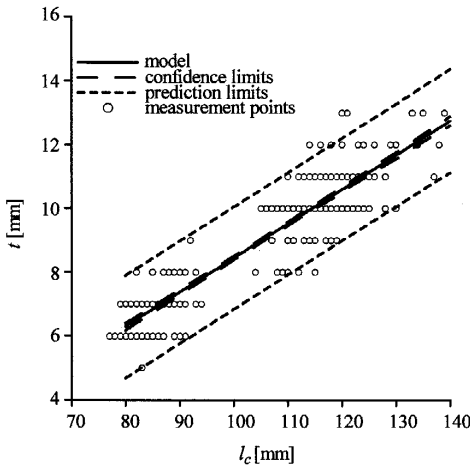


Fig. 3. Graphic presentation of morphometric equation $t = 0.107 l_c - 2.32$

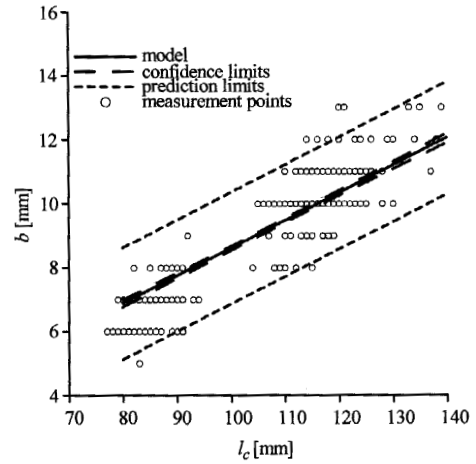


Fig. 4. Graphic presentation of morphometric equation $t = 0.086 l_c$

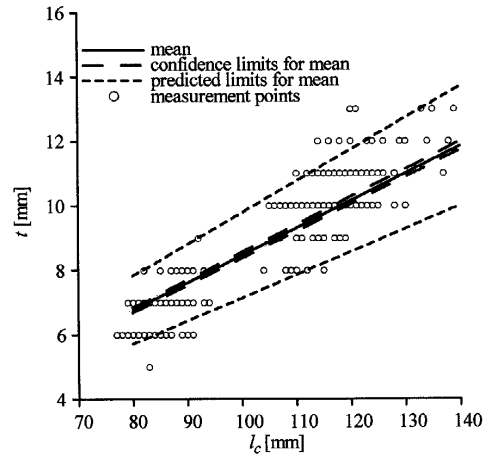


Fig. 5. Graphic presentation of morphometric equation $t = 0.085 l_c$, obtained from invariant transformation

$y_i = b_i \cdot l_{ci}$ dependence obtained from the invariant. Therefore, the inclusion of $l_c = 140$ mm (the longest fish in the size range) to [4] yields $Db_i = d = 0.00357$.

After determining parameter d and by employing results of measurements for the 10-element random sprat sample, the mean values and variances of particular invariants were calculated. Based upon these and by applying formula [1], the minimum values of samples n_{imin} were calculated. These values allow for the determination of the mean values of invariants with set accuracy d . Since the minimum number of measurements varies in different cases, the greater value of n_{imin} was used in further measurements instead of n_{min} . The number of measurements of the random sample was increased by $n = n_{imin} - 10$ and then the invariants were determined again. The results of calculations for the minimal 10-element sample and, for the purpose of comparison, for the 300-element sample are presented in Table 2. The results reveal that the differences among values of related invariants of the minimal and the 300-element samples range from 0 to 0.05, i.e. by 0 to 6%. Therefore, particular parameters of the sprat body, with respect to mechanization and fish processing and which were estimated using

Table 2. Invariants determined for random, minimum and the whole sprat sample

Invariant	Sample				
	Random ($n = 10$)			Random ($n_{min} = 28$)	Total ($n = 300$)
	b_i	σ^2	n_{imin}	b_i	b_i
b_t	0.092	0.00004	13	0.090	0.085
b_c	0.187	0.00007	22	0.187	0.188
b_h	0.190	0.00006	19	0.189	0.189
b_{l_o}	0.623	0.00009	28	0.627	0.621

Table 3. Results of morphometric measurements of freshwater fish

Fish species	Invariant	Sample					
		Random ($n = 10$)			Random ($n = n_{min}$)		Total ($n = 55$)
		b_i	σ^2	n_{imin}	n_{min}	b_i	b_i
Common bream	b_t	0.112	0.00006	18	36	0.110	0.110
	b_c	0.177	0.00005	15	36	0.178	0.179
	b_h	0.315	0.00010	28	36	0.308	0.307
	b_{l_o}	0.493	0.00013	36	36	0.500	0.502
Roach	b_b	0.124	0.00004	5	20	0.124	0.127
	b_c	0.165	0.00017	19	20	0.165	0.167
	b_h	0.263	0.00017	19	20	0.267	0.269
	b_{l_o}	0.597	0.00018	20	20	0.601	0.598

invariants, vary insignificantly (compare Fig. 3). The absolute value of the greatest difference, which occurs in estimations of the distance from the anus to the beginning of the head, was 0.8 mm for the longest sprats from the investigated size range ($l_c = 140$ mm).

Additionally, the verification of the proposed method for obtaining morphometric dependencies of fish bodies involved measuring two freshwater fish species, common bream and roach.

Preliminary morphometric measurements were carried out using fresh fish of various lengths, as follows:

- 180–535 mm (common bream),
- 180–335 mm (roach).

The number of each sample was $n = 10$. Basic invariants (b_p , b_c , b_h and b_{l_o}) were calculated along with variance and a minimum number of independent measurements at the level $1 - \alpha = 95\%$, by assuming that using the invariant value of morphometric parameter y_i must be derived with an error not exceeding $\Delta y_i = d = \pm 2$ mm. Then, the measurements were supplemented, while their minimum number for each species was determined by a maximum value among the invariants determined. Next, their new values were calculated. The results obtained are presented in Table 3, which also gives the results of invariant calculations for samples of $n = 55$. Similarly to the morphometric measurements of sprats, differences between invariants of the minimum sample and the 55-element sample are 0 to 0.03, i.e. 0 – 2.4%. The parameters

estimated vary by a maximum of 1 mm. This difference, when related to the size of the measured fish, is lower than it is with sprats.

The results of the analyses indicate that the application of geometric fish invariants to the determination of morphometric equations is a much more effective method than the previous method, which was based on the approximation of measurement results with linear functions. Utilizing invariants allows for the description of the sample size on the basis of introductory measurements, while assuring the accuracy of the estimation of the desired morphologic parameter.

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Preliminary investigations of the mechanical properties of meshes turned through 90°

Waldemar Moderhak

Sea Fisheries Institute, Kołłątaja 1, 81-332 Gdynia, Poland

Abstract. This paper presents preliminary results of investigations of the breaking resistance of both standard and turned through 90° meshes. Due to the inappropriate load on the knots during standard breaking, the tests were carried out in a different manner than standard tests. It was determined that turning meshes through 90° does not result in the decrease of their resistance to breaking force. An increase of tensile force was registered by about 15% to 25% for meshes with 1.1 mm and 1.8 mm bar thickness, respectively.

Key words: breaking strength, netting, meshes turned through 90°

INTRODUCTION

Attempts to introduce new constructions in fisheries are questioned in terms of usability and, especially, strength. Such was the case with codends with meshes turned through 90°. The most common question after its presentation concerned its strength. The preparation of this type of codend requires turning the netting through 90° which results in changing the direction of stretching forces acting on the turned meshes. It must be verified that this type of netting is stable, i.e. that when stretched its knots will not move, which could result in changes of mesh size and mesh deformation. These questions can be answered by experimental investigations of the mechanical properties of the turned netting. Observations of reactions of this type of netting has shown it has different mechanical properties than traditional netting. The differences are so significant that netting with meshes turned through 90° must be regarded as a new type of material. Employing a new type of material with turned meshes in netting constructions requires many investigations and tests which will determine the mechanical properties of this material.

Until now, netting with meshes turned through 90° has been applied to selectivity investigations of cod codends made of polyamide (Moderhak 1997 and 1999) in which a very good level of protection of juvenile cod and other small fish species was demonstrated.

Further investigations of the mechanical properties of meshes turned through 90° required developing an original method of sample preparation and new investigation methods which vary from the accepted standard.

MATERIALS AND METHODS

The resistance to breaking strength of both meshes turned through 90° and standard meshes was tested along with tests of the influence of mesh location in netting on their strength. The investigations were focused on the application in fisheries of netting with meshes turned through 90° .

As is mentioned above, samples for investigations of the strength of meshes turned through 90° were prepared in a special way. The samples were cut from a piece of traditionally folded material with an adequate surplus of twine so that none of the four twines secured in the gripping jaw of the tensile testing machine slipped out during stretching. Depending on the mesh stretching direction, additional knots were made of the two twines of adjacent mesh bars (from the same row of meshes) for the sample of mesh turned through 90° or of the twines of meshes above and below (from the same mesh column) for the standard mesh sample. These knots were made in order to protect the sample from slipping away from the gripping jaws during the breaking tests.

Polyamide (PA) meshes made of knot netting with 1.1 mm (dtex 235/30) thick twine with a standard mesh bar length of 20 mm and with 1.8 mm (dtex 940/18) thick twine with a mesh bar length of 100 mm were used for the investigations.

In order to determine the differences in resistance to tensile forces of both standard and turned meshes, the tests were performed differently from the standard procedure. These standards can not be applied to testing turned meshes since they require that the tensile force be applied from the inside. The application of the standard method does not permit the determination of differences in the resistance of standard and turned through 90° meshes since hooks installed inside the mesh change the mesh knot load which plays a very important role in the breaking process.

Breaking was carried out with an Instron tensile testing machine which is used to test the mechanical properties of various types of materials. The breaking mesh was mounted in the gripping jaws of the testing machine by clamping two outside twines from opposite sides of the tested mesh. The side knots of the investigated mesh were free, i.e. not mounted. Mesh sample stretching was done by moving the upper gripping jaw while the lower gripping jaw remained immobile. A detector for measuring the stretching force was attached to a registering device and to a computer. The additional registration of tensile force on paper permitted the stretching process to be observed and to ensure that the acceptable device force was not exceeded. Various tensile force sampling frequencies can be set on the tensile testing machine depending on requirements. Measurements were registered frequently during the tests. On average, the whole process of breaking involved from 2,000 to 2,500 measurements of instantaneous stretching force.

The tests were carried out on samples of meshes turned through 90° and, for the purpose of comparison, on samples of standard meshes. Recording the stretching data on a computer is very a convenient way of pooling test results. The high frequency of sampling allowed for the precise reproduction of the whole process of sample breaking and the presentation of the process in graphic form. The Borland Quatro Pro program was applied to process the data obtained from the tests. The processing involved choosing measurement ranges which were of interest with regard to the analyzed problem and transforming the data from all the individual tests to the zero point (the determination of force increase momentum). The application of this procedure allowed all the curves to be placed on one graph and then to compare them. The average values of tensile forces for particular sample types were also determined with this

program. This data was used to prepare graphs and to analyze mesh resistance to tensile force.

RESULTS AND DISCUSSION

Figure 1 presents curves which illustrate the dependence of the breaking processes in time for both standard and turned meshes. The samples in this case were made of netting with a bar length of 100 mm and with 1.8 mm thick twine. Figure 2 presents curves which describe the breaking of meshes of netting made with 1.1 mm twine and a 20 mm bar length. Both twine thicknesses demonstrated slightly different curves for the increase of stretching forces in both standard and turned meshes. In the case of breaking meshes made of 1.8 mm thick twine, the process of increasing the stretching forces in the whole range was faster for standard meshes than for turned meshes. Whereas, with meshes made of 1.1 mm twine, the stretching force increase for the first half of the process was the same for both types of meshes, but then the increase of stretching force was faster for turned meshes. All the mesh samples were destroyed by the breaking of one mesh bar and not by the twine on the outside of the tested mesh.

The results obtained were surprising since it commonly was believed by fishing technique specialists that meshes turned through 90° should have a slightly lower resistance to tensile force than standard meshes do. The breaking forces acting on the turned meshes were slightly higher than those on the standard meshes. A different increase was observed in different twine thickness. For mesh made of 1.8 mm thick twine, the resistance to tensile forces was about 15% higher with turned meshes than with standard meshes, while the resistance of turned meshes made of 1.1 mm thick twine was higher by 25%.

Breaking force is practically not affected by mesh bar length and only the material type and its quality and the finishing on the sample netting influenced tensile force. It would be expected that the resistance to tensile forces is mainly influenced by the way the stretching

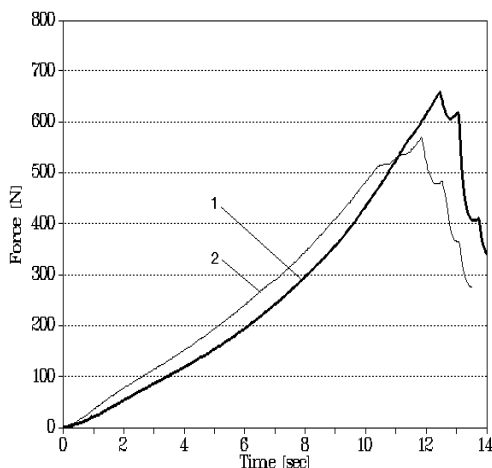


Fig. 1. Mesh tensile force curves; turned (1) and standard (2) with 100 mm mesh bar length and 1.8 mm twine thickness

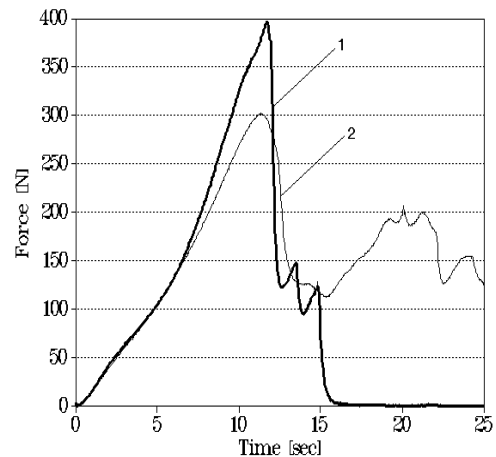


Fig. 2. Mesh tensile force curves; turned (1) and standard (2) with 20 mm mesh bar length and 1.1 mm twine thickness

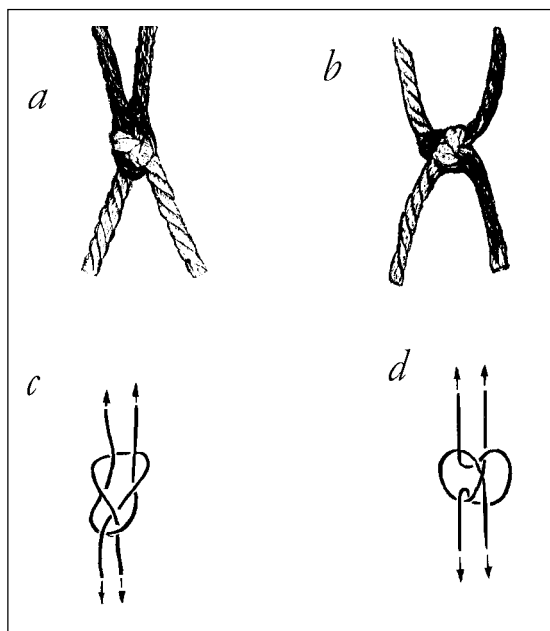


Figure 3. Twine configuration in netting knots with standard meshes (a, c) and with meshes turned through 90° (b, d).

forces affect the knot. Figure 3 illustrates standard mesh (a) and turned mesh (b) knots, as well as an example of twine placing in a standard mesh knot (c) and a turned mesh knot (d). Forces acting on particular parts of the knot may influence total mesh strength. These forces act differently on different parts of the knot twines, which is illustrated in Figures 3 (c) and (d). Standard mesh knot tightening should occur faster than that in turned mesh since, for turned mesh knot, stretching forces initially should not allow for knot tightening and then,

when the stretching force exceeds the friction force generated between knot twines, the knot tightens fast. Plot curves of mesh breaking presented in Figures 1 and 2 confirm this interpretation. The graph which illustrates the breaking of meshes made of thicker twine (1.8 mm) clearly reveals the described process of knot tightening. This process is not as obvious for thinner twine (1.1 mm) due to the smaller differences between forces. The differences in the placing of both twines versus the stretching forces in the standard mesh knot and the turned mesh knot cause, in one case, the shearing force to play a more important role and, in the other case, the tensile force to be decisive in the breaking process. This can be assumed if an analogy is made between the properties of meshes with those of other materials, for example, steel (the resistance of steel samples to tensile forces is greater than their resistance to shearing forces).

In order to determine the influence of these forces on processes of mesh destruction, additional tests on the breaking of simple configurations of twines must be done. These tests would explain the registered differences in breaking of meshes turned through 90° and standard, diamond meshes.

Netting knots play a special role in the processes of stretching as they delay the moment of mesh bar breaking and create a sort of buffer.

The following conclusions can be drawn from the results of preliminary investigations of the resistance to tensile force of meshes made with 1.1 mm and 1.8 mm thick twine:

1. Meshes turned through 90° demonstrate greater resistance to tensile force than standard meshes by about 15% to 25%.
2. Netting knots play an important role in the process of netting mesh breaking by accumulating a large portion of the stretching energy.

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Selectivity tests of polyamide and polyethylene codends made of netting with meshes turned through 90°

Waldemar Moderhak

Sea Fisheries Institute, Kołłątaja 1, 81-332 Gdynia, Poland

Abstract. In 1998, selectivity tests were carried out on codends with meshes turned through 90°:
– made of polyamide (PA) with 85 mm mesh openings in commercial catches,
– two polyethylene codends (PE) with 120 mm and 100 mm mesh openings.
The polyethylene codends tests were carried out jointly by the Sea Fisheries Institute in Gdynia and the Institut für Fischereitechnik (IFH) in Hamburg.

In both cases, the test results of the new codend constructions proved their significant protective properties. Codends with meshes turned through 90° have many advantages and their application may help to restore fish resources in the Baltic Sea.

Key words: trawl catches, codend, cod, protection, selectivity, turned meshes

INTRODUCTION

Selectivity tests of trawl codends have been carried out for many years in order to determine the influence of technical and biological conditions on the selectivity of fishing gear. These investigations cover both technical elements which have an impact on fishing processes, i.e. fishing boats and trawling sets, and the object of the catch itself. The influence of technical parameters of the fishing process and their interrelations and, additionally, hydrological and meteorological conditions causes the selectivity process, i.e. the process of retaining fish in the codend, to differ depending on conditions at a particular place and time. Humans affect these parameters by creating fishing boats and gear. Nature influences them by creating hydrological and meteorological conditions and, especially, through the biological condition of the fish. Comprehensive investigations of the influence of such conditions on selectivity are becoming more frequent. An example of this is the EU project BACOMA (Improving Technical Management in Baltic Cod Fishery).

The selectivity of the fishing gears is determined mainly by humans whose technological activities may result in the retention of only those fish which are abundant and, thus, their fishing activities will not endanger the state of resources. Determining these resource limit magnitudes is left to experts who deal with resource estimations.

The protection means for particular fish species which have been created to date are insufficient to ensure that juvenile fish are really well-protected. It is necessary to continue large-scale investigations and technical tests in order to create and introduce to commercial

fishing effective technical means of protecting resources from excessive catch mortality. The Baltic cod, whose resources have been diminishing in recent years, is under special protection. There is why so much attention is paid to this species in order to protect it from over-fishing.

The investigations of polyamide cod codends with meshes turned through 90° which have been carried out over the last few years at the Sea Fisheries Institute are aimed at determining the protective properties of this gear during cod catches. From the tests which have been carried out so far, it has been concluded that it is possible to create better protective fishing gear than the existing type. The introduction of new solutions to commercial catches may significantly help to restore Baltic cod resources. This paper is a continuation of publications focused on the selective properties of cod codends with meshes turned through 90° (Moderhak 1997, 1999).

Investigations of codends with meshes turned through 90° which were carried out in 1998 were focused on determining which mesh opening would secure proper codend selectivity during commercial catches without reducing catch rates. Investigations which have been carried out so far on this type of codend with 120 mm, 105 mm and 90 mm sized mesh openings have revealed that their protective abilities are higher than those stipulated by the regulatory requirements.

Further investigations, carried out in co-operation with the IFH will focus on the influence of netting material (polyamide, polyethylene) on selectivity and on collecting the maximum amount of data to confirm better protective parameters of codends with meshes turned through 90°. With these goals in mind, investigations of codends with meshes turned through 90° were carried out in 1998 from aboard the German R/V SOLEA. The results, as yet, have not been published.

MATERIALS AND METHODS

A polyamide (PA) codend with 3.5 mm thick twine and 85 mm nominal mesh opening was designed and constructed for testing purposes. In 1998 a commercial codend with meshes turned through 90° was tested. The differences in the mesh shape of both standard and turned 90° construction was presented in an earlier publication (Moderhak 1997). The mesh size and total length of the codend varied from the codends made in 1997. The investigated codend had a total length of about 22 m and a circumference of about 6.7 m (dimensions in the stretched state). It was three times longer than the codends which had been tested up to that point on the R/V BALTICA. The length of the test codend was adjusted to the fishing set-up of a B-403 fishing cutter. Arrangements were made with the ship's owner for the codend to be fitted with length reinforcement on both sides of the codend and to be attached in such a way so as not to distort the natural opening of the meshes turned through 90°. The codend was attached directly to a WP-53/64x4 pelagic rope trawl without an extension. The lack of a typical extension distinguishes the construction of the Polish selective codend with turned meshes from other codends used in both commercial fisheries and testing purposes.

According to the most popular selectivity test methodology, a small sized mesh hooped cover, which prevents fish from leaving the cover, should be used. This allows for easy calculation of the selectivity of the codend being tested. As previously mentioned, the 1998 tests were carried out during commercial catches; this made the use of both a cover and the alternate haul method impossible. This last method requires each haul with a test codend to be followed by a

haul with a codend with a small sized mesh insert in order to determine the fish length distribution at a particular fishing ground. Alternate hauls cannot be carried out from B-403 and B-410 cutters since they are equipped with only one net drum. This is why catch selectivity tests were reduced to comparisons of the number of captured cod in each lengths classes in codends during commercial exploitation. Therefore, the protective parameters of two different codends were tested. The first had meshes turned through 90° and the second had standard diamond meshes which comply with international regulations regarding good selectivity in commercial cod catches. The results of these investigations (without the use of either a cover or an insert) reflect the real fish size selection processes in codends during commercial fishing.

Due to the commercial character of the tests which were carried out in subsequent cruises (May-June 1998), only one type of codend was used, this being either the codend with turned or standard meshes without the small sized mesh cover.

Depending on the catch size, either all cod or only 50% of the cod, if the catch exceeded 1000 kg, were measured. Traditional fish length measurement methodology was applied, which means that fish length in centimeters was rounded down. Also, measurements of mesh opening in codend with meshes turned through 90° were made in two stages, before testing (dry) and after testing (wet after 100 h of work). The values obtained are presented in Table 1.

Marine investigations and their results

The tests were carried out aboard a B-403 (Hel-125) commercial fishing boat in fishing grounds in the Gulf of Gdańsk near the eastern border of the Polish economic zone (fishing ground T6). The selectivity of the codend with meshes turned through 90° was tested at the end of May 1998, while at the beginning of June the standard codend was tested. The tests were carried out in a system of semi-pelagic hauls with a WP-53/64x4 pelagic rope trawl at depths of about 100 m and at speeds of about 1,5 m/s (2,8 knots). A total of 10 measurement hauls were made, five with the codend with meshes turned through 90° and 85 mm nominal mesh opening and five with the standard codend with diamond meshes and 120 mm nominal mesh opening. The duration of hauls varied significantly and reflected commercial fishing practice. The haul times ranged from 6 to 14,5 h (a total of 48 h) for the codend with meshes turned through 90°, and varied from 10 to 11,5 h (a total of 53,5 h) for the standard codend. A total of 3361 cod were measured from the codend with meshes turned through 90° and 4551 cod were measured from the standard codend. The total mass of cod caught with both the turned codend and the standard codend was about 5,7 t and 6,0 t respectively. The catch rate per hour of trawling was about 119 kg/h for the codend with meshes turned through 90°, and about 112 kg/h for the standard codend. Thus, the catch

Table 1. Mesh opening of polyamide netting of codend with meshes turned through 90°

Measurement means	Nominal mesh opening [mm]	Real mesh opening [mm]				Nominal twine diameter [mm]
		on dry		on wet		
		average	min./max.	average	min./max.	
Along codend	85	84.9 (1.66)*	81/88	86.6 (1.78)*	82/91	3.5
Across codend		85.6 (1.9)*	82/90	88.7 (1.79)*	85/92	

*Numbers in parentheses refer to standard deviations of mesh openings derived from measurements

Table 2. Catch data obtained during commercial cod codend tests

Haul No	Type of codend	Date	Duration of haul [h]	Mass of cod caught [kg]	Catch rate [kg/h]	Number of measured cod [spec.]	Cod up to 34 cm class in codend [%]
1	with meshes	26.05.98	6	785	131	646	1.86
2	turned	26.05.98	9	1,390	154	1,070	3.08
3	through 90°	27.05.98	7	362	52	350	2.29
4	with 85 mm mesh	28.05.98	14.5	2,145	148	828*	3.50
5	opening	29.05.98	11.5	997	87	467*	4.50
6	with diamond	7.06.98	10	1,344	134	1,215	9.62
7	meshes,	8.06.98	11.5	1,238	108	1,055	8.63
8	(standard) ;	8.06.98	11.5	1,721	150	654*	7.65
9	with 120 mm mesh	9.06.98	11.5	785	68	714	8.12
10	opening	9.06.98	9	921	102	913	8.76

*About 50% of fish from the catch were measured

rates obtained during commercial investigations were even slightly higher (about 6%) for the more selective codend with turned meshes than for the standard codend. The basic catch data for both codends are presented in Table 2.

On the basis of hauls in which all cod were measured, it was determined that the average unit mass of cod caught with the standard codend with 120 mm mesh opening was 1.1 kg and that for the codend with meshes turned through 90° with 85 mm mesh opening it was 1.23 kg. In standard codend catches, a certain amount of herring were caught in addition to cod, while only cod were caught with the codend with meshes turned through 90°. Also the condition of the fish in the catch was observed on board after hauling. As is presented in Table 2, the hauls were very long, ranging from six to almost 15 hours. These long hauls revealed another advantage of the turned codends in addition to their good selective properties. The majority of the cod from this codend were still alive, unlike those from the standard codend. This must be related to better water flow in the turned codend. As was observed in other investigations, the turned codends are more opened than standard codends. This very much influences flow parameters e.g. water velocity and flow intensity through the codend (Moderhak 1993). Yet another advantage of the turned codend was the shorter time required to haul it aboard. It was not necessary to shake down (move the fish concentrated along the whole codend and extension to its end) the turned codend; after partial winding on the drum all the cod were in the end of the codend with meshes turned through 90° which allowed for the immediate landing of the fish on board.

Length distribution of caught cod

Investigations of the length distribution of caught cod create a basis for comparing the selective properties of the tested codends. The selective properties of codends can be compared with a high degree of probability if sample numbers are adequately large. The comparison of the ability of the codend to protect juvenile fish with the properties of previously tested codends is also possible. As stated above, large samples were collected and large numbers of cod from both codends were measured during the investigations in 1998, and thus, they must be regarded as

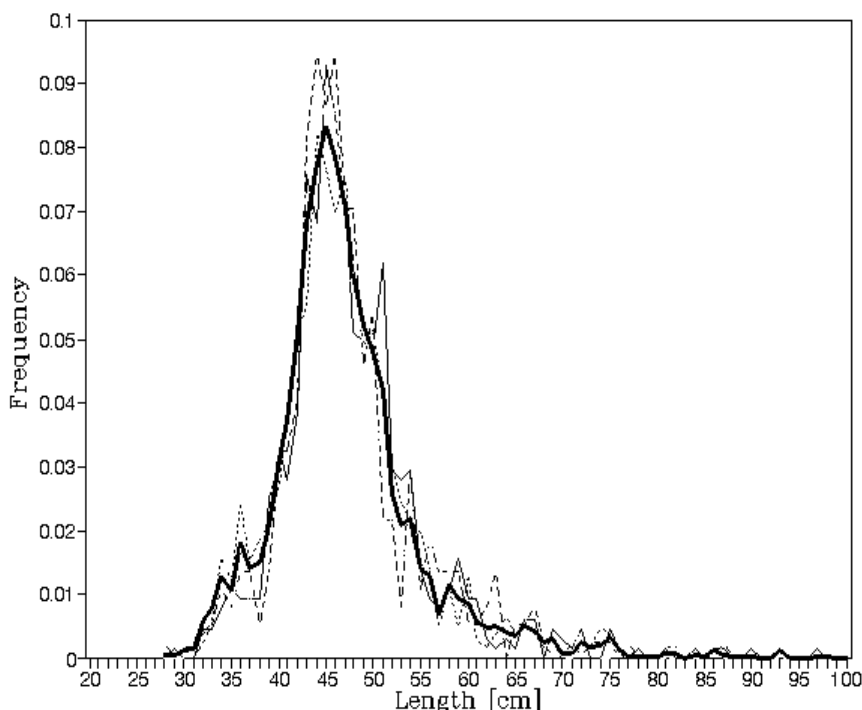


Fig. 1. Cod length distribution in hauls made with turned meshes codend with 85 mm opening and the cumulative distribution of all hauls (thick line)

representative.

Figures 1 and 2 present the frequency curves of cod caught during individual hauls which were carried out with both types of codends and the summary frequency, i.e. the frequency of cod from all hauls made with one type of codend. These curves reveal a slight divergence of frequencies of fish in each length class in individual hauls made with both the standard and turned through 90° codends.

Figure 3 presents curves of the total number of cod caught of both types of codends and the related frequencies in particular length classes. Despite decreasing mesh size by 25%, the codend with meshes turned through 90° with 85 mm nominal mesh opening protected juvenile cod better than the standard codend with diamond meshes with 120 mm nominal mesh opening. The turned codend let more cod up to 36 cm in length escape, while simultaneously capturing more marketable sized fish from 38 cm to 48 cm (see curve in Figure 3). Both codends captured the same number of fish exceeding 48 cm, as is revealed by overlapping frequency curves (Fig. 3). The results of commercial investigations from 1998 reveal that codends with meshes turned through 90° present a significant ability to protect juvenile cod while simultaneously capturing a greater number of marketable cod.

Codend testing on R/V SOLEA

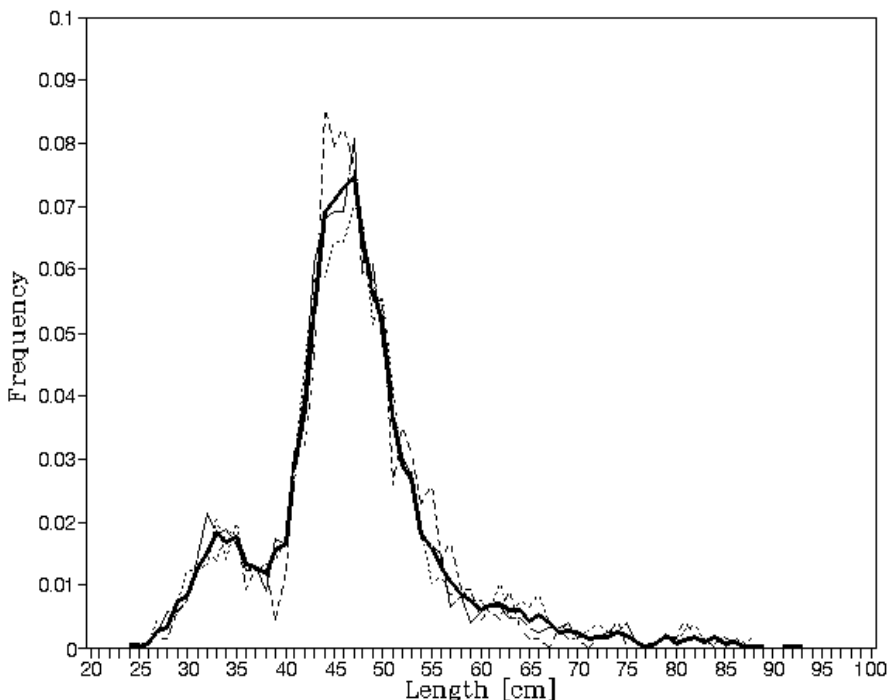


Fig. 2. Cod length distribution in hauls made with a standard codend with 120 mm mesh opening and the cumulative distribution of all hauls (thick line)

In September 1998 investigations with underwater observations were carried out aboard the German R/V SOLEA on two polyethylene (PE) codends with meshes turned through 90° (the nominal and real mesh openings were 120/112 mm and 105/103 mm), one standard codend (120/117 mm) and a multi-panel Swedish codend (with three selective windows with square meshes) made of Ultra-Cross type netting (105/105 mm). The latter was an improved version of a codend which has been under investigation for three years, while the codend with diamond meshes is well-known and is regarded as a standard codend. Polyethylene codends with meshes turned through 90° were made in the traditional manner as two-panel constructions of single twine and were tested using the hooped cover method.

The investigations were carried out during the day in the Arkona fishing grounds (C5,D5) at depths of about 30 m. A bottom trawl was used and its speed was measured by a SCANMAR device attached to the trawl headline. The average trawl speed was about 1,95 m/s (3,8 knots). An underwater manoeuvrable cable camera was used for transmitting pictures of the working trawl and the codends to two monitors located on the ship's bridge.

The codends with meshes turned through 90° were about 11 m long and their circumference was about 6 m (stretched state dimensions) and were made of 4 mm polyethylene twine. The construction of these codends varied significantly from the Polish constructions which were tested at the Sea Fisheries Institute. Aside from netting material, the main difference was that these codends were two-panelled with a 15 m long codend extension made of standard (diamond) meshes with 120 mm mesh opening. This extension was also used in other types of

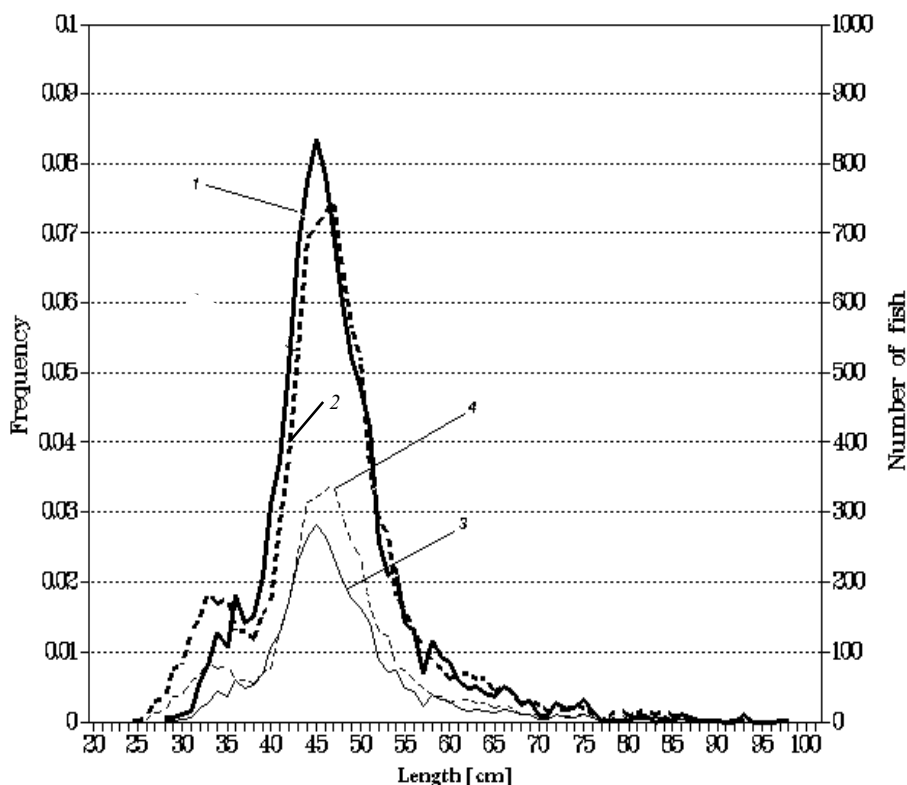


Fig. 3. Cod length distribution in hauls made with turned meshes codend (freq. 1 and number of fish 3) and a standard codend (freq. 2 and number of fish 4) during commercial catches in May and June 1998

codends. Underwater observations of free codends, those without small-sized mesh covers, revealed certain disadvantages. The traditionally constructed extension made it impossible to deploy the main codends and this was clearly observed in the case of the turned codend. Both the codend and the extension of the standard types were almost completely closed along 60% of their lengths. Codends with selective windows and turned codends were better to deploy. It must be noted that the natural opening of meshes turned through 90° is over two times greater than that of diamond meshes. Therefore, the traditional two-panel extension actually acted as a tightening element and created folds on the surface of the netting with meshes turned through 90° resulting in a complete change of the hydrodynamic conditions of water flow in this codend.

The codends were equipped with lifting ropes whose lengths were adjusted to the circumferences of both the standard codend and the multi-panel codend made of Ultra-Cross. On the turned codends they were too short and caused the ends part of the codend closed. The turned codend with 120 mm mesh opening was tested first with the lifting rope which was still too short. After underwater observations it was decided that the following codends would be tested without the lifting rope. Therefore, results obtained for the turned codend with 120 mm mesh opening were corrupted by the short lifting rope since they included lowered protective parameters. Other codends (turned with 100 mm mesh opening, standard with 120 mm mesh opening and multi-panel with 105 mm mesh opening) were tested without the lifting rope. The underwater camera allowed the investigators to determine that the lifting rope was the wrong length in

Table 3. Selective parameters of polyethylene (PE) codends tested on the R/V SOLEA

Codend type	Nominal mesh opening [mm]	Real mesh opening [mm]	Diameter of mesh bar [mm]	L_{50} [cm]	S_R [cm]	S_F [cm]	Cod up to 34 cm class in codend* [%]
With meshes turned through 90°	100	103	4	38	5.5	3.69	32.2
	120	112	4	40.5**	7.5**	3.62**	22.6**
Standard with diamond meshes	120	117	4	40	7.5	3.42	29.7
Multi-panel with 3 selective windows	105	105	6.7	39	5.5	3.71	–

*Determined based on data obtained while an SFI scientist was aboard the R/V SOLEA – percentages determined with respect to the total number of cod captured in the codend

**Results refer to codend with lifting rope which was too short

relation to the turned codend circumference in the deployed state.

The results of investigations obtained during the 431st research cruise of the R/V SOLEA must be mentioned, despite the fact that they describe codends which were not functioning properly. It is especially important in the cases of codends with meshes turned through 90° and a standard mesh extension. Probably, the processes observed (nearly closed codends during towing) using the underwater camera are typical for the a majority of codends currently in use. The results of preliminary analyses carried out at the Institut für Fischereitechnik in Hamburg were made available to the Sea Fisheries Institute in the form of accumulated plots of selectivity curves. The selectivity coefficients of the tested codends were obtained from these plots and are presented in Table 3.

The results of the investigations reveal that, despite improper hydro-mechanic functioning (underwater observations), all the codends comply with the current regulations regarding the protection of cod. However, juvenile cod still constitute a significant percentage of the total number of cod caught (see Tabele 3). These may be affected by the depth of the fishing ground, for example. The investigations on the commercial B-403 cutter were carried out in the Polish economic zone in the Gulf of Gdańsk at depths of about 100 m, while investigations on the R/V SOLEA were carried out at fishing grounds near Arkona at depths of about 30 m. The influence of fishing ground depth on the number of juvenile cod captured was observed by Zaucha (Zaucha *et al.* 1997). In shallower waters he obtained a lower selective factor than in deeper waters. Thus, relatively large numbers of juvenile fish caught in the codends during investigations carried out from aboard the R/V SOLEA might have been caused by different environmental conditions in the shallow fishing grounds of the German economic zone.

A significant improvement in the state of natural resources can be obtained through the application of technical means for juvenile fish protection. The results of investigations which have been carried out thus far, both from research vessels and commercial cutters, reveal that the application of codends with meshes turned through 90° to trawl fishing may provide better protection of juvenile fish. This type of fishery still catches too many juvenile cod which results in the decrease of the mass of fish which could be potentially fished in subsequent years.

As a result of the 1998 investigations of selective properties of cod codends with meshes

turned through 90°, the following conclusions can be drawn:

1. The commercial version of a codend with meshes turned through 90° and nominal 85 mm mesh opening has greater selective properties than the standard codend (with diamond meshes) with nominal 120 mm mesh opening in the range of cod protective length, which is currently up to 34 cm length class.

2. The turned codend with 85 mm mesh opening complies with regulations regarding the protection of juvenile cod.

3. After long hauls, cod in the turned codend were in much better condition than those from the standard codend.

4. The fish are located at the rear of the codend with meshes turned through 90°, thus landings are quicker and easier.

5. No damage to the codend with meshes turned through 90° was observed after over 100 hours of commercial catches. (Additionally, this codend was used aboard the cutter Hel-101 during one cruise.)

6. Investigations carried out on the R/V SOLEA, confirmed the high protective potential of polyethylene (PE) codends with meshes turned through 90°.

7. Underwater observations of two-panel polyethylene cod codends with standard extensions confirmed their improper hydrodynamic functioning. More investigations should be focused on constructions which would ensure that both the codend and the extension remain wide open along their length during whole fishing.

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Levels and trends of changes in heavy metal concentrations in Baltic fish, 1991 to 1997

Lucyna Polak-Juszczak

Sea Fisheries Institute, Kołłątaja 1, 81-332 Gdynia, Poland

Abstract. Among pollutants occurring in the marine environment, toxic metals occupy a special place. The laboratory has a special interest in monitoring the contamination of commercial fish stocks in the Baltic. It is of particular importance to provide information on toxic metals to Polish exporters who will use it to demonstrate the quality of their products and to health authorities to assist in determining the dietary exposure of Polish consumers to toxic metals. This research was conducted on the muscle tissue of the basic species of fish taken from the southern Baltic (herring, sprat, cod and flounder) in 1995-1997. The results were compared with data which have been recorded since 1991. In the period examined, changes in some heavy metal concentrations in the fish were observed. Currently, these concentrations are at a low level. Differences in toxic metal content depends on the fish species as well as on their habitat.

Key words: Baltic fish, heavy metals, concentrations, trends

INTRODUCTION

From an economic point of view, herring, sprat, cod and flounder are the predominant Baltic fish species in Polish catches. From 1991 to 1997, 100 to 130 thousand tons of fish were caught in Baltic waters, of which herring constituted about 20%, sprat about 50%, cod about 20% and flounder about 6% (Szostak *et al.* 1999).

Analyses of fish contamination with metals have been carried out by the Sea Fisheries Institute since 1991. Initially, however, they were unsystematic, and samples were collected only from certain southern Baltic fishing grounds. The systematic monitoring of Baltic fish contamination has been conducted by the Sea Fisheries Institute since 1995. The research comprises the analyses of trace metals, organochlorine pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons and biogenic amines.

The aim of this study was to compare the concentrations in recent data with those of previously reported data (De Cleark *et al.* 1995, Guns *et al.* 1992, Luten *et al.* 1987, Polak-Juszczak and Domagała 1994, Polak-Juszczak 1996) in order to cover the period from 1991 to 1997.

MATERIALS AND METHODS

Herring, sprat, cod and flounder samples were collected from the following southern Baltic fishing grounds: the Gulf of Gdańsk, the Gdańsk Deep, Władysławowo, Kołobrzeg-Darłowo, Bornholm, the Słupsk Furrow and the Pomeranian Bay.

Fish samples, weighing from 3 to 5 kg, were collected from the southern Baltic from a vessel, they were frozen and stored at a temperature of -18°C until analysis could be carried out. Muscle tissue was used for the analyses. The research material consisted of adult fish of the following lengths: herring 18-26 cm, sprat 12-15 cm, flounder 24-35 cm and cod 30-80 cm.

Trace metals were determined by atomic absorption. About 2 grams fish muscle tissue was mineralized with 6 ml nitric acid in microwave ovens. The residue was deluted to 25 ml. The zinc concentration was determined by the flame method, while the concentrations of cadmium, lead and copper were determined by the flameless method in a graphite furnace.

Material for determining arsenic was mineralized with an ashing slurry of $\text{Mg}(\text{NO}_3)_2$ and MgO_2 in an electric oven at 500°C . The residue was dissolved in 6 M hydrochloric acid. The arsenic was reduced to arsine by 0.2% sodium tetrahydridoborax in 0.2 N NaOH. The hydride is transported by argon into the absorption cell maintained at 900°C where the hydride is decomposed and the atomic As is determined at a wave length of 193.7 nm. The mercury concentration was measured by the vapor generation method in a gold amalgam by a mercury analyzer (AMA 254). Tissue was mineralized inside the analyzer at a higher temperature with oxygen in three steps: sample drying, sample decomposition and reading the analytic signal.

A visual assessment of the results and an analysis of the variance between the groups was performed with the STAT GRAPHICS program for Windows. The significance level was set at a 95% minimum. The figures show the upper and lower quartiles (0.75 and 0.25 fractiles) at the top and bottom of a *box* with a parallel line corresponding to the median and a + indicating the mean value. A *whisker* or *vertical* line is then drawn from the upper quartile to the maximum from the lower quartile to the minimum (De Clerck *et al.* 1995).

RESULTS AND DISCUSSION

Research conducted so far has shown that trace metal concentrations in fish change with time (Protasowicki *et al.* 1993). The research results for 1995-97 presented in this study and the author's own earlier results (1991-1994, literature data; Polak-Juszczak and Domagała 1994) confirm changes in the trace metal content in Baltic fishes and statistical analysis indicates the trends of these changes. The results are presented in Figures 1 through 6.

Copper (Fig. 1)

Copper occurred to a varied degree in the tissues of the examined fish species. The highest concentration of this element was found in sprat tissue (the mean concentration in 1997 was 0.56 mg/kg), herring tissue had a lower concentration (0.46 mg/kg) and cod and flounder tissues

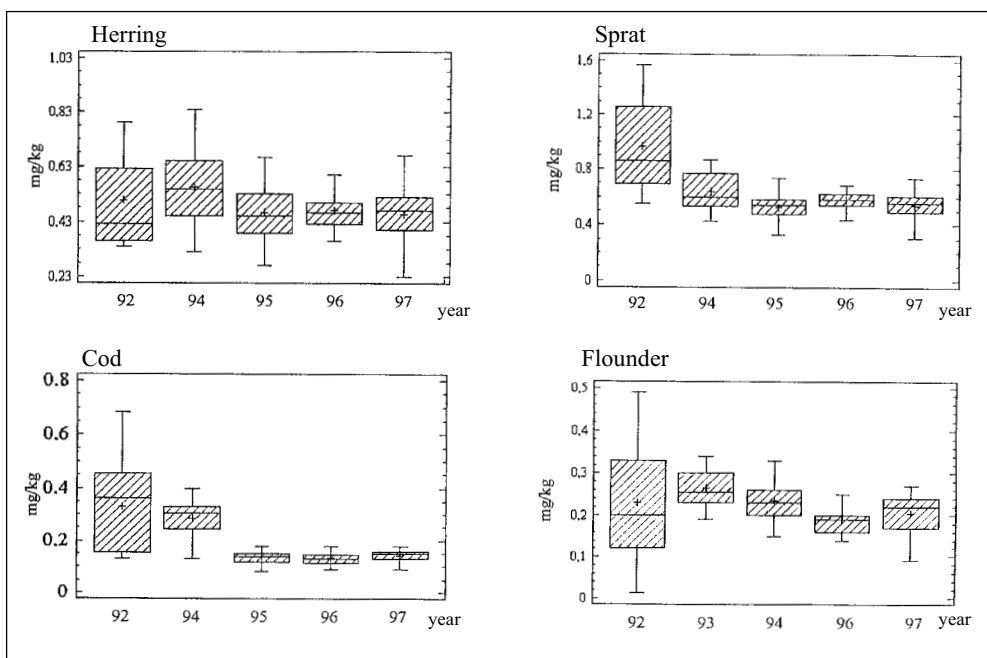


Fig. 1. Copper concentrations in Baltic fish [mg/kg w.w.]

(0.14 mg/kg and 0.20 mg/kg, respectively) had the lowest concentrations. No significant trends of change in copper concentration were observed during the research period. In 1992-1997, the copper concentration in herring and flounder tissues remained at a similar level. In cod tissues, slight decreases in the concentration of this element were observed; however, these changes were statistically insignificant.

Zinc (Fig. 2)

Based upon the research results, it can be concluded that zinc is an element that, depending on the fish species, occurs over the widest range of concentrations of the elements analysed in this review. This results mainly from the way fish feed. Herring and sprat are plankton eating fish, whereas cod are predators (Ostrowski 1993). The highest zinc concentration was found in sprat tissue. The mean concentration in 1997 was 29.51 mg/kg. It was about three times lower in herring tissue (11.42 mg/kg) and about five times lower in cod (4.97 mg/kg) and flounder tissue (6.82 mg/kg). Upward trends were observed in the zinc concentration in sprat and herring tissues. A significant increase in the zinc content of herring and sprat has occurred since 1993. Despite fluctuations in zinc concentration, no significant changes were observed in flounder tissue during the research period.

Cadmium (Fig. 3)

The cadmium concentration in Baltic fishes in 1991-1997 fluctuated; nonetheless, no significant trends of change were observed. Slight decreases in the concentration of cadmium were noted in cod tissue; however, these changes were statistically insignificant. Differences in the cadmium

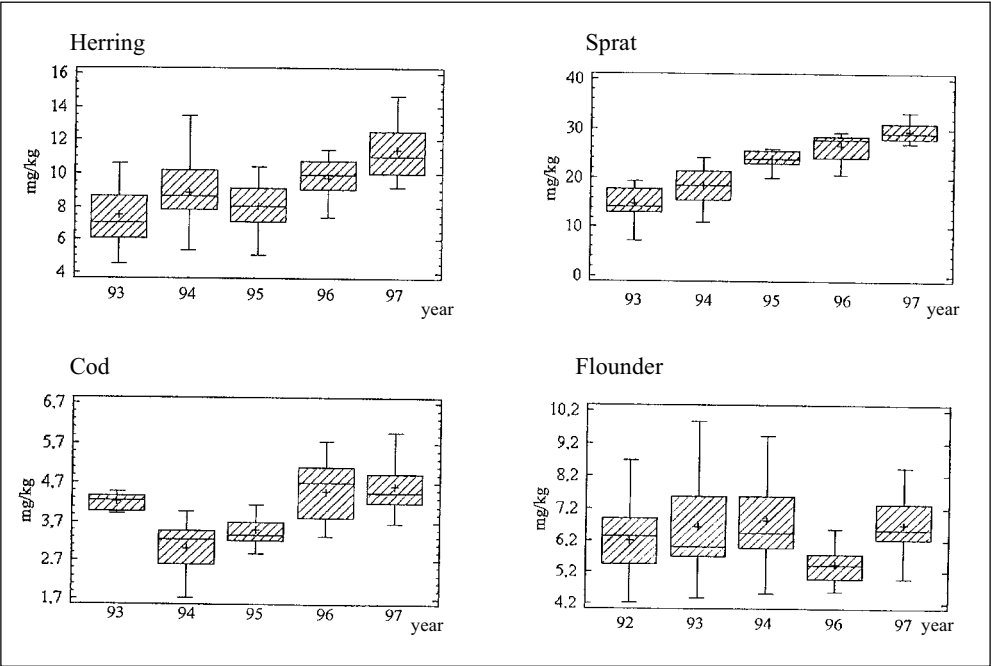


Fig. 2. Zinc concentrations in Baltic fish [mg/kg w.w.]

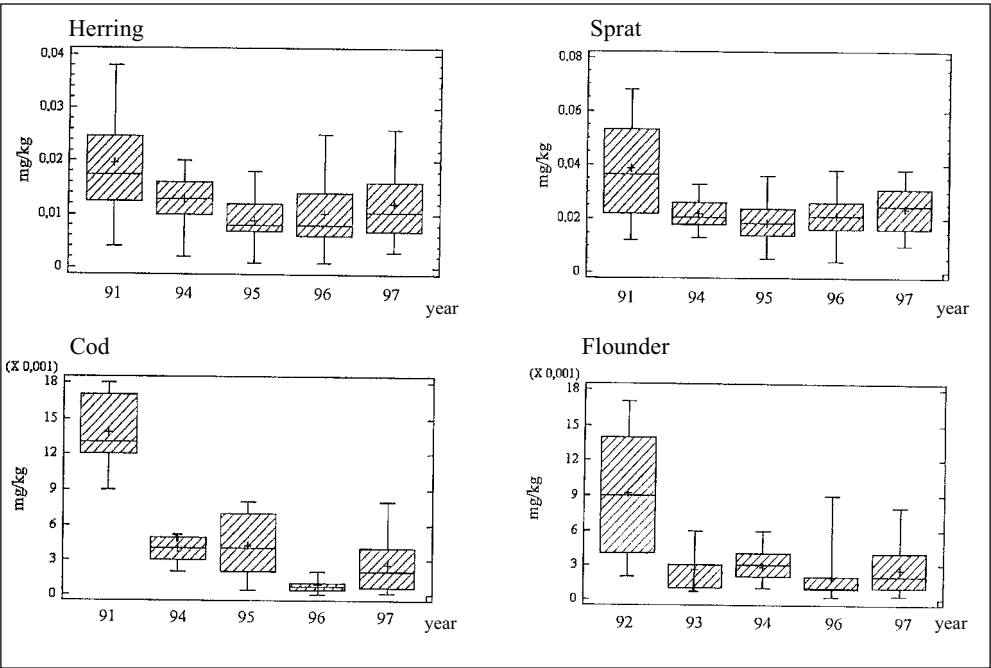


Fig. 3. Cadmium concentrations in Baltic fish [mg/kg w.w.]

content were, however, observed between the examined fish species. The highest cadmium concentration was recorded in sprat tissue. Its mean value in 1997 was 0.024 mg/kg of tissue, which was twice more than in herring (0.012 mg/kg of tissue) and five times more than in cod and flounder (0.002 mg/kg).

Lead (Fig. 4)

The research results for 1993-1997 point to a progressive decrease in the lead concentration in Baltic fish tissues. The greatest changes in lead content were observed in herring, flounder and sprat tissues. The mean concentration in 1995-1997 decreased from 0.034 mg/kg of tissue to 0.010 mg/kg of tissue in herring, and from 0.036 mg/kg of tissue to 0.012 mg/kg of tissue in sprat. Smaller, yet significant, changes in the lead content were observed in cod and flounder tissues. The concentration of this element in flounder fell from 0.050 mg/kg to 0.010 mg/kg, and from 0.022 mg/kg to 0.012 mg/kg in cod. Differences in the lead content between fish species were insignificant. The mean lead concentrations were at a similar level.

Arsenic (Fig. 5)

During the research period, the mean arsenic concentration in Baltic fish tissues ranged from 0.221 mg/kg to 1.423 mg/kg. The highest arsenic content was recorded in sprat tissue. A considerably lower concentration occurred in herring, cod and flounder tissues. Based on the research results for 1995-1997, downward trends were observed in the arsenic content in the examined Baltic fish species.

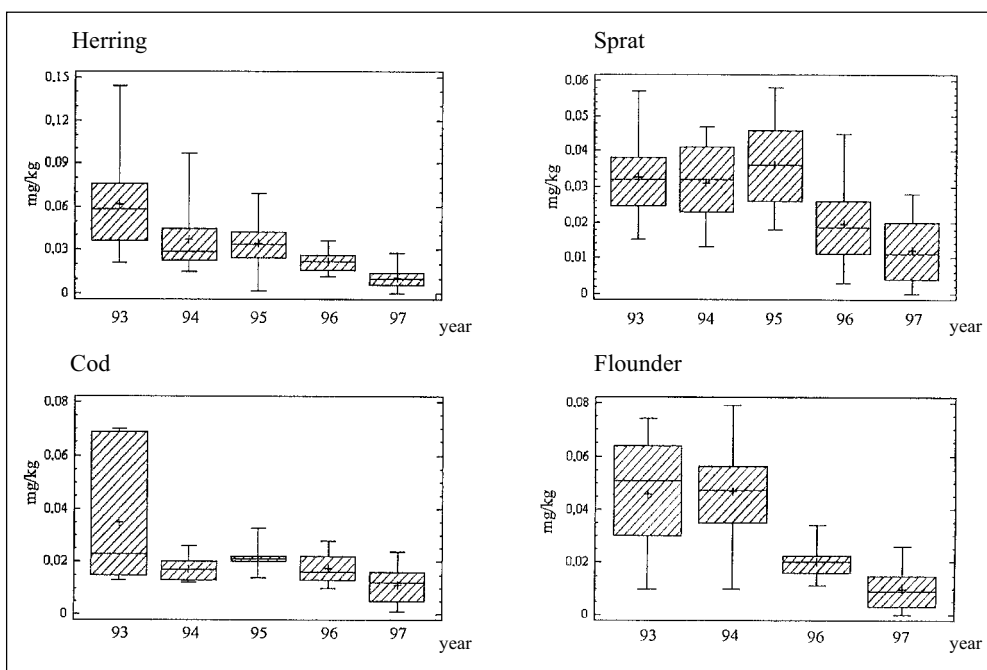


Fig. 4. Lead concentrations in Baltic fish [mg/kg w.w.]

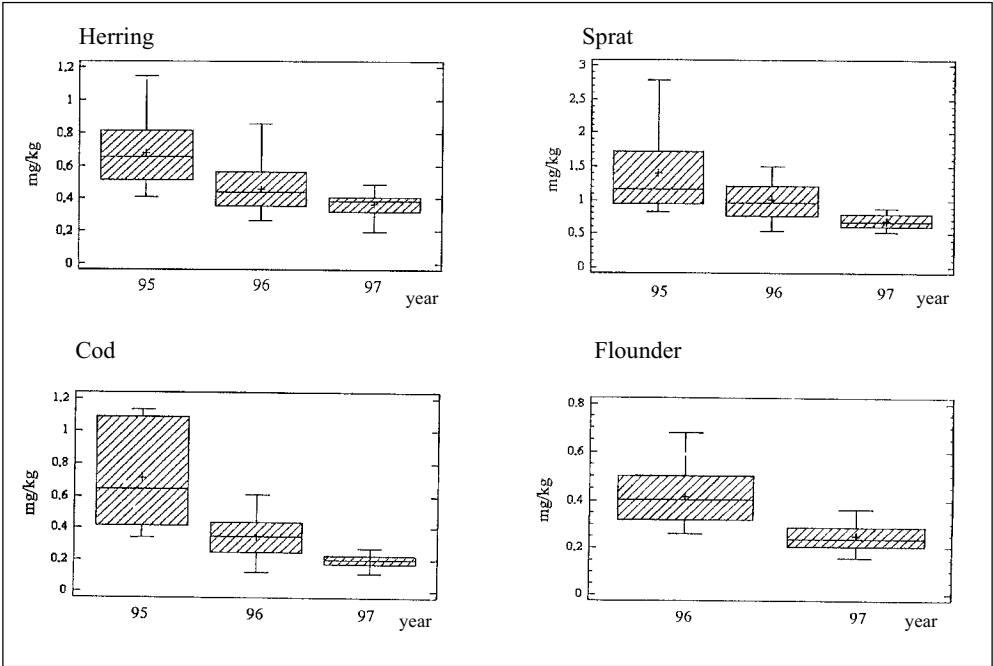


Fig. 5. Arsenic concentrations in Baltic fish [mg/kg w.w.]

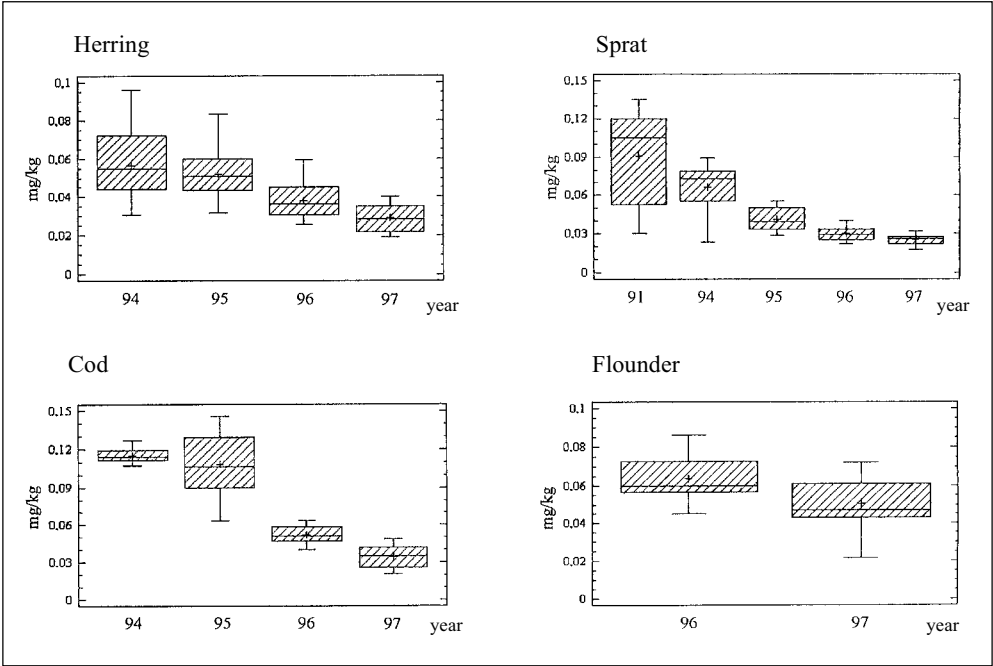


Fig. 6. Mercury concentrations in Baltic fish [mg/kg w.w.]

Mercury (Fig. 6)

During the research period, the highest mercury concentration was recorded in the tissue of cod caught in the southern Baltic in 1994. Its mean concentration was 0.118 mg/kg, which was twice more than in other fish species. Such a high mercury content stemmed from its accumulation in the tissue of the adult specimens that were taken for analysis (fish over 60 cm in length). In 1994-1997, a decrease was observed in the concentration of this element in Baltic fish tissues. In herring tissue, the mean mercury concentration fell from 0.056 mg/kg to 0.029 mg/kg, in sprat tissue from 0.066 mg/kg to 0.025 mg/kg, and in cod tissue from 0.118 mg/kg to 0.035 mg/kg. Mercury in flounder tissue was determined only in 1996 and 1997, and mean values of its concentration were 0.064 mg/kg and 0.050 mg/kg.

From 1991 to 1997, heavy metal concentrations changed; however, change trends were observed only in the case of some elements. A downward trend was recorded in the contents of lead, mercury and arsenic, and an upward trend was noted in zinc content. No change trends were observed in the concentrations of copper and cadmium even though they underwent fluctuations.

The research results confirm that the heavy metal content in tissue depends on the fish species. Particularly large differences were observed in zinc concentration.

The mean concentrations for all fish species analysed for mercury, lead and cadmium in this work met the European limits set for mercury (0.5 mg/kg), European Commission Decision 93/351, and the proposed limits for lead and cadmium, 0.05 mg/kg. All concentrations in the upper range found for all species from 1996 onwards were below the EU limits and proposed limits.

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The carotenoid content in certain fish species from the fisheries of New Zealand

Bazyli Czczuga¹, Bernard Kłyszajko² and Ewa Czczuga-Semeniuk¹

¹Medical University, Kilińskiego 1, 15-230 Białystok, Poland

²Academy of Agriculture, K. Królewicza 4, 71-550 Szczecin, Poland

Abstract. Using column and thin-layer chromatography, carotenoid content was examined in the fillets (muscles with skin), liver and gonads (in a few species) of twenty-two species of fish from the fisheries of New Zealand. Twenty-six carotenoids were found, with a predominance of canthaxanthin and astaxanthin. The total carotenoid content in the examined material ranged from 0.281 (carcasses of *Zeus faber*) to 6.384 $\mu\text{g} \cdot \text{g}^{-1}$ wet mass (liver of *Scomber japonicus*).

Key words: New Zealand, fishes, carotenoids

INTRODUCTION

Fish meat owes its nutrient value not only to protein and fats but also to other biologically active substances, including carotenoids which are the source of vitamin A. Thus, the knowledge of carotenoid content in meat of respective fish species in new fisheries seems to be of great importance.

We have already investigated some of the species from the Baltic Sea (Czczuga 1976, 1980b,d, Czczuga and Bartel 1998a), the Black Sea (Czczuga 1973), fishing areas off the western coasts of Africa (Czczuga and Kłyszajko 1979) and the Antarctic (Czczuga 1978a,b, 1982c, Czczuga and Kłyszajko 1978, 1986). Moreover, studies have been performed on some other sea fish species (Czczuga 1980c, 1982a, b, Czczuga and Czczuga-Semeniuk 1998a).

The present paper discusses the results of the analysis of carotenoid content in fish from the fisheries of New Zealand.

MATERIAL AND METHODS

The study population included 22 fish species (see Table 2) from the fisheries of New Zealand (42°-44° S, 169°-177°E), caught in June-September 1996.

The investigation used the muscles with skin (fillets) of all species, the livers of over half of the species and the female gonads of a few species. The material has been analysed from three specimens.

After three weeks of storage, the refrigerated materials (-4°C) which were used for the analysis was sent by air to the department laboratory where they were analyzed a week later.

The carotenoid pigments were isolated using column and thin-layer chromatography. Prior to chromatography, the material was homogenized and then subjected to hydrolysis in a 10% KHO solution in nitrogen atmosphere and at room temperature for 24 hours. Then, the extract was placed on the column of Quickfit Co. filled with Al_2O_3 . The particular fractions were eluted using various solvent systems (Czeczuga 1980a). The eluent was evaporated, and the remainder was dissolved in a suitable solvent to draw the maximum of absorption, necessary, among other things, to identify a particular carotenoid.

Irrespective of column chromatography, the acetone extract was divided into fractions by means of thin-layer chromatography. Silicon gel-covered glass plates (Merck Co.) and various solvent systems (Czeczuga and Czerpak 1976) were used. The R_f values were established according to commonly accepted criteria.

Carotenoids were identified based on the absorption maximum in different solvents, R_f values according to the standards of F. Hoffman-La Roche Co., Basel and Sigma Chemical Co. USA, and the obtained ratios of epiphase to hypophase. In order to distinguish tunaxanthin from lutein and norastaxanthin from astaxanthin, spectral analysis was used to determine to presence of hydroxyl and epoxide groups at the iononic rings (end group) of these carotenoids (Wetter *et al.* 1971). The absorption maxima were determined with a spectrophotometer Spektromom-203 and Specol.

Quantitative ratios of the respective carotenoids were estimated according to the Davies method (Czeczuga 1988), while the structure of carotenoids was estimated according to Straub (1987).

The identification of respective species of fish was based on the Jackowski (1994) key.

RESULTS

Twenty-six carotenoids were found in the material examined. Most of them are common to fish, but some, such as deepoxyneoxanthin, antheraxanthin, mutatoxanthin, violaxanthin, phoenicoxanthin and rhodoxanthin are rather rare. The finding of 2 norcarotenoids and 2 apocarotenoids (Table 1 and Fig. 1) is worth noting.

In most species, canthaxanthin, a ketocarotenoid, appeared predominant. In the fillets, the lowest carotenoid content was revealed in *Zeus faber* individuals (0.281), the highest in *Centriscoops obliquus* ($5.955 \mu\text{g g}^{-1}$ wet mass). In the liver, the lowest carotenoid content was noted in *Merlucius australis* (0.915), while the highest in *Scomber japonicus* ($6.384 \mu\text{g} \cdot \text{g}^{-1}$ wet mass). In all cases, the livers were more abundant in carotenoids than the fillets. In the gonads of some fish species, carotenoid content ranged from 0.383 (*Macruronus novaezelandiae*) to $1.029 \mu\text{g} \cdot \text{g}^{-1}$ wet mass (*Scomber japonicus*) (Table 2).

DISCUSSION

Apart from the carotenoids which are common to fish, rare carotenoids were found. One of them is deepoxyneoxanthin, a derivative of neoxanthin, frequently found in plants. It was first isolated from the cells of *Euglena gracilis* (Nitsche 1974). It has been encountered in several

Table 1. Carotenoid list from the investigated material

Carotenoids	Summary formula	Structure (see Fig. 1)	Semisystematic name
α -Carotene	$C_{40}H_{56}$	A - r - B	β , ϵ -carotene
β -Carotene	$C_{40}H_{56}$	B - r - B	β , β -carotene
ϵ -Carotene	$C_{40}H_{56}$	A - r - A	ϵ , ϵ -carotene
β -Cryptoxanthin	$C_{40}H_{56}O$	B - r - C	β , β -caroten-3-ol
α -Carotene	$C_{40}H_{56}O$	A - r - D	ϵ , ϵ -caroten-3-ol
Lutein	$C_{40}H_{56}O_2$	C - r - D	β , β -carotene-3,3'-diol
Calthaxanthin	$C_{40}H_{56}O_2$	C - r - D	β , ϵ -carotene-3,3'-diol (stereoisomeric)
Zeaxanthin	$C_{40}H_{56}O_2$	D - r - D	β , β , -carotene,3,3'-diol
Tunaxanthin	$C_{40}H_{56}O_2$	C - r - C	ϵ , ϵ -carotene-3,3'-diol
Deepoxyneoxanthin	$C_{40}H_{56}O_3$	C - r ₁ - E	6,7-didehydro-5,6-dihydro- β , β -carotene-3,5,3'-triol
Lutein epoxide	$C_{40}H_{56}O_3$	D - r - F	5,6-epoxy-5,6-dihydro- β , ϵ -carotene-3,3'-diol
Antheraxanthin	$C_{40}H_{56}O_3$	C - r - F	5,6-epoxy-5,6-dihydro- β , β -carotene-3,3'-diol
Mutatoxanthin	$C_{40}H_{56}O_3$	C - r ₁ - G	5,8-epoxy-5,8-dihydro- β , β -carotene-3,3'-diol
Violaxanthin	$C_{40}H_{56}O_4$	F - r - F	5,6,5',6'-diepoxy-5,6,5',6'-tetrahydro- β , β -carotene-3,3'-diol
Echinenone	$C_{40}H_{54}O$	B - r - H	β , β -caroten-4-one
3'-Hydroxyechinenone	$C_{40}H_{54}O_2$	C - r - H	3-hydroxy- β , β -carotene-4,4'-one
Adonixanthin	$C_{40}H_{54}O_2$	C - r - I	3,3'-dihydroxy- β , β -caroten-4-one
Idoxanthin	$C_{40}H_{54}O_4$	I - r - K	3,3',4'-trihydroxy- β , β -caroten-4-one
Canthaxanthin	$C_{40}H_{52}O_2$	H - r - H	β , β -carotene-4,4'-dione
Astaxanthin	$C_{40}H_{52}O_4$	I - r - I	3,3'-dihydroxy- β , β -carotene-4,4'-dione
Rhodoxanthin	$C_{40}H_{50}O_2$	L - r ₂ - L	4',5'-didehydro-4,5'-retro- β , β -carotene-3,3'-dione
Phoenicoxanthin	$C_{40}H_{50}O_3$	H - r - I	3-hydroxy- β , β -caroten-4,4'-dione
2'-Norastaxanthin ester	$C_{39}H_{50}O_4$	I - r - N	3,3'-dihydroxy-2-nor- β , β -carotene-4,4'-dione-3-acylate
2'-Norastaxanthin diester	$C_{39}H_{50}O_4$	M - r - N	3,3'-dihydroxy-2-nor- β , β -carotene-4,4'-dione-3,3'-diacylate
β -Apo-2'-carotenal	$C_{37}H_{48}O$	B - r - O	3',4'-didehydro-2'-apo- β -caroten-2'-al.
β -Apo-10'-carotenal	$C_{27}H_{36}O$	B - r ₃ - P	10'-apo- β -caroten-10'-al

salmonids, including the rainbow trout *Oncorhynchus mykiss* (Schiedt *et al.* 1985, Torrissen *et al.* 1989, Czczuga and Czczuga-Semeniuk 1998b) and in river lamprey (*Lampetra fluviatilis*) individuals (Czczuga and Bartel 1999). Antheraxanthin, a zeaxanthin derivative, has been observed in salmonids of the genus *Oncorhynchus* (Matsuno *et al.* 1980, Kitahara 1983, 1984a,b, 1985) and in *Salmo trutta* m. *trutta* individuals during the spawning season (Czczuga and Bartel 1989). It has been also encountered in rainbow trout (Czczuga and Czczuga-Semeniuk 1998b) and in a few fish species from the Szczecin Lagoon (Czczuga and Kłyszczko 1996). In plants, antheraxanthin, together with zeaxanthin and violaxanthin, take part in the so-called violaxanthin cycle. Zeaxanthin is predominant at intensive insolation, but when the exposure to the sun's rays is poor, zeaxanthin becomes converted through antheraxanthin into violaxanthin, and vice versa. Mutatoxanthin is common in plants, particularly in the terminal stage of vegetation and fruit ripening (Goodwin 1980). In fish, it has been encountered in the *Gymnocephalus cernus* from the Szczecin Lagoon (Czczuga and Kłyszczko 1996). Violaxanthin is also one of the most common plant carotenoids; in fish it has been found in *Oncorhynchus nerka* (Matsuno *et al.* 1980) and *Abramis brama* (Czczuga and Kłyszczko 1996). Phoenicoxanthin, a canthaxanthin derivative, is frequently called adonirubin. Although in the material examined canthaxanthin was found in all fish species, and was dominant in most, phoenicoxanthin was observed only in 8 out of 22 species and was predominant only in *Prionace glauca* and *Raja nasuta* individuals. Phoenicoxanthin has been found in certain algae and fungi (Goodwin 1980), while in animals it is common to insects (Kayser 1982), particularly butterflies (Czczuga

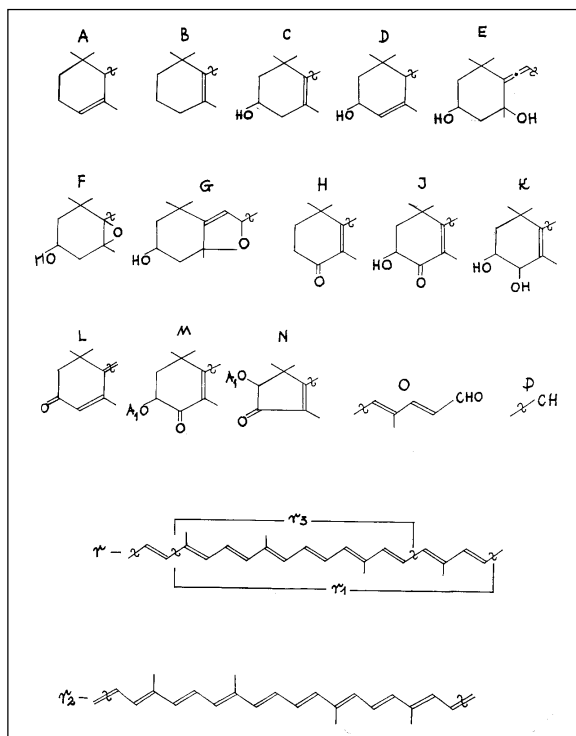


Fig. 1. Structural features of carotenoids from investigated materials

A-P – end group designation of carotenoids;
 A_1 in end of M and N – Acyl;
 R – polyene chain

1990). In fish, phoenicoxanthin occurred in the grass carp-*Ctenopharyngodon idella* (Czeczuga 1981). Rhodoxanthin is quite common to gymnosperms, flower petals, fruits and water plant leaves. It also occurs in animals and sporadically in fish. Katsuyama and Matsuno (1979) were the first to find this carotenoid in *Tilapia nilotica*, where it turned out to be predominant. *Tilapia mossambica* individuals contain smaller amounts of rhodoxanthin (Matsuno and Katsuyama 1979). Two years later, the presence of rhodoxanthin was established in *Ctenopharyngodon idella* individuals (Czeczuga 1981). Experiments have confirmed that rhodoxanthin in fish is of plant origin and accumulates in respective body parts without transformations (Czeczuga and Kiziewicz 1985). In plants, however, zeaxanthin is produced as the result of oxidative transformations. Norcarotenoids, such as 2'-norastaxanthin ester and 2'-norastaxanthin diester are the astaxanthin derivatives. They have been found in highly red aquatic organisms, in fish with highly-coloured fins or skin (Czeczuga and Czeczuga-Semeniuk 1998a). Species from the fishing areas of New Zealand which contained two norcarotenoids are not characterized by highly red fins or skin. Apocarotenoids in plants occur mainly at the end of the vegetation period as the result of degradation of less oxidized carotenoids. They may originate in the same way in fish. Apocarotenoids, and particularly β -apo-2'-carotenal, have been added to fodder on fish farms to give natural colour to fish meat, particularly to salmonids (Hirae *et al.* 1962, Storebakken *et al.* 1987), since apocarotenals, like canthaxanthin and astaxanthin, give a red colour to organisms.

All fish individuals examined contained ketocarotenoids such as astaxanthin and canthaxanthin, except for *Helicolenus maculatus* and *Prionace glauca*, in which only astaxanthin was noted. However, in 16 species canthaxanthin and in 3 species astaxanthin appeared predominant.

Table 2. Carotenoid content in investigated body parts of body particular species of fish (major carotenoid underlined and in parenthesis % of this carotenoid)

Species	Skin and muscles		Liver		Gonads	
	Carotenoid (see Table 1)	Total content ($\mu\text{g} \cdot \text{g}^{-1}$ wet mass)	Carotenoid (see Table 1)	Total content ($\mu\text{g} \cdot \text{g}^{-1}$ wet mass)	Carotenoid (see Table 1)	Total content ($\mu\text{g} \cdot \text{g}^{-1}$ wet mass)
<i>Beryx splendens</i> Lowe	4,9,16, <u>19</u> (58.3),20	1.536				
<i>Brama brama</i> Bonnaterre	2,4,5,8,11,16, <u>19</u> (36.1),20,22	0.704	1,2,9,11,12, <u>19</u> (45.2),20,22,25	1.645		
<i>Centriscoops obliquus</i> Waite	2,5,8,9,11,14,16, <u>19</u> (28.4),29,22	5.955				
<i>Coelorhynchus</i> sp.	2,4,5,8,9,11,17, <u>19</u> (61.1),20,21,25	1.353	4,11,12,14, <u>19</u> (40.2),20,25	4.562		
<i>Cyttus travesi</i> Gunther	2,3,4,5,8,11,16, <u>19</u> (48.6),20,23	2.675	2,8,9,12, <u>19</u> (40.6),22	5.794		
<i>Genypterus blacodes</i> Bloch et Schneider	2,4,5,8,9,10,11,16,18, <u>19</u> (48.9),20	0.589	2,10,16, <u>19</u> (66.8),20,21	1.624	2,9,11,12,16,18,19, <u>20</u> (55.6)	0.475
<i>Helicolenus maculatus</i> Cuvier	2,4,9,13,16, <u>20</u> (25.7),21	0.342				
<i>Katheostoma giganteum</i> Haast	4,9,11, <u>19</u> (58.1)	0.577	11,12,17, <u>19</u> (54.6),20	2.583		
<i>Lepidotus caudatus</i> Euphrasen	4,9, <u>19</u> (58.1),20	0.947			2,8,10,11, <u>19</u> (63.5),20	0.385
<i>Macruronus novaezelandiae</i> Hector	2,4,8,9,10,11,15,16,19, <u>20</u> (51.5)	0.971	4,8,9,11,16, <u>19</u> (61.5),20,25	5.824	1,2,6,8,11,13, <u>19</u> (38.6)	0.591
<i>Merlucius australis</i> Hutton	3,4,6,9,11,16, <u>19</u> (49.6),20	0.591	5,6,9,11, <u>13</u> (24.0),15,19,20	0.915		
<i>Micromesistius australis</i> Norman	4,8,9,11, <u>19</u> (34.9),20	0.707				
<i>Peltorhamphus novaezelandiae</i> Gunther	8,11, <u>19</u> (65.4),20,22	1.261				
<i>Petropinna semoni</i> Weber	5,6,9,11,12,19, <u>20</u> (25.6)	0.716				
<i>Prionace glauca</i> Linnaeus	4,10,11,20, <u>22</u> (43.4),23,24	0.862		1.909		
<i>Raja nasuta</i> Muller et Heule	1,2,4,5,8,11,16,19,20, <u>22</u> (24.5)	0.645	2,4, <u>19</u> (26.6),20,21,22	0.954	2,4,5,8,9,10,11,12,16, <u>19</u> (42.4),20	0.878
<i>Rexea solandri</i> Cuvier	4,7,11,12, <u>13</u> (37.5),19,20	0.545	4, <u>19</u> (71.7),20	1.133	4,7,11,12, <u>19</u> (29.1),20	1.029
<i>Soriolella punctata</i> Bloch et Schneider	2,4,5,8,9,10,11,12,18, <u>19</u> (42.3),20	0.331	2,4,6,11, <u>19</u> (33.5),20	6.384	1,2,3,4,5,8,9,11, <u>19</u> (37.2),23	0.397
<i>Scomber japonicus</i> Hutton	4,8,9,11, <u>19</u> (30.6),20	1.207	4,8, <u>19</u> (57.8),20	4.971		
<i>Thyrstites atun</i> Euphrasen	1,4,11,18, <u>19</u> (26.5),20,22,26	0.498	1,2,8,9,11, <u>19</u> (29.9),20,22	3.004		
<i>Trachurus novaezelandiae</i> Richardson	2,4,11,16, <u>19</u> (55.7),20,21	0.505	2,4,11, <u>19</u> (72.6),20,22			
<i>Zeus faber</i> Linnaeus	4,5,6,7,9,10,11,18, <u>19</u> (24.4),20	0.281				

Total carotenoid content was higher in livers than in fillets. However, the carotenoid content in the fillets of the species from New Zealand fishing areas in comparison with species from other fisheries should be considered high (mean $1.082 \mu\text{g} \cdot \text{g}^{-1}$ wet mass).

The mean value for 9 species from the region of the Falkland Islands was 0.08 (Czczuga and Kłyszejko 1978) and for 10 species from the Szczecin Lagoon $0.230 \mu\text{g} \cdot \text{g}^{-1}$ wet mass (Czczuga and Kłyszejko 1996). In the fillets of *Salmo trutta* m. *trutta* individuals carotenoid content ranged from 0.525 to 3.034 (Czczuga and Chelkowski 1984), and in *Salmo trutta* m. *lacustris* from 0.256 to $0.777 \mu\text{g} \cdot \text{g}^{-1}$ wet mass (Czczuga and Bartel 1989). The muscles of *Oncorhynchus keta* during anadromous migration contained $0.330\text{--}0.347 \mu\text{g} \cdot \text{g}^{-1}$ wet mass (Kitahara 1983). In *Oncorhynchus mykiss*, carotenoid content in the fillets was 0.339 (ordinary form) and $0.668 \mu\text{g} \cdot \text{g}^{-1}$ wet mass (golden form) (Czczuga and Czczuga-Semeniuk 1998b).

Among the individuals examined, the species which lead a pelagic mode of life (Rutkiewicz 1982) were the most abundant in carotenoids. They contained over $1 \mu\text{g} \cdot \text{g}^{-1}$ of wet mass, pink individuals of *Centriscops obliquus* over $6 \mu\text{g}$. Plankton, mainly crustaceans, is known to have higher carotenoid content than bottom organisms in conversion to mass unit.

This could explain the accumulation of larger amounts of carotenoids from the consumed food.

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