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Bulletin

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of the Sea Fisheries Institute

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GENERAL INFORMATION

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Papers submitted to the Bulletin are classified according to the following three categories: 1) scientific papers, 2) short communications, 3) varia.

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2 (150) 2000

Biological characteristics of walleye pollock *Theragra chalcogramma* in the Cape Navarin area (Bering Sea) based on Polish catches from 1995-1998

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Abstract. This work presents the results of investigations from 1995 to1998 which were focused on the walleye pollock stock in the Cape Navarin area (Bering Sea). The length, age, weight and sexual maturity of the fish of the exploited stock were determined. The length and age frequencies of walleye pollock varied throughout the investigations. The average walleye pollock lengths from 1996 to1998 were almost the same, 40.4 cm, as was the average age, 5.3 years. Only in 1995 was the average length shorter, 39.0 cm, and the average age lower, 4.6 years. The parameters of the von Bertalanffy growth equation were determined using average lengths in age groups from four years and the relationship between fish weight and length were determited. The results of the investigations indicate a high degree of similarity between the biological features of walleye pollock in the Cape Navarin area in comparison with results of investigations in the northeastern Bering Sea shelf.

Key words: walleye pollock, *Theragra chalcogramma,* Bering Sea, Russian shelf, fish biology

INTRODUCTION

Resources of walleye pollock *Theragra chalcogramma* are the greatest among all fish in the North Pacific. The Bering Sea is the main walleye pollock harvest area. Average annual catches of pollock in this area in 1995-1998 were about 1.85 million tons. Five stocks of walleye pollock have been designated for the management of the fisheries. There are three stocks in the US EEZ of the Bering Sea – a stock over the shelf and slope up to the border of the Russian EEZ, the stock near the Aleutian Islands and that in the Aleutian Basin, with spawning grounds near Bogoslof Island. In the Russian EEZ there are two stocks – one in the western part of the Bering Sea (Olyutorsky Bay) and the other in the northern Navarin region which extends up to the border of the US EEZ.

Despite wide-scale biological and genetic investigations, the walleye pollock stock near Cape Navarin has not yet been unequivocally identified. American scientists (Wespestad 1993;

Ianelli *et al.* 1999) believe that this is a mixture of two stocks, the eastern and the western, while Russian scientists (Borets *et al.* 1998) state that this stock is separate from other stocks in the Bering Sea and that it has its own spawning and feeding grounds.

The problem is not easy to solve since walleye pollock migrates widely. Japanese tagging studies revealed that walleye pollock migrates throughout the Bering Sea, especially while feeding (Dawson 1989). Wide-scale genetic investigations, which have been carried out in the past few years, have also failed to produce an unequivocal walleye pollock population structure (Bailey *et al.* 1999).

From 1995 to1998 Polish fishing vessels exploited the walleye pollock stock in the Russian EEZ in the Bering Sea near the border with the US EEZ. Simultaneously, biological investigations were carried out on the Polish vessels and the results are presented in this paper. The aim of this paper has been to describe the biological characteristics of the commercially exploited walleye pollock stock and to determine changes which occurred in it during this period.

MATERIALS AND METHODS

The materials were collected in the Cape Navarin area between August and October from 1995 to 1998 during catches carried out by Polish fishing vessels (Fig. 1). A total of 35,130 walleye

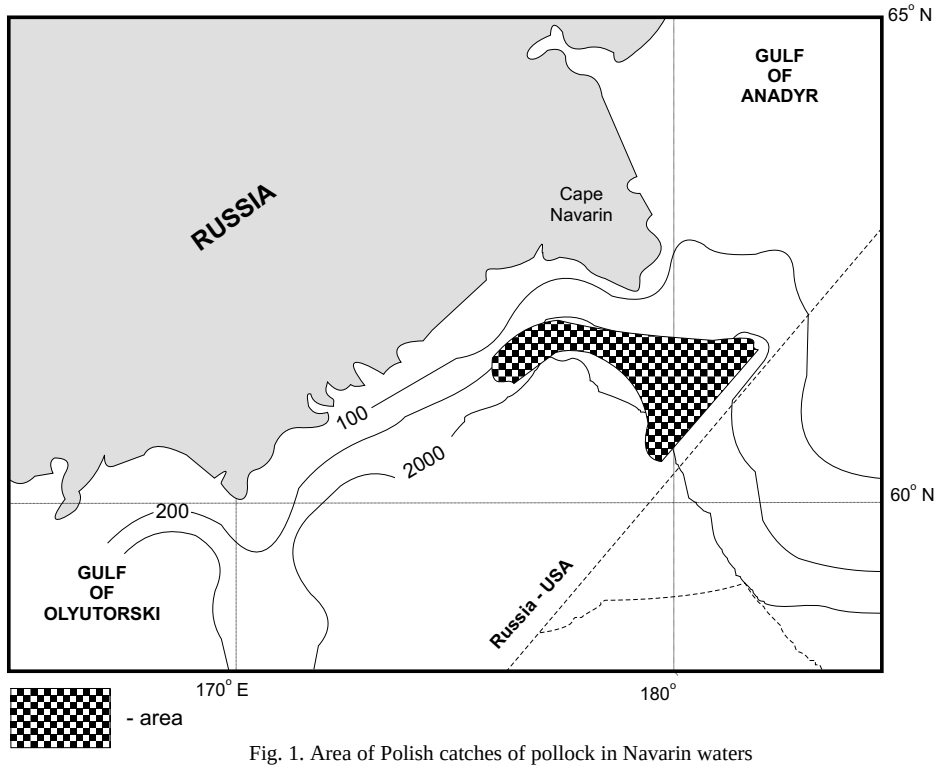

Fig. 1. Area of Polish catches of pollock in Navarin waters
during the time of biological observations in August-October 1995-1998

pollock individuals were measured, 2,987 underwent further biological analysis, including 1,816 individuals whose age was determined from otoliths. For length measurements fishes were chosen randomly (taken from random samplings) directly from the catches. The fish were divided according to their sex and measured from the tip of the snout to the fork in the caudal fin (fork length) and the measurement was rounded down to the nearest centimeter. The sex of the fish was also determined at the time of measurement. During detailed analyses, the following parameters were determined: length, sex, body weight, degree of gonad maturity according to the eight degree Maier Scale (Anon. 1965) and otoliths were collected in order to determine fish age.

Walleye pollock growth was determined using the von Bertalanffy equation:

$$L_t = L_\infty \ (1 - e^{-K(t - to)})$$

where:

L_t – total fish length at age t;
L_∞ – fish asymptotic length;
K – growth rate;
t_o – theoretical age of fish when its length is zero.

The growth curve was determined based on average fish lengths in age groups over the four years of investigations. The relationship of body weight to fish length was determined as follows:

$$W = k \cdot L^n$$

in which data from 1995 to 1998 were also used,

where:

W – fish weight (g);
L – fish length (cm);
k, n – constant values.

RESULTS

The frequency of fish in length classes varied significantly in different years (Fig. 2). Fish from 24 to 72 cm in length occurred in the catches, however, fish longer than 63 cm occurred sporadically. Fish in the length classes from 32 to 48 cm dominated and they constituted over 90% of the catches. Fish under 32 cm in length constituted only 4% of the catches. This resulted from using nets with 110 mm mesh sizes in the codends. In 1997 and 1998 the contribution of fish exceeding 53 cm in length decreased in the catches, while the contribution of fish from the 24 to 26 cm length classes increased. Despite the fact that frequency of fish in length classes was different in the various years of the investigations, the average lengths (for both males and females) from 1996 to1998 were almost identical at 40.4 cm and only in 1995 was the average fish length lower at 39.0 cm. The average female length was about 1 cm longer than that of the males.

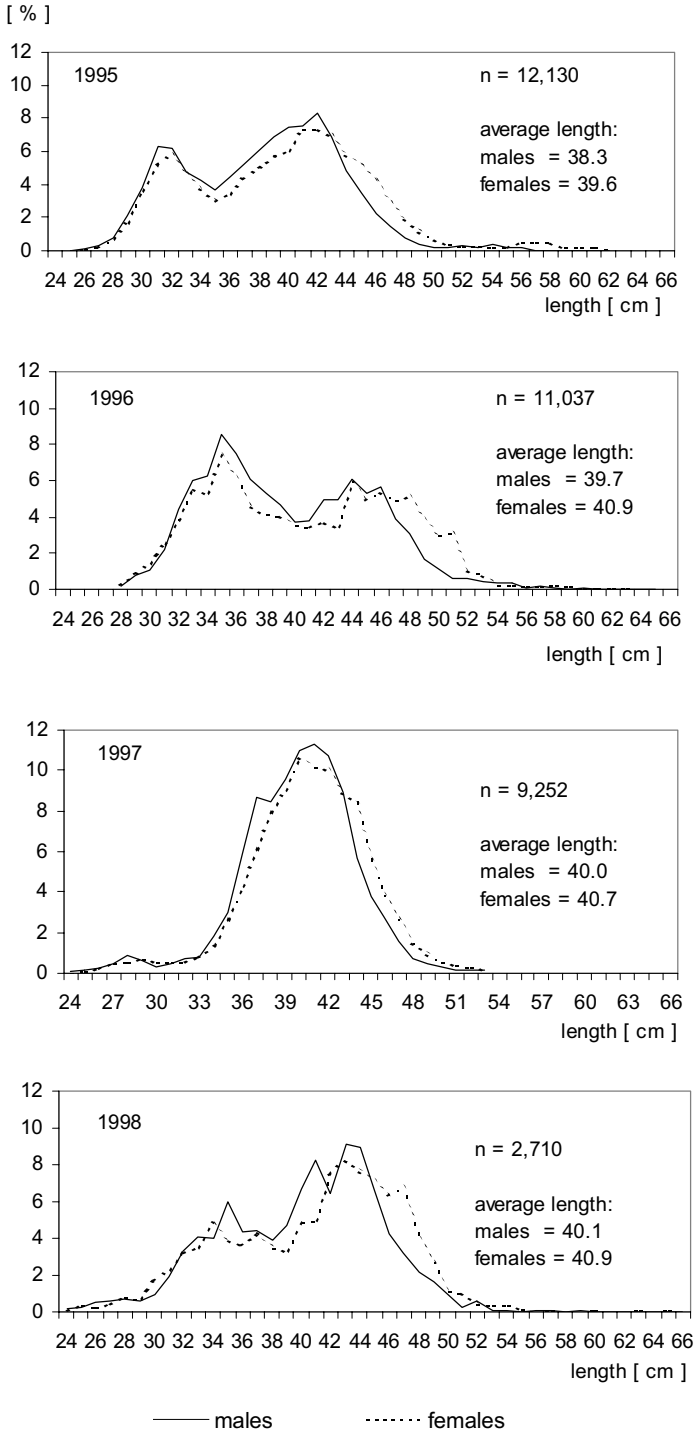

Fig. 2. Length frequencies of pollock in Polish catches in the Navarin area in 1995-1998

Walleye pollock age was determined based on annual otolith growth. Fish from 2 to 21 year age groups occurred in the catches. Only a few fish older than 15 years were observed. The age structure of walleye pollock varied from year to year (Fig. 3). In the exploited stock in1995, fish in the 3 to 6 year age groups occurred almost equally (a total over 74%) and a relatively high contribution of young, two-year old fish was observed (11.9%). In 1996, fish in the 3 and 4 years age groups constituted the basis of the catches (45.1%), with a significant contribution of 6 and 7 year old fish (26.4%). In 1997, fish in the 5 to 6 year age groups constituted almost 75% of the catches. In 1998, fish from the 3 to 7 year old age groups dominated the catches (83.9%).

Throughout the investigation period, the most abundant cohort of walleye pollock in the catches was that from the1992 year class (Fig. 2). The 1993 cohort, which in 1995 and 1996 (two and three years old) was anticipated to become abundant, but was not very common in the following years. In 1995 and 1996, the cohort from 1989 (6 and 7 year old fish) still played an important role. In 1998, a significant contribution of fish from 1995, which were in the 3 year age group, was observed in the catches.

Despite the differences in the structure of age groups of walleye pollock in particular years of exploitation, the average age (for both males and females) from 1996 to 1998 was very similar at 5.3. Only in 1995 was the average fish age lower at 4.6 years.

The average fish lengths in age groups in particular years does not differ significantly (Table 1). The growth curve parameters for the walleye pollock, obtained from averages of four years of exploitation for both males and females, are as follows:

parameter	males	females
L_∞(cm)	54.46	62.12
K	0.205	0.156
t_o	– 1.641	– 1.886

Table 1. Mean length (F.L. cm) at age of pollock in Navarin area in 1995-1998

	Males				Females				Males and females			
Age	1995	1996	1997	1998	1995	1996	1997	1998	1995	1996	1997	1998
2	30.4	30.1	26.9	28.6	30.4	29.4	27.5	28.9	30.4	29.6	27.3	28.7
3	33.4	33.5	30.9	33.9	33.2	33.2	30.9	33.7	33.3	33.3	30.9	33.8
4	37.7	36.7	36.2	37.7	37.9	36.9	35.5	38.8	37.8	36.7	35.9	38.3
5	41.2	41.4	39.3	41.4	41.2	40.3	39.6	41.4	41.2	40.5	39.5	41.4
6	42.7	43.5	42.5	42.3	43.4	43.9	42.9	43.5	43.1	43.7	42.7	42.8
7	44.7	45.2	46.0	45.1	45.7	46.3	46.1	45.7	45.2	45.9	46.2	45.4
8	46.4	46.1	46.5	46.8	46.9	48.0	46.8	47.3	46.6	47.6	46.8	47.2
9	48.0	47.9	47.0	49.4	50.1	48.9	50.5	51.0	49.0	48.5	50.2	50.7
10	49.4	47.7	52.0	49.0	53.9	50.1		51.3	52.7	49.6	52.0	48.9
11	50.3	57.0		53.5	52.6	51.4			51.1	51.6		54.4
12		49.5		51.0	57.7	51.5			58.1	51.0		51.0
13	53.0	46.6			59.5	54.9			56.8	50.9		68.0
14	56.0	47.1			57.5	59.4			57.4	51.4		
15	54.9	52.4			62.0	58.0			56.9	53.2		

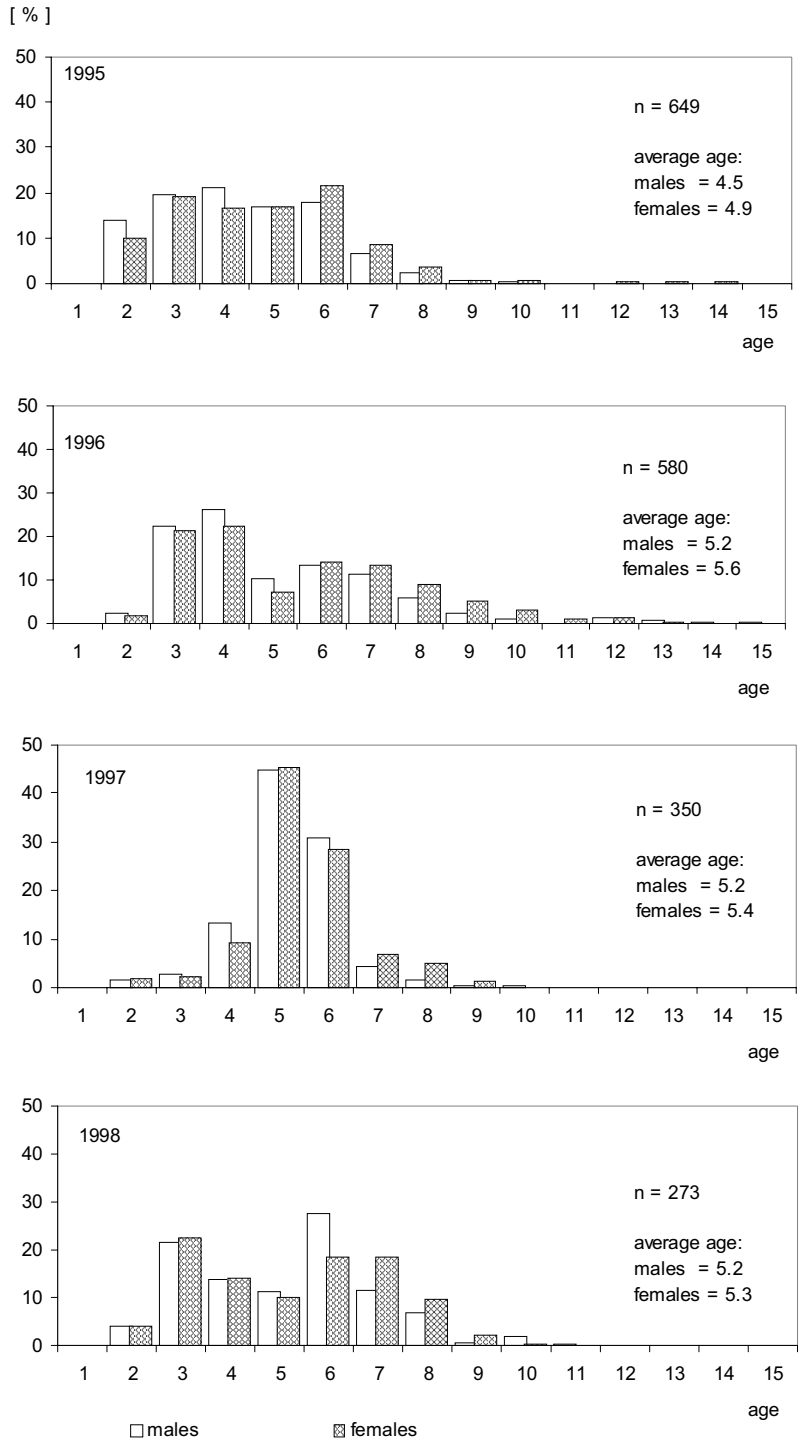

Fig. 3. Age composition of pollock in Polish catches in Navarin area in 1995-1998

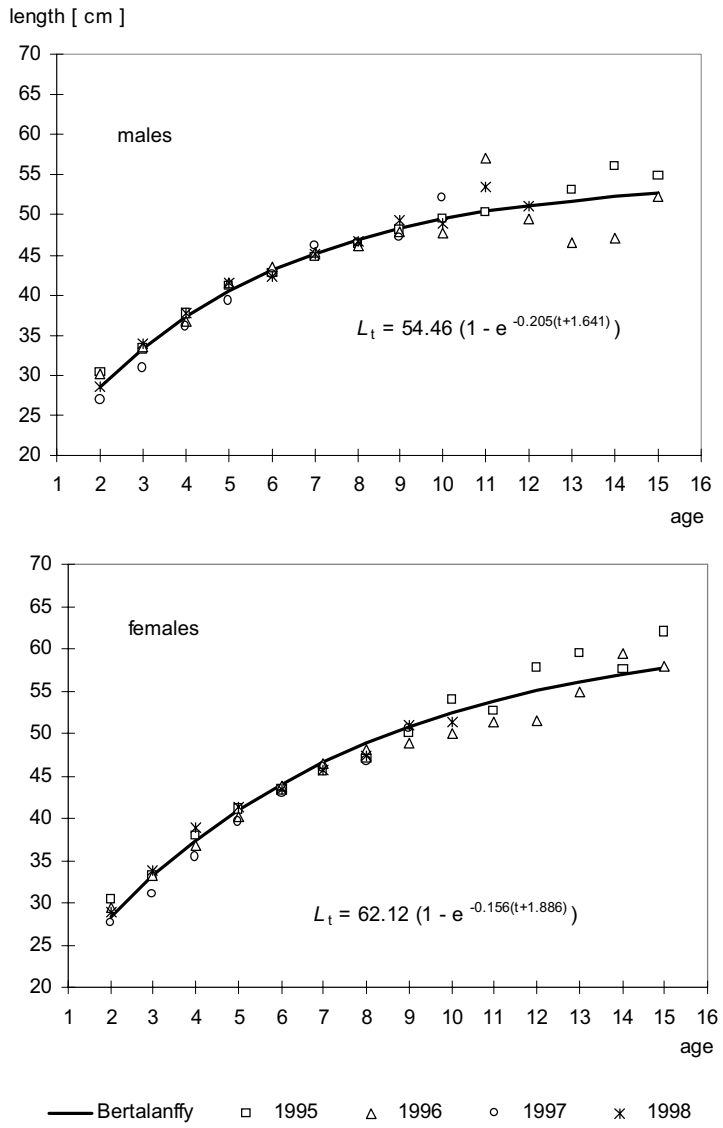

Fig. 4. Mean length age and the von Bertalanffy growth curves for pollock in Polish catches in the Navarin area in 1995-1998

The curves obtained using these parameters (Fig. 4) reveal that the growth of walleye pollock males and females, despite slight differences, show similar tendencies.

In the period of investigations the individual weight of the fish varied from 100 to 2,750 g. There were no significant differences of the average fish weight in particular length classes. The exception was 1995 when it was lower than in other years (Fig. 5). Parameters of the walleye pollock weight-length relationship for the average from four years are as follows:

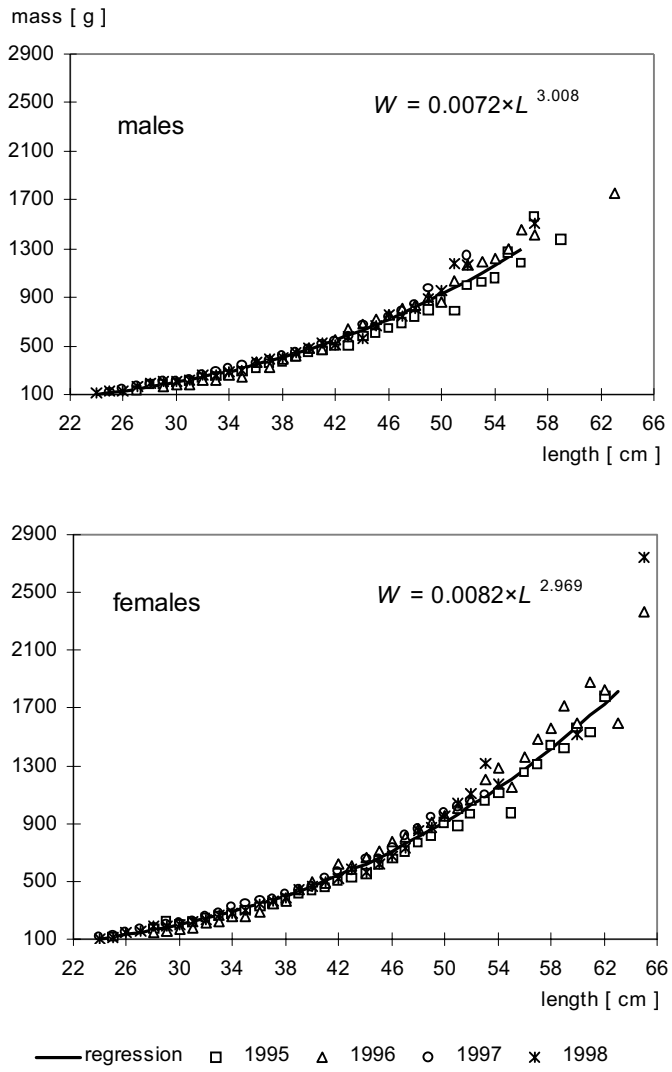

Fig. 5. Length-weight relationship of pollock in Polish catches in the Navarin area in 1995-1998

parameter	males	females
k	0.0072	0.0082
n	3.008	2.969

The average walleye pollock weight (in grams) of both males and females from 1996 to 1998 was similar, but it was much lower in 1995. The average weights are as follows:

year	males	females	total
1995	381.7	433.2	409.4
1996	485.2	547.7	522.7
1997	486.7	523.3	509.6
1998	491.0	518.8	508.0

Observations of gonad maturity indicate that from August to October the exploited walleye pollock stock near Navarin was rather homogeneous in terms of gonad maturity. Again, the exception is 1995, when about 80% of males and females had gonads in the developing stage (III) and many of them were determined to be in the virgin stage (I). In the following years, about 50% of the males and 80% of the females had gonads in the recovering spent stages (II). In 1996, a relatively high contribution of males, about 20%, were in the partly spent stage (VII) and the resting stage (VIII) (Fig. 6).

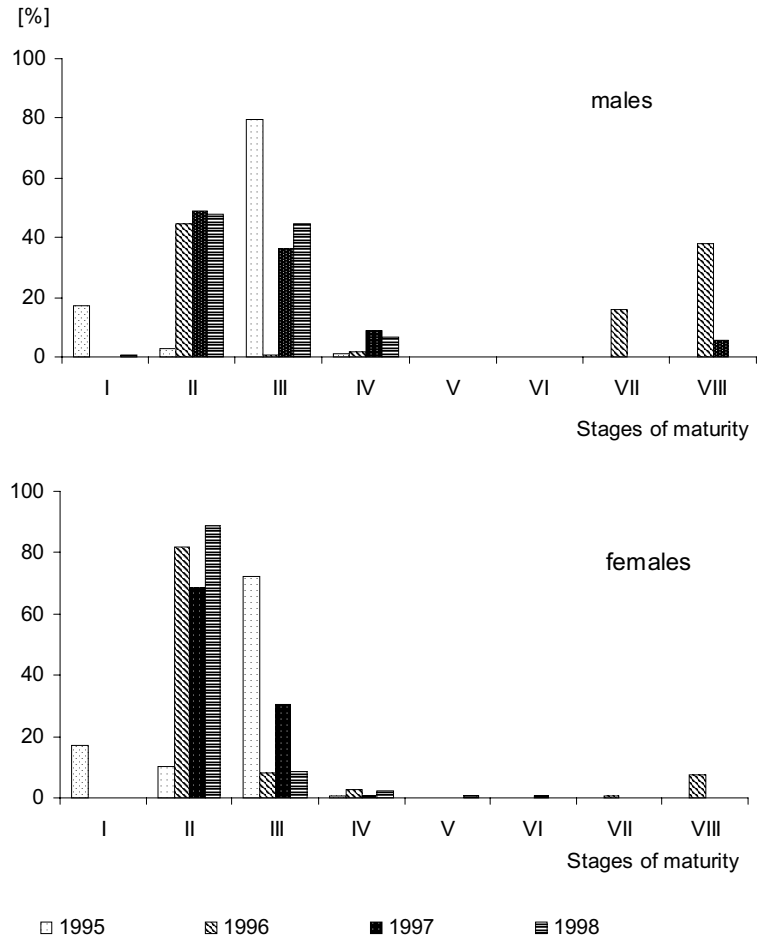

Fig. 6. Maturity stages of pollock observed in Polish catches in the Navarin area in 1995-1998

DISCUSSION

The investigations reported here were carried out each August to October period from 1995 to 1998 in the Navarin area close to the border between the EEZs of the Russian Federation and the USA. Determining the population structure of this stock is quite complicated because the walleye pollock cross the borders of economic zones during migrations. The biological investigations of both the Russians and the Americans are usually limited to their respective economic zones, which makes solving the problem even more difficult. Extensive migrations of walleye pollock throughout the Bering Sea, and especially in its northern part, to a great extent depend on oceanographic conditions and the abundance of year classes of fish which most actively migrate (Stepanenko1989). Significant variations in annual walleye pollock catches near Navarin, sometimes varying by 50% (Ianelli *et al.* 1999), may also result from the year class strength of fish which constitute the exploited stock and from fish migrations between the economic zones of the Russian Federation and the USA.

Walleye pollock spawning in the Russian zone occurs on the shelf and the Olyutorski-Navarin slope from April to June (Balykin 1989). In the US zone, walleye pollock spawning occurs on the northern shelf and slope from June to August (Hinckley 1987). During the investigations from August to October, the fish gonads indicated that spawning had been completed and a new maturing process (stages II and III) had begun. Only in 1996 did fish with gonads which appeared to have just completed spawning (VII and VIII spent, resting) occur in the catches. It may be assumed that the walleye pollock spawning in 1996 was delayed in comparison to other years of the investigations.

From June to October, walleye pollock feeds intensively and at this time populations from the western (Russian) and eastern parts (American) of the Bering Sea occurs near Navarin (Balykin 1989). Fadeev (1988) estimated that in summer concentrations of walleye pollock near Navarin are constituted mainly of the American population. According to American scientists (Ianelli 1999), the Navarin stock is supplemented with young specimens from the American shelf and the size of this resource depends mainly on the spawning intensity of fish from the American shelf. On the other hand, fish from the Navarin area, as mature specimens, supplement the spawning stock on the American shelf (Shuntov *et al.* 1993). It was not, however, determined that the catches near Navarin have a negative influence on the size of the walleye pollock stock on the American shelf (Katugin 1999).

Based on recent investigations, the Russians (Borets *et al.* 1998) state that the Navarin stock is separate from others in the Bering Sea and that it has its own spawning and feeding grounds. They also believe that, due to changes in hydrological conditions, currently only about 5% of the American shelf stock migrates towards the Navarin area.

By comparing the results of Polish investigations from the Navarin area with results obtained on the northwestern American shelf, the great similarity of walleye pollock biological features can be observed. In 1995, the length distribution of walleye pollock on the American shelf was characterized by two peaks and two modal lengths, 41 cm and 32 cm (Wespestad *et al.* 1996). Similar modal lengths (with a greater number of smaller fish) were confirmed while investigating length class frequencies in the Polish catches near the border between the Russian and US EEZs. The parameters of walleye pollock growth from the American shelf show a great similarity with Polish results and for both sexes they are as follows: $L_{inf} = 56.6$ cm; $k = 0.217$; $t_o = -0.814$ (Wespestad *et al.* 1997).

Despite the great similarity between the stocks exploited near Navarin and those from the American shelf, it cannot be assumed that the stock which is fished is part of the American

stock. The age structure and distribution of fish frequency in length classes show significant diversity within the same stock depending on spatial distribution. This was revealed by Ianelli (1999) on the American shelf, where the age structure and length frequency of walleye pollock belonging to the same stock are varied and depend on whether the investigations were carried out on the inner, central or outer shelf regions.

Throughout the investigation period (1995-1998), the 1992 year class dominated in Polish commercial catches. The same year class was found to be very abundant near the US-Russia boundary (Wespestad *et al.* 1997). The 1995 and especially the 1996 year classes are also regarded as abundant (Borets *et al.* 1998). The abundance of the 1995 year class was confirmed by the results of Polish investigations in 1998, when these 3 year old fish constituted over 20% of the catches.

Over the course of the four-year Polish investigations, which were carried out at the same time of year and in the same area, significant changes in the walleye pollock stock were not observed. Despite the fact that the investigations took place during theoretical stock mixing feeding period, the results indicate the homogeneity of the exploited stock.

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Deacetylation of chitin in a two-stage chemical and enzymatic process

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Abstract. The two-stage chemical and enzymatic process may be used for preparing chitosans with different deacetylation degrees. The first, chemical stage of processing in a hot NaOH solution should only lead to ensure solubilization of chitin in slightly acid solutions. For further deacetylation of the substrate a chitin deacetylase preparation can be used. In case of crude deacetylase extracts from *Mucor rouxii* mycelium and 0.25% solution of the substrate with initial deacetylation degree of 68%, the enzymatic deacetylation reached 90% at optimum conditions after 6 h. In 1.2% solution of this substrate the highest degree of deacetylation was 85% after at least 10 h. The use of a crude enzyme preparation containing chitosanolytic and chitinolytic enzymes leads to partial hydrolysis of the polysaccharide. A substantial inhibition of the hydrolytic activity was achieved when the deacetylase extract was pre-purified by acidification to pH 4 and pre-incubated with 1 mM Zn^{2+} or by adding these ions to the reaction mixture. However, the viscosity of the products of enzymatic deacetylation was at least about 10 times lower than that of the substrates. This shows, that even in the most purified preparation the minute residual amounts of hydrolytic enzymes can effectively degrade the polysaccharide chains. For obtaining highly viscous chitosans the enzyme preparation should be purified in order to completely remove the chitino- and chitosanolytic enzymes.

Key words: chitin, chitosan, chitin deacetylase, chitosanolytic enzymes

INTRODUCTION

The products of partial or almost complete deacetylation of chitin – chitosan can find various applications, e.g. in biotechnology, agriculture, environment protection, in the fiber and paper industry, in cosmetics, veterinary medicine, dentistry, medicine, and in the food industry (Knorr 1984, Haard *et al.*1994).

At present, chitosan is produced by deacetylation of chitin at 60-140°C using 30-50% aq. NaOH solution. The highest degree of deacetylation (DD), about 98%, can be achieved in a two-stage chemical process, at 140°C and high concentration of NaOH. Recently, the suitability of enzymatic procedures carried out in mild conditions are being investigated. The enzyme that catalyses the reaction, chitin deacetylase (EC 3.5.1.41), was first demonstrated in

extracts of the fungus *Mucor rouxii* (Araki and Ito 1975) and purified to homogeneity by Martinou *et al*. (1993). Deacetylase activity was also found in other *Zygomycetes* (Trudel and Asselin 1990, Dunkel and Knorr 1994) and in a culture filtrate of *Colletotrichum lindemuthianum* (Kauss and Bauch 1988, Tsigos and Bouriotis 1995). *Zygomycetes* might be a source of commercial deacetylase preparations. Furthermore, the biomass of these fungi, after enzyme isolation, might be used for obtaining chitosan, which is present in the cell wall (Arcidiacono *et al*. 1989, Synowiecki and Al-Khateeb 1997), or as a component of the culture medium for their cultivation (Malesa-Ciećwierz *et al*. 1997).

Insoluble crystalline chitin is resistant to enzymatic deacetylation (Davis and Bartnicki-Garcia 1984, Tsigos *et al*. 1996, Kołodziejska *et al*. 1997). However, chitin deacetylated partially by chemical treatment at 60°C can be further deacetylated by chitin deacetylase (Wojtasz-Pająk *et al*. 1998) .

According to Martinou *et al*. (1995) almost all N-acetylglucosamine residues in partially deacetylated chitin were deacetylated by the enzyme isolated from *Mucor rouxii* and purified to homogeneity. It would perhaps be more reasonable to use crude deacetylase preparations for various practical applications. It was considered interesting to investigate the possibility of enzymatic deacetylation of partly deacetylated chitin using the crude and pre-purified extracts of deacetylase from *Mucor rouxii* mycelium.

MATERIALS AND METHODS

Reagents

The following compounds were used: peptone and yeast extract, Difco; Alumina A-5, Sigma; Tris, Serva; neocuprione hydrochloride, Merck; kit for determination of acetic acid, Boehringer. Other chemicals used were also of analytical grade.

Microorganism and culture conditions

About 6×10^7 spores of *Mucor rouxii* ATCC 24905 prepared according to Bartnicki-Garcia and Nickerson (1962) were inoculated per 600 ml of liquid YPG medium (0.3% yeast extract, 1% peptone, and 2% glucose), pH, 4.5 in 3 l conical flasks. The cultures were incubated in a reciprocating shaker at 220 rpm at 28°C for 48 h.

Preparation of the mycelial extract

The mycelium was separated from the culture medium by filtration on a Büchner funnel with filter paper, washed with cold distilled water, and frozen at –20°C. The frozen mycelium was ground with Alumina A-5 (1:2, w/w) in a mortar, at 0°C for 15 min. During grinding, 0.025 M Tris-HCl buffer pH 7.2, was gradually added (wet mycelium:buffer = 1:7, w/v). The homogenate was centrifuged at 0°C for 10 min. at 4,000 × g, and for 60 min. at 15,000 × g. The supernatant was used as the crude enzyme extract.

For pre-purification, the extract was acidified to pH 4 using 0.2 M HCl solution, incubated for 30 min at 25°C, and centrifuged for 15 min. at 15,000 × g. The protein concentration in the supernatant was determined according to the method of Lowry *et al*. (1951) with bovine serum albumin as a standard.

Table 1. The degree of enzymatic deacetylation of chitosans

Preparing conditions of chitosans by chemical methods[a]	Initial deacetylation degree of chitosans [%]	Deacetylation degree after enzymatic reaction[b] [%]
75°C, 15 min.	55	88
80°C, 15 min.	64	90
60°C, 90 min.	68	90
85°C, 15 min.	73	89
60°C, 180 min.	76	92
115°C, 30 min	82	96
115°C, 2 x 20 min.	87	94
115°C, 3 x 20min.	95	97
140°C, 1 x 60 min. and 140°C, 1 x 90 min.	98	99

[a] chitin was treated with 50% aq. NaOH solution (1:10 w/w)
[b] the reaction mixture contained 40 mU of deacetylase of crude extracts in 1 ml 0.1 M Tris-HCl solution, pH 5.8, temp. 50°C, 24h. The deacetylation degree was calculated from the quantity of released acetic acid.

The extract pre-purified at pH 4 was incubated with $ZnCl_2$ in concentration 1 mM for 30 min at 25°C for further reduction of hydrolytic enzymes.

Substrates

Chitin from frozen shells of krill (*Euphausia superba*) was used for the preparation of chitosan (Brzeski *et al*. 1985). Chitosans of the DD of 55-98% were obtained by treating chitin with 50% aq. NaOH solution (1:10) in conditions shown in Table 1, washing exhaustively with water after each step of deacetylation (1:10, w/v), and drying at 80°C. Each product obtained by alkaline deacetylation of chitin to a specific degree is referred to in this paper as chitosan with a superscript denoting the DD, e.g. chitosan^{68}.

DD was determined by potentiometric titration according to Broussignac (Roberts, 1992) and was calculated using the equation:

$$DD\ (\%) = 2.03\ (v_2 - v_1) / [m + 0.0042\ (v_2 - v_1)]$$

where:

v_1 and v_2 – ml of 0.1 M NaOH corresponding to the two inflection points on the titration curve

m – weight of the sample [g].

Enzyme assays

The activity of the chitin deacetylase was assayed by determining acetic acid released from the substrate. The enzymatic method of Bergmeyer and Möllering (1974) was used.

The reaction mixture, containing 2.5 mg of the chitosan^{73} and 0.1 mg of the extracts' protein in 1 ml of 0.1 M Tris-HCl, was incubated at pH 5.8 for 30 min. at 50°C. The reaction was terminated by heating the samples for 3 min. in a boiling water bath. The control sample was prepared in the same way, but the substrate and extract were incubated separately and combined when the reaction was stopped. The samples were cooled to room temperature. The pH in each sample was adjusted to 8-9 by NaOH addition in order to avoid chitosan precipitation during the determination of acetic acid. Subsequently, the samples were centrifuged for 20 min. at 15,000 × g and acetic acid was determined in the supernatant. The specific activity of the enzyme was expressed as mU mg^{-1} protein. One unit is defined as the amount of enzyme that produces 1 μmole of acetic acid from the substrate in 1 min under the conditions of the assay.

The reaction mixture for determination of the chitosanolytic activity, containing 2.5 mg of chitosan^{98} and 0.1 mg of the extracts' protein in 1 ml of 0.1 M Tris-HCl, was incubated at pH 5.8 for 60 min. at 40°C. Further procedure was the same as described above, however, the pH was not adjusted. The reducing sugars were determined in the supernatant according to Dygert *et al*. (1965).

Enzymatic deacetylation of chitosans

Deacetylation of chitosans by the crude and pre-purified deacetylase extract was carried out in 0.1 M Tris-HCl solution at pH 5.8 and temp. 50°C. The released acetic acid was determined according to Bergmeyer and Möllering (1974). The hydrolysis of chitosan was followed by determining the reducing sugars (Dygert *et al*. 1965) and viscosity at temp. 50°C. Detailed conditions of these experiments are given in Tables and Figures illustrating the results.

Chitosan was precipitated at pH 11 using 2 M aq. NaOH solution. The precipitate was filtered off, washed several times with hot water, and freeze-dried. The following properties of the product were determined: dry weight, ash, solubility in 1% acetic acid, the DD by potentiometric titration, and viscosity of 1% solution of chitosan in 0.85% acetic acid at 25°C.

Viscosity at 25°C and 50°C was measured in Brookfield DV II viscosimeter with the SC-4 small adapter working unit using different spindles and chambers and at changing rpm from 0.3 to 60. The plot of viscosity vrs. calculated shear rate for temp. 25 and 50°C was made. The viscosity at 25 and 50°C was read from the appropriate plots at shear rate 6.6^{-s} and 15.8^{-s}, respectively.

The standard deviation of all results carried out in triplicate was less than 5% of the mean value.

RESULTS AND DISCUSSION

The effect of the crude deacetylase extract

Preliminary experiments showed, that when soluble forms of chitin, i.e. partially deacetylated, were used, the initial rate of enzymatic deacetylation of the substrate was related to the initial

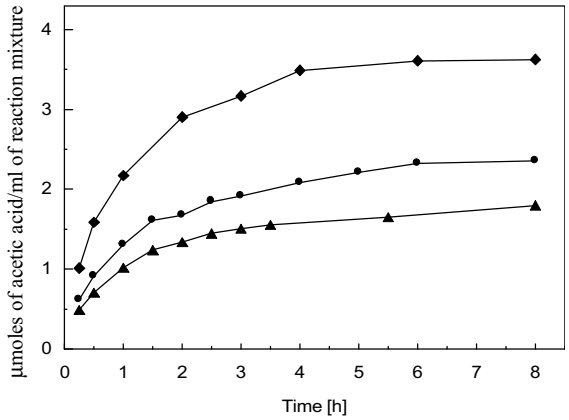

Fig. 1. The effect of incubation time and degree of deacetylation of chitosan on the release of acetic acid by the deacetylase in crude extract from *M. rouxii* mycelium. The reaction mixture, containing 2.5 mg of chitosan and 40 mU of deacetylase in 1 ml of 0.1 M Tris-HCl, was incubated for an appropriate time at pH 5.8 at 50°C. Chitosan [55] (◆), chitosan[73] (●), chitosan[82] (▲)

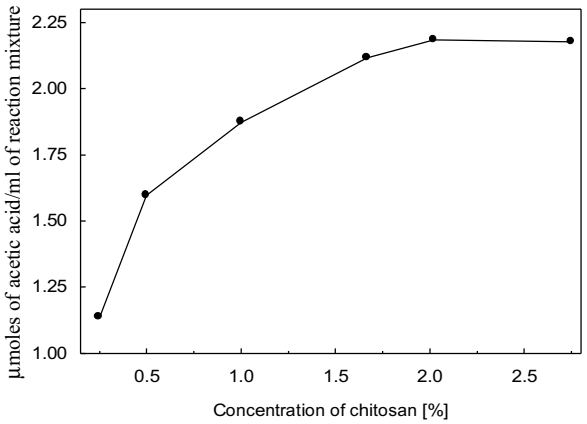

Fig. 2. The effect of the concentration of chitosan[68] on the release of acetic acid by the deacetylase in crude extract from *M. rouxii* mycelium. The reaction mixture, containing an appropriate concentration of chitosan[68] and 40 mU of deacetylase in 1 ml of 0.1 M Tris-HCl, was incubated for 30 min at pH 5.8 at 50°C

content of the acetyl groups in the polymer (Fig. 1). This indicates, that the first, chemical stage of processing should only lead to solubilization of the substrate. In further experiments mainly chitosan[68] was used.

The activity of the crude enzyme reached at the given reaction conditions a plateau at chitosan concentrations above 1.7% (Fig. 2). However, the high viscosity of chitosan[68] makes the use of such a high concentration of the substrate impractical. In experiments with chitosan[68] the highest DD, 90%, was reached at optimum conditions after 6 h in a 0.25% solution of the substrate. This is, however, a very diluted solution. For practical purposes it would be recommended to apply a 1.2% solution of chitosan. In this case the highest DD, 85%, was reached not

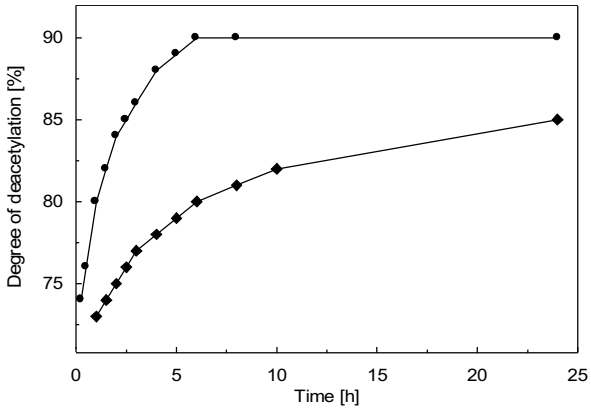

Fig. 3. The effect of incubation time on the degree of deacetylation of chitosan[68] in concentration 1.2% (◆) and 0.25% (●) by the deacetylase in crude extract from *M. rouxii* mycelium.
The reaction mixture, containing chitosan[68] and 40 mU of deacetylase in 1 ml of 0.1 M Tris-HCl, was incubated for appropriate time at pH 5.8 at 50°C

earlier than after 10 h (Fig. 3). A higher degree of enzymatic deacetylation, of about 97%, was achieved by using highly purified chitin deacetylase and a 0.5% solution of chitosans of 58% and 72% deacetylation (Martinou *et al*. 1995). In our experiments, in which 0.25% solution of chitosans were used as the substrates, almost complete deacetylation was obtained when the initial DD of the substrates was above 82%. Below this value the DD of the products did not exceed 92% (Table 1). Most probably the use of the crude enzyme preparation containing chitosanolytic and chitinolytic enzymes leads to the advanced degradation of the substrate into short fragments which are not attacked by the deacetylase. These hydrolytic enzymes were found in the preparation earlier (Kołodziejska *et al*. 1996). The deacetylase is inactive against substrates containing less than four N-acetylglucosamine residues (Kafetzopoulos *et al.* 1993, Kołodziejska *et al*. 1997).

The effect of pre-purified deacetylase

The deacetylase is stable in a broad range of pH 4.1 - 8.9, while the chitosanolytic and chitinolytic enzymes are less stable (Kołodziejska *et al*. 1996, 1999). By acidification of the enzyme extract to pH 4.0 the activity of the chitosanolytic enzymes is decreased to 10% of the initial value, while the deacetylase retains about 80% of its original activity. This is accompanied by precipitation of about 75% of the total protein and results in about a four-fold increase in the specific activity of the enzyme preparation (Kołodziejska *et al*. 1999). However, such a decrease in the hydrolytic activity of the pre-purified enzyme was not effective enough, as with prolonged reaction time the accumulation of reducing sugars in the reaction medium remained significant (Fig. 4). A substantial inhibition of the hydrolytic activity was achieved when the extract pre-incubated with 1 mM Zn^{2+} was used and by adding these ions to the reaction mixture (Fig. 4). Zn^{2+} is known not to inhibit the deacetylase activity (Araki and Ito 1975, Kołodziejska *et al.* 1999). The decrease in the accumulation of the reducing sugars achieved by these treatments, however, had no practical effect on the DD of the substrate (Table 2). It has been found, that the viscosity of the chitosan solution decreased with the time of treatment with the enzyme extract,

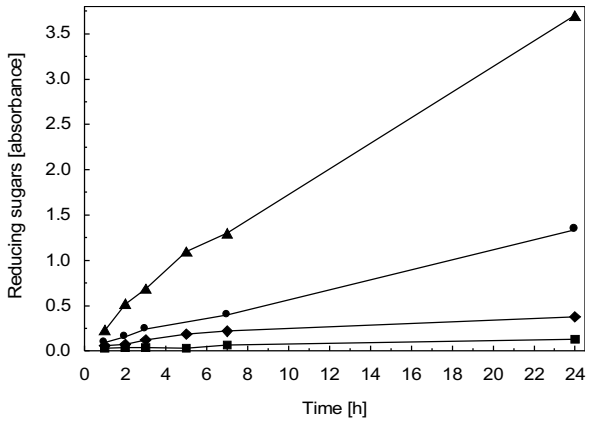

Fig. 4. The effect of pre-purification of the crude ezyme extract from *M. rouxii* mycelium and Zn^{2+} on the release of reducing sugars from chitiosan[68]. The crude enzyme extract (▲), the extract pre-incubated without substrate at pH 4 for 30 min at 25°C and centrifuged at 0°C 15,000 x g for 15 min (●), extract pre-purified at pH 4 and pre-incubated with $ZnCl_2$ (1mM) for 60 min at 25°C (◆), extract pre-purified at pH 4, pre-incubated with $ZnCl_2$ (1mM) for 60 min at 25°C and Zn^{2+} (1 mM) present in reaction mixture (■) The reaction mixture, containing 2.5 mg of chitosan[68] and 55 µl of the extract in 1 ml of 0.1 M Tris-HCl, was incubated for an appropriate time at pH 5.8 at 50°C. The reducing sugars were determined as described under Materials and Methods. The absorbance given in the graph has been computed from measurements made in diluted samples.

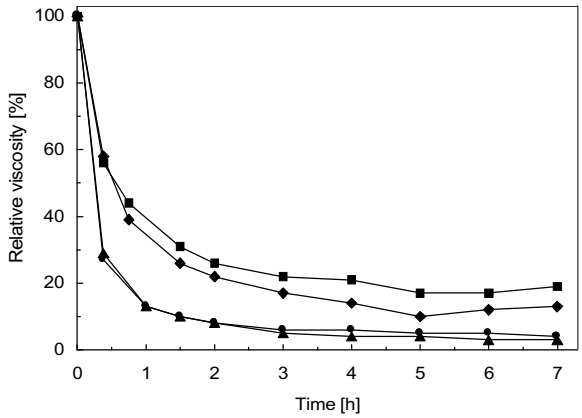

Fig. 5. The effect of pre-purification of the crude enzyme extract from *M. rouxii* mycelium and Zn^{2+} on the viscosity of 1.2% chitosan[68] solution. The crude enzyme extract (▲), the extract pre-incubated without substrate at pH 4 for 30 min at 25°C and centrifuged at 0°C 15,000 x g for 15 min (●), extract pre-purified at pH 4 and pre-incubated with $ZnCl_2$ (1mM) for 60 min at 25°C (◆), extract pre-purified at pH 4, pre-incubated with $ZnCl_2$ (1mM) for 60 min at 25°C and Zn^{2+} (1 mM) present in reaction mixture (■). The reaction mixture, containing 12 mg of chitosan[68] and 55 µl of the extract in 1 ml of 0.1 M Tris-HCl, was incubated for an appropriate time at pH 5.8 at 50°C. The viscosity of 1.2% chitosan[68] solution with addition of thermally inactivated enzymes was used as reference of 100% .

the intensity of the loss in viscosity being highest when the crude and pre-purified enzyme extract was used (Fig. 5). This suggests, that even in the most purified preparation the minute amounts of residual hydrolytic enzymes can effectively degrade the substrate. Thus, depending

Table 2. The effect of partly purified deacetylase extract and Zn^{2+} on the deacetylation degree of chitosan[68] after 24 h enzymatic reaction at 50°C

Chitosan[68] treated with	Deacetylation degree[a] [%]
crude enzyme extract	85.2
pre-purified enzyme extract at pH 4	85.9
pre-purified enzyme extract incubated with $ZnCl_2$ (1 mM) for 30 min. at 25°C	86.2
pre-purified enzyme extract incubated with $ZnCl_2$ (1 mM) for 30 min. at 25°C and $ZnCl_2$ (1 mM) present in the reaction mixture	86.1

[a] the reaction mixture contained 40 mU of deacetylase in 1 ml 0.1 M Tris-HCl solution, pH 5.8. The deacetylation degree was calculated from the quantity of released acetic acid.

Table 3. The physico-chemical properties of chitosans prepared by enzymatic deacetylation

Material	Dry weight [%]	Ash [% dry weight]	Insoluble material [% dry weight]	Viscosity [mP x s]	Deacetylation degree [%]
Substrate: Chitosan[68]	98.4	0.05	1.5	2440	68
Product[a]	93.4	0.12	1.1	297	82 (83)[b]
Substrate:Chitosan[76]	98.9	0.06	0.6	1720	76
Product[a]	91.4	0.17	0.6	93	86 (89)[b]

[a] the substrate was treated 16 h at 50°C with pre-purified enzyme extract
[b] the deacetylation degree was calculated from the quantity of released acetic acid

on the expected end use of the deacetylated chitosan, the product can be "tailor-made" in respect to viscosity in mild conditions by using enzyme preparations of different degrees of purification.

Physico-chemical properties of chitosans obtained on a laboratory scale by enzymatic deacetylation of two products of alkaline treatments of chitin at temp. 60°C are shown in Table 3. The DD calculated from the quantity of released acetic acid was similar to that determined by potentiometric titration. The viscosity of the products of enzymatic deacetylation measured in standard conditions at temp. 25°C was at least about 10 times lower than that of the substrates. This confirmed the results obtained for the reaction mixture at temp. 50°C.

The two-stage chemical and enzymatic process may be effectively used for preparing chitosans with the required DD. For producing chitosans of low viscosity solution, pre-purified deacetylase preparation can be used. However, for obtaining highly viscous chitosans the enzyme preparation should be purified in order to completely remove the chitino- and chitosanolytic enzymes from the extracts.

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Infection variability of the parasitic copepod *Eubrachiella antarctica* (Quidor, 1906) on fishes in the Atlantic sector of the Antarctic

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Abstract. *Eubrachiella antarctica* (Quidor, 1906) is the most commonly occurring ectoparasite in different fish species in the Atlantic sector of the Antarctic. The present work summarises results of studies carried out by various authors from 1975 to 1988 and is devoted to the prevalence, intensity and distribution of parasites on 12 species of fishes. The results of the authors' own studies carried out during the cruise of R/V PROFESOR SIEDLECKI in the 1978/79 season were used to statistically evaluate the significance of differences of infection parameters of the studied fish species sampled at five locations. It was demonstrated that the parasite invasion level on some fish species varied significantly depending on the areas where the fishes were caught, which suggests the possible existence of distinct stocks of some fish species.

Key words: Antarctica, Atlantic sector, fishes, parasites, *Eubrachiella antarctica*, infection levels

INTRODUCTION

Parasitic infections of fishes can be regarded as a sanitary and veterinary problem, because the consumption of such fishes can threaten consumer health while at the same time lowering the consumption suitability of these fish as well as their aesthetic value. In particular, this refers to fish species infected by endoparasites such as *Anisakis simplex* (Nematoda), present in herring and other fishes in the North Atlantic, or *Kudoa alliaria* (Myxozoa), commonly found in southern blue whiting. The decrease of the overall fish performance and its survivability is not insignificant and such effects were observed in Atlantic cod infected with the ectoparasite *Clavella adunca* (Copepoda) (Janusz 1980).

Parasitic infections can also be understood in an ecological context. For the parasite, the fish as a host constitutes the primary environment, while the host's surroundings should be regarded as the secondary environment. The secondary environment is common for the host and the parasite and it effects the mutual development of the parasite and host populations (Grabda 1981).

Water body environmental factors have an effect not only on the composition of the parasite assemblages, but also on the infection parameters in the case of a single, very abundant parasite species. These phenomena make parasites good tools for confirming results obtained

by other research methods in relation to stock separation and their migrations. They constitute biological tags (Kabata 1963, Mac Kenzie 1986, Grabda 1974).

In the 1970s, research on living resources of the Antarctic significantly intensified. Within the framework of these studies parasitological investigations were also conducted. It turned out that the most frequently occurring and exceptionally abundant parasite was *Eubrachiella antarctica* (Quidor, 1906), a member of the subphylum Copepoda and the family Lernaeopodidae.

A number of researchers focused their attention on the occurrence of this parasite and its distribution on the fish body. Among them were: Kock and Möller (1977), Kock (1979), Siegel (1980, 1980a), Sosiński and Janusz (1986), Rokicki and Skóra (1986), Rokicki and Zdzitowiecki (1991), Rokicki *et al*. (1993), El Mehlawy *et al.* (1993), Janusz and Sosiński (1999). It is evident from the completed studies to date that *Eubrachiella antarctica* is present in the large area of the Scotia Sea and the adjacent waters on a large number of fish species. The infection level varies from subarea to subarea, species to species and even locations of the fish body. In some species, the parasite is predominately found in the mouth cavity and in others primarily on the fins.

It can be assumed that the conditions of the secondary environment, affecting the prevalence of a given parasite, are also among the factors which cause differences between populations of the same fish species. If so, the parasite species fulfils the requirements of a good biological tag for particular fish populations. To be useful, however, the prevalence differences between various areas must be statistically significant.

There were two principal aims of the present work. The first one was to summarise the results of the studies published by different authors on the infestation rate of fishes from the Atlantic sector of the Antarctic with the parasite *Eubrachiella antarctica* and its distribution on the fish body. The second was to determine the possible significance of the differences between infection levels of the fish of a given species from different areas.

MATERIAL AND METHODS

The selection of the fish species and the selection of the areas where the parasitological studies were conducted were associated with the specificity of the research cruises performed in the 1970s and 1980s in the Atlantic sector of the Antarctic. They were associated with the exploration of the Antarctic as a potential area of fishing exploitation. The studies were carried out mainly on the shelf waters of islands and on the fishes which represented commercially caught species. Except for the Burdwood Bank, the studied areas (Fig. 1) were situated south of the Antarctic Convergence. Research in the Burdwood area in the sub-Antarctic zone focused on parasitic infections of *Dissostichus eleginoides* which, similarly to *Patagonotothen brevicauda guntheri* caught near Shag Rocks, could be considered a wider ranging species. The remaining fish species under study are endemites of the Antarctic zone which do not cross the limits of the Antarctic Convergence.

The infection levels of fishes from the Atlantic sector of the Antarctic with the copepod *Eubrachiella antarctica* presented here constitute a synthesis of the data from published sources. These papers covered investigations conducted at a time of intensive exploration and exploitation of Antarctic fish resources between 1975 and 1988. The papers and the numbers of the fishes studied are listed in Tab. 1.

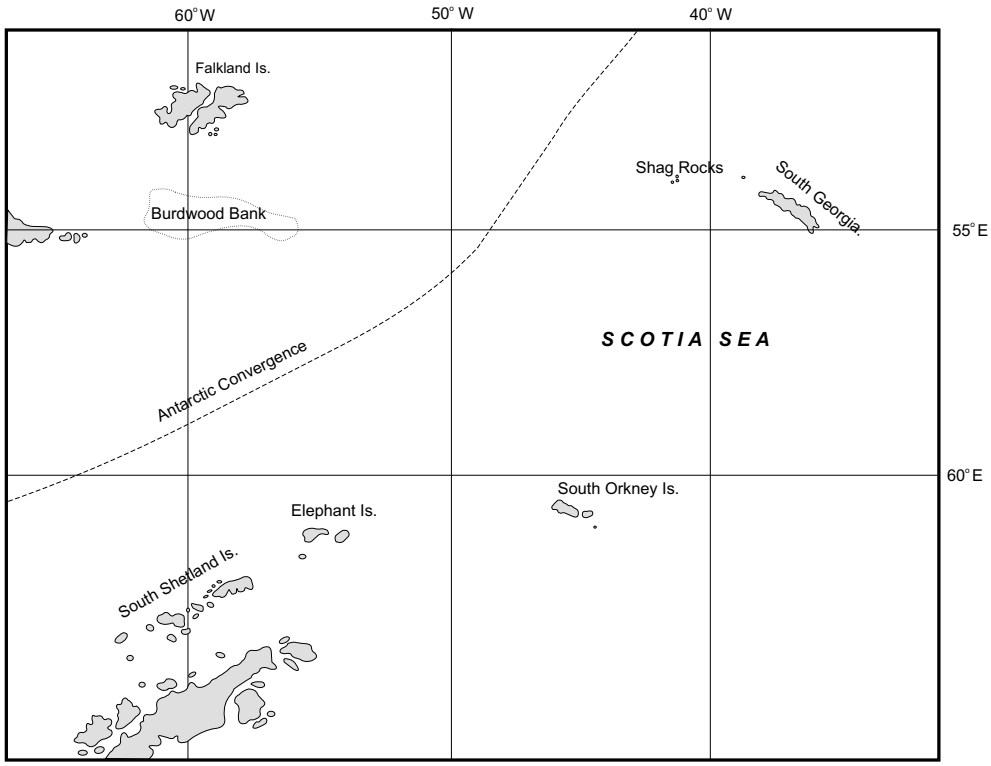

Fig. 1. The areas of parasitological study

During this period, the most extensive parasitic studies were carried out in the area of South Georgia. The fewest fishes were studied for parasites at the fishing grounds of Burdwood Bank (outside the Antarctic) and Shag Rocks. The most frequently studied fishes were: *Champsocephalus gunnari* Lönneberg, 1905, *Notothenia* (*Gibionotothen*) *gibberifrons* Lönneberg, 1905, *Chaenocephalus aceratus* Lönneberg, 1906, *Chionodraco rastrospinosus* de Witt at Hureau, 1979, *Pseudochaenichthys georgianus* Norman, 1937, and *Dissostichus eleginoides* Smitt, 1898. The intensity of research in individual areas and of individual fish species directly reflects their importance in fishing. The infection levels were described using two parameters: prevalence – invasion incidence *P* and intensity – mean invasion intensity *I*. A total of 25,240 fishes of 12 species were examined (Tab. 1). The fish names followed the FAO/CCAMLR key (Fischer and Hureau 1985).

The significance of differences in the prevalence levels of individual fish species occurring in the areas of the Atlantic sector of the Antarctic was determined based on material collected during the R/V PROFESOR SIEDLECKI cruise in the 1978/1979 season (Tab. 1, bold print).

During this cruise, 12 fish species were studied for the presence of *Eubrachiella antarctica* and eight of them were studied on at least two fishing grounds. Namely, these species were *Champsocephalus gunnari*, *Chaenocephalus aceratus, Pseudochaenichthys georgianus*, *Chionodraco rastrospinosus*, *Chaenodraco wilsoni* Regan, 1914, *Dissostichus eleginoides*, *Notothenia* (*Gobionotothen*) *gibberifrons*, *Notothenia* (*Lepidonotothen*) *kempi* Norman, 1937.

Table 1. Number of fishes studied for presence of *Eubrachiella antarctica* (Quidor, 1906) in 1975-1988

Species	Area							Source
	Season	B. B.	S. R.	S. G.	S. O.	E.	S. S.	
Champsocephalus	1975/76	–	–	998	46	–	–	Kock, Moller 1977
gunnari	1977/78	–	–			2013		Kock 1979; Siegel 1980
	1978/79	–	**200**	**759**	**297**	**600**	**484**	Sosiński, Janusz 1986
	1981/82	–	–	1575	–	–	–	Rokicki *et al.* 1993
	1983/84	–	–	1402	–	–	–	
	1986/87	–	–	–	–	2205	–	
		–	49	112	–	89	–	Zdzitowiecki (unpubl.)
	1987/88	–	–	425	–	–	–	Rokicki *et al.* 1993
Chaenocephalus	1975/76	–	–	–	–	–	96	Kock, Moller,1977
aceratus	1977/78	–	–	–	1560	–	–	Siegel 1980, 1980a
	1978/79	–	–	**700**	–	**200**	**539**	Sosiński, Janusz 1986
Pseudochaenichthys	1977/78	–	–	636	–	–	–	Siegel 1980
georgianus	1978/79	–	–	**576**	**100**	–	**83**	Sosiński, Janusz 1986
Chionodraco	1975/76	–	–	–	99	–	61	Kock, Moller 1977
rastrospinosus	1977/78	–	–	**–**	**–**	950	**–**	Siegel 1980
	1978/79	–	–	–	**190**	**141**	**752**	Sosiński, Janusz 1986
Chaenodraco wilsoni	1978/79	–	–	–	**100**	–	**600**	Sosiński, Janusz 1986
Dissostichus	1975/76	121	–	21	–	–	–	Kock, Moller 1977
eleginoides	1977/78	–	551	–	–	–	–	Siegel 1980
		–	–	51	–	–	–	El Mehlowy *et al.* 1993
	1978/79	–	**68**	**101**	–	–	–	Janusz, Sosiński 1999
	1986/87	–	–	56	–	–	–	El Mehlowy *et al.* 1993
Notothenia	1977/78	–	–	101	–	–	–	Rokicki, Zdzitowiecki 1991
(Notothenia) rossi	1978/79	–	–	202	–	–	–	Janusz, Sosiński 1999
	1986/87	–	–	514	–	–	–	Rokicki, Zdzitowiecki 1991
Notothenia (Notothenia) neglecta	1978/79	–	–	–	–	–	31	Janusz, Sosiński 1999
Notothenia (Gobiono-	1977/78	–	–	1304	–	–	–	Rokicki, Skóra 1986
tothen) gibberifrons	1978/79	–	–	**999**	**300**	**406**	**889**	
Notothenia (Lepido-notothen) kempi	1978/79	–	–	–	–	**100**	**300**	Janusz, Sosiński 1999
Patagonothen brevi-cauda guntheri	1978/79	–	200	–	–	–	–	Janusz, Sosiński 1999
Pagothenia hansoni	1978/79	–	–	288	–	–	–	Janusz, Sosiński 1999

Bold print indicates data considered in determination of the statistical differences in the prevalance values.
B. B .– Burdwood Bank, S. R. – Shag Rocks, S. G. – South Georgia, S. O. – South Orkney Is.
S. S. – South Shetland Is.

From the combined total of 9,484 specimens, the statistical significance of differences in the infection levels between different areas was studied. The infection levels were determined on fresh fishes. They were immediately studied after capture in the course of routine ichthyological examinations.

To determine the significance of differences in the infection level P of the fish populations caught on different fishing grounds a significance test u for the difference of two fractions of large samples (Oktaba 1996) was used. The prevalence of first population is repre-

sented by p_1 and the prevalence of the second population by p_2. A null hypothesis of the following form was assumed:

$$H_0 : p_1 - p_2 = 0$$

This means that no differences between prevalence values of infection of both populations exist.

A test function was used in the following form:

$$u^0 = \frac{p_1^* - p_2^*}{\sqrt{p^* * q^* * (1/n_1 + 1/n_2)}}$$

where:
p^* – prevalence of both populations
q^* – percentage of uninfected individuals in both populations,
p_1^* – prevalence of first sample;
p_2^* – prevalence of second sample;
n_1, n_2 – numbers of fish studied in the respective samples.
The hypotheses were verified at a significance level of 1%.

RESULTS

An overview of the results of other authors studying the infection of 12 species of fishes with *Eubrachiella antarctica* in individual areas of the Atlantic sector of the Antarctic is provided in Tab. 2. The highest prevalence was observed in *Dissostichus eleginoides* and *Notothenia* (*Notothenia*) *rossii* on the fishing ground of South Georgia. In three cases, the studied fish species did not yield parasites, namely: *Champsocephalus gunnari* from Shag Rocks (twice in the 1978/79 and 1986/87 seasons), *Notothenia* (*Gobionotothen*) *gibberifrons* from the South Orkney Islands, and *Notothenia* (*Lepidonotothen*) *kempi* in the area of Elephant Island.

Infection intensity *I*)in the majority of the studied species was relatively low and ranged from 1 to 7 parasites in a single fish. The exception was *Notothenia* (*Notothenia*) *rossii* where the intensity of infection was 19 to 43 parasites and *Chaenocephalus aceratus* in the 1975/76 season on the fishing ground in the South Shetland Islands.

The parasites were distributed in the buccal cavity (including the gills) (Bc), on the skin (S), and on the fins (F). The distribution of the parasites on a given species of fish was similar in consecutive years of the studies. In *Chionodraco rastrospinosus*, *Dissostichus eleginoides*, *Notothenia* (*Notothenia*) *rossi,* and *Notothenia* (*Notothenia*) *neglecta* almost all parasites were attached to the buccal cavity and a few on the fins. On the fishes of the remaining species ,the majority of parasites was found on the fins. Rarely were parasites found on the skin of fishes (Tab. 2).

The reference data used for calculating *u* value for the test of significance of differences between prevalences of fishes from different areas in the 1978/79 season are listed in Tab. 2. The materials gathered allowed the significance of the differences in infection levels of *Champsocephalus gunnari*, between five areas and in the case of *Notothenia* (*Gobionotothen*) *gibberifrons* – between four areas to be determined. For two species (*Chaenocephalus aceratus*, *Pseudochaenichthys georgianus*) sufficient material was collected only in three areas, while for *Notothenia* (*Lepidonotothen*) *kempi* – in two areas only, despite of the presence of those species in all areas. For three species (*Chionodraco rastrospinosus*, *Chaenodraco wilsoni*, and

Table 2. Infection parameters and distribution of *Eubrachiella antarctica*

Species	Season	Area					
		Burdwood Bk.		Shag Rocks		S. Georgia	
		P	I	P	I	P	I
Champsocephalus gunnari	1975/76	–	–	–	–	0-3	1-2
	1977/78	–	–	–	–	–	–
	1978/79	–	–	0	0	17	2
	1981/82	–	–	–	–	5	1
	1983/84	–	–	–	–	7	1
	1986/87	–	–	–	–	–	–
	1986/87	–	–	0	0	13	1
	1987/88	–	–	–	–	23	2
Chaenocephalus aceratus	1975/76	–	–	–	–	–	–
	1977/78	–	–	–	–	0–4	–
	1978/79	–	–	–	–	2	2
Pseudochaenichthys georgianus	1977/78	–	–	–	–	–	1-2
	1978/79	–	–	–	–	5	2
Chionodraco rastrospinosus	1975/76	–	–	–	–	–	–
	1977/78	–	–	–	–	–	–
	1978/79	–	–	–	–	–	–
Chaenodraco wilsoni	1978/79	–	–	–	–	–	–
Dissostichus eleginoides	1975/76	82-96	2-3		–	56-67	2
	1977/78	–	–	82	2-7	–	–
	1977/78	–	–	–	–	86	4
	1978/79	–	–	79	4	84	4
	1986/87	–	–	–	–	66	3
Notothenia (Notothenia) rossii	1977/78	–	–	–	–	93	43
	1978/79	–	–	–	–	59	19
	1986/87	–	–	–	–	9	2
Notothenia (Notothenia) neglecta	1978/79	–	–	–	–	–	–
Notothenia (Gobionotothen) gibberifrons	1977/78	–	–	–	–	12	2
	1978/79	–	–	–	–	6	–
Notothenia (Lepidonotothen) kempi	1978/79	–	–	–	–	–	–
Patagonothen brevicauda guntheri	1978/79	–	–	1	2	–	–
Pagothenia hansoni	1978/79	–	–	–	–	5	2

P – prevalence (%), I – Intensity (no), Bc – buccal cavity and gills, S – skin, F – fins.

Dissostichus eleginoides) the calculations were conducted between two or three areas, because of the limited presence of those species in the Atlantic sector of the Antarctic.

The calculated u values for the significance test for differences of prevalences of fishes occurring in different areas of the study are listed in Tab. 3. The values printed in bold indicate that in the studied relationships there is no reason to reject the zero hypothesis with the error risk of 1%, which means that the differences in the infection level of a given species between

on the fishes from the Atlantic sector of the Antartic

Area						Distribution [%]			
S. Orkney Is.		Elephant I.		S. Shetland Is.					Source
P	I	P	I	P	I	Bc	S	F	
37-80	2-5	–	–	–	–	0	0	100	Kock, Moller 1977
5-70	1-4	–	–	–	–	+	1	99	Kock1979; Siegel 1980
30	2	13	2	37	2	+	1	99	Sosiński, Janusz 1986
–	–	–	–	–	–	4	3	94	Rokicki *et al*. 1993
–	–	–	–	–	–	2	2	96	Rokicki *et al*. 1993
–	–	6	1	–	–	1	0	100	Rokicki *et al*. 1993
–	–	20	2	–	–	–	–	–	Zdzitowiecki (unpubl.)
–	–	–	–	–	–	–	–	–	Rokicki *et al*. 1993
–	–	–	–	30-57	15-30	0	0	100	Kock, Moller 1977
4-21	–	23-77	–	31-59	–	+	3	97	Siegel 1980, 1980a
–	–	34	2	30	2	0	1	99	Sosiński, Janusz 1986
–	–	–	–	–	–	2	18	80	Siegel 1980
1	2	–	–	7	1	0	4	96	Sosiński, Janusz 1986
10-31	1-3	–	–	0-69	1-3	99	0	1	Kock, Moller1977
–	–	10-50	1-5	–	–	93	7	1	Siegel 1980
2	1	33	3	25	3	98	1	1	Sosiński, Janusz 1986
4	1	–	–	1	1	0	0	100	Sosiński, Janusz 1986
–	–	–	–	–	–	100	0	0	Kock, Moller 1977
–	–	–	–	–	–	93	6	1	Siegel 1980
–	–	–	–	–	–	100	0	0	El Mehlowy *et al*. 1993
–	–	–	–	–	–	100	0	0	Janusz, Sosiński 1999
–	–	–	–	–	–	–	–	–	El Mehlowy *et al*. 1993
–	–	–	–	–	–	100	0	0	Rokicki, Zdzitowiecki 1991
–	–	–	–	–	–	100	0	0	Janusz, Sosiński 1999
–	–	–	–	–	–	–	–	–	Rokicki, Zdzitowiecki,1991
–	–	–	–	29	3	100	0	0	Janusz, Sosiński 1999
–	–	–	–	–	–	–	–	–	Rokicki, Skóra 1986
0	–	1	–	3	–	–	–	–	
–	–	0	0	1	2	–	–	–	Janusz, Sosiński 1999
–	–	–	–	–	–	0	0	100	Janusz, Sosiński 1999
–	–	–	–	–	–	0	15	85	Janusz, Sosiński 1999

those areas were incidental. It means that there are no significant differences in the infection levels of the fish populations between the areas studied.

The materials collected in the 1978/79 season and which were subjected in this work to statistical analysis showed both significant and insignificant differences in the infection levels of the studied fish species in different areas (Tab. 3, Fig. 2).

According to the test used, there are no grounds for rejecting the null hypothesis, which means that there are no significant differences between the following pairs of populations:

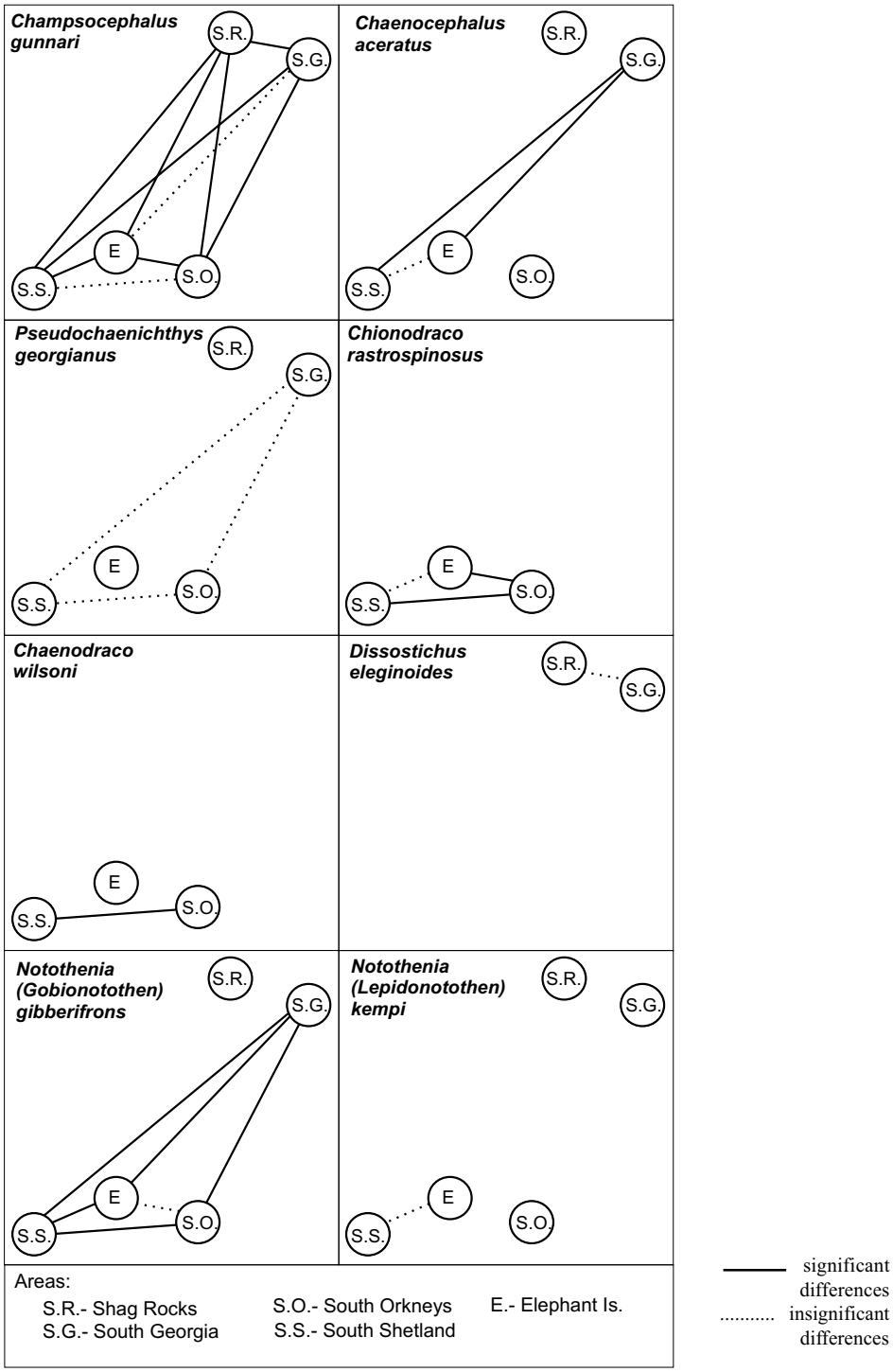

Fig. 2. Prevalence variability of fish populations from different areas infected with *Eubrachiella antartica* (Quidor, 1906)

Table 3. Calculated u values for test of significance of differences in prevalence values of fishes from different areas

Species	Area	Shag Rocks	S. Georgia	S. Orkney Is.	Elephant I.
Champsocephalus gunnari	S. Georgia	6.2670			
	S. Orkney Is.	8.5444	4.6820		
	Elephant I.	5.4054	**1.9470**	6.0692	
	S. Shetland Is.	9.9335	7.8115	**1.8905**	9.0190
Chaenocephalus aceratus	Elephant I.		13.869		
	S. Shetland Is.		13.905		**1.1284**
Pseudochaenichthys georgianus	S. Orkney Is.		**1.6564**		
	S. Shetland Is.		**1.0759**	**2.1871**	
Chionodraco rastrospinosus	Elephant I.			7.6671	
	S. Shetland Is.			6.8909	**2.0324**
Chaenodraco wilsoni	S. Shetland Is.			**2.9034**	
Dissostichus eleginoides	S. Georgia	**0.7919**			
Notothenia (Gobionotothen) gibberifrons	S. Orkney Is.		4.4961		
	Elephant I.		4.7488	**1.2174**	
	S. Shetland Is.		3.4118	3.0554	2.8710
Notothenia (Lepidonotothen) kempi	S. Shetland Is.				**0.5781**

Value from t-Student tables equals 2.580 (with the error risk of 1%)

- *Champsocephalus gunnari* – South Georgia and Elephant Island, South Orkney and South Shetland Islands;
- *Chaenocephalus aceratus* – Elephant Islands and South Shetland Islands;
- *Pseudochaenichthys georgianus* – South Georgia and South Shetland Islands, South Georgia and South Orkney, South Shetland Islands and South Orkney;
- *Chionodraco rostrospinosus* – Elephant Islands and South Shetland Islands;
- *Dissostichus eleginoides* – Shag Rocks and South Georgia;
- *Notothenia* (*Gobionotothen*) *gibberifrons* – South Orkney and Elephant Islands;
- *Notothenia* (*Lepidonotothen*) *kempi* – Elephant Islands and South Shetland Islands.

Firm reasons for rejecting the null hypothesis (when the differences between u^o value and the value from the tables were the highest) means that statistically significant differences were observed between the following pairs of populations:

- *Champsocephalus gunnari* – Shag Rocks and South Shetland Islands, Elephant Islands and South Shetland Islands, Shag Rocks and South Orkney Islands, South Georgia and South Shetland Islands;
- *Chaenocephalus aceratus* – South Georgia and Elephant Islands, South Georgia and South Shetland Islands;
- *Chionodraco rastrospinosus* – South Orkney and Elephant Islands, South Orkney and South Shetland Islands.

Statistically significant differences (with small level disagreement between u^0 and the values form the tables) were also apparent between the following populations:

- *Champsocephalus gunnari* – Shag Rocks and South Georgia,
  South Orkney and Elephant Islands,
  Shag Rock and Elephant Islands,
  South Georgia and South Orkney Islands;
- *Chaenodraco wilsoni* – South Orkney and South Shetland Islands;
- *Notothenia* (*Gobionotothen*) *gibberifrons* – from four areas studied except for relation between South Orkney and Elephant Islands.

DISCUSSION

The areas of the Atlantic sector of the Antarctic listed in Tab. 1 belong to two provinces differing in their fish fauna (Andriashev 1965, Kock 1992, Eastman 1993). The areas of the South Orkney Islands, Elephant Island and Shetland Islands belong to the Continental Province, while Shag Rocks and South Georgia belong to the South Georgia Province. This arrangement of the areas into ichthyofauna provinces was confirmed by Kulesz (1998) who revised the individual ichthyocoenoses. Therefore, the specific composition of the studied fishes in selected areas differs slightly. It is particularly evident in the absence of *Chionodraco rastrospinosus* and *Chaenodraco wilsoni* on the fishing grounds of South Georgia and Shag Rocks and *Dissostichus eleginoides* and *Patagonotothen brevicauda guntheri* on the fishing grounds of the Continental Province.

Parasitic studies in the Antarctic were carried out by different authors in different years and usually during the Antarctic summer. This season of the year has a particular influence on the infection level because of the life cycle of the parasite (hatching period of copepod larvae) and the fishes (spawning concentrations) (Siegel 1980a).

The results of studies on the prevalence and infection intensity of the parasite *Eubrachiella antarctica* on fish species in the Atlantic sector of the Antarctic, which were carried out over 13 years by different researchers, demonstrated a distinct variability depending on season and study area. No parasite infestation was found in three fish species, namely the population of *Champsocephalus gunnari* in the area of Shag Rocks (investigated in the 1978/79 and 1986/87 seasons), the population of *Notothenia* (*Gibionotothen*) *gibberifrons* in the area of the South Orkney Islands and the population of *Notothenia* (*Lepidonotothen*) *kempi* in the area of Elephant Island.

The highest infection levels (Tab. 2) were observed in *Dissostichus eleginoides* and *Notothenia* (*Notothenia*) *rossi*. The latter was different from the others in its relatively high infection intensity. On the other hand, the infection of *Notothenia* (*Notothenia*) *rossii* was relatively low in the 1986/87 season. Most probably this was affected by the fact that the fishes were frozen prior to examination. Rokicki and Zdzitowiecki (1991) speculated that some copepods might have been dislodged during mechanical manipulations associated with freezing and thawing. Examining previously frozen fish possibly could have contributed to the underestimation of the prevalence values in *Champsocephalus gunnari* in the 1981/82, 1983/84, and 1986/87 seasons (Rokicki *et al.* 1993).

Data on the infection levels of 12 fish species with *Eubrachiella antarctica* are listed in Tab. 2. The presence of this parasite on some other host species cannot be ruled out. Also, this

number of species is large enough to qualify *Eubrachiella antarctica* as a species of wider host-specificity (Niewiadomska *et al.* 1986). The above mentioned fish species belong to two families: Notothenidae and Chaenichthyidae. The importance of individual fish species to parasitic copepods is different, depending on environmental conditions. As it is evident from the data in Tab. 2, in the Shag Rocks and South Georgia areas belonging to the South Georgia province, *Dissostichus eleginoides* and *Notothenia* (*Notothenia*) *rossii* were the principal hosts, while *Champsocephalus gunnari* was an auxiliary host and the remaining fish species studied were accidental hosts.

In the area of South Orkney Islands the principal host was *Champsocephalus gunnari*. In the areas of Elephant Islands and South Shetland Islands the principal hosts were *Champsocephalus gunnari*, *Chaenocephalus aceratus*, and *Chionodraco rastrospinosus*.

One characteristic feature observed during the study was the parasite distribution on the fish. The studied fish species were characterised by either the occurrence of the parasites in the buccal cavity and on the gills or on the fins (Tab. 2). These observations confirm the results obtained by various researchers in various years. On three species of the family Notothenidae, *D. eleginoides*, *N.*(*N.*) *rossii* and *N.*(*N.*) *neglecta,* the parasites were attached to the buccal cavity and on the gills. In the remaining species of this family [*Notothenia* (*Lepidonotothen*) *kempi*, *Patagonotothen brevicauda guntheri* and *Pagothenia hansoni*] they were on the fins. The three former fish species are larger.

Parasites were usually situated on the fins of fishes from the family Channichthyidae. This can be associated with a peculiarity of this family; they are white-blooded. These fishes have proportionally larger, abundantly vascularised fins, which reflect their alternative way of oxygen distribution (Jakubowski 1971). This feature may be preferred by the parasites as their settlement sites. The exception of this explanation was *Chionodraco rastrospinosus*, which, as the only white-blooded fish, had parasites in the buccal cavity and on the gills. This fact was confirmed by the studies of Kock and Möller (1977) and Siegel (1980).

An important issue in parasitology has been the significance of differences between the infection levels of the same fish species from different areas. The possibility of using *Eubrachiella antarctica* in different areas of the Antarctic as a biological tag, helpful in differentiating between different stocks, was suggested by specialists in this subject (Kabata 1963, Siegel 1980a).

Studies on the variability of infection levels with a given parasite are more reliable, the more areas, fish species, and fish specimens they cover. In order for studies to be comparable, the observations should be carried out at the same time of year, because infection levels are very much dependent on environmental conditions associated with season and the physiological state of the fishes (Noble 1960). Another asset can be the coverage of the same time-period and area by the same team of researchers. The conditions which most closely met the ideal were those of studies carried out in the 1978/79 season during the R/V PROFESOR SIEDLECKI cruise (Sosiński 1979). The studies were conducted in the defined fishing areas of Shag Rocks, South Georgia, South Orkney Islands, Elephant Island, and South Shetland Islands (Fig. 1). They were carried out mostly in the January-March period, which is the Antarctic summer. The populations of the studied fish species of different areas were similar with respect to their length distribution and sexual maturity (Skóra 1979).

The analysis of the infection level of the fish populations in the studied areas and the significance level of the area variability yielded different results in individual fish species.

Champsocephalus gunnari

This species occurs in all areas of the Atlantic sector of the Antarctic. The infection levels can be dealt with on three levels. In the area of Shag Rocks, there were no parasites found on the fish studied. In the areas of Elephant Islands and South Georgia the prevalence was moderate (13.2 and 17.0%, respectively), whereas it was the highest off the South Orkney and South Shetland Islands (30.0 and 36.6%, respectively) (Tab. 2).

Statistical analysis demonstrated the lack of statistical differences between South Georgia-Elephant Islands and South Orkney-South Shetland. In the remaining relationships there were statistically significant differences (Tab. 3, Fig. 2). The result obtained can suggest the tightest isolation of the *Champsocephalus gunnari* stock in the area of Shag Rocks, where, despite the proximity to the South Georgia area with fish with moderate parasite infections, no presence of the parasites was found on the fish of this species.

It is difficult to explain why there was no statistical variability between fish with higher parasite infection from the South Shetland and South Orkney Islands and the fish from Elephant Islands area which is situated between these two areas. The fish from the Elephant Islands area exhibited statistically significant differences, both in relation to South Shetland and South Orkney Islands, which, in the case of migrations of the fish between these areas, should not happen. Kock and Möller (1977) indicated the presence of two separate stocks of *Champsocephalus gunnari* in the area of South Georgia and South Orkney Islands. Siegel (1980a) demonstrated statistically significant variability in larger fish from the areas of South Georgia, South Orkney and South Shetland Islands.

The biologically separate identity of *Champsocephalus gunnari* stocks in different areas of the Atlantic and Indian Ocean sectors of the Antarctic was also demonstrated by Kock (1981) based on the analysis of the morphometric and meristic features. He distinguished five populations from the areas of South Georgia (including the Shag Rocks), South Orkney Islands, Elephant Island, South Shetland Islands, and Kergulen Islands. Sosiński (1985), based on meristic and biological features concluded that *Ch. gunnari* is a polytypic species consisting of two subspecies occurring in the Atlantic and Indian Ocean sectors of the Antarctic. Within the subspecies from the Atlantic sector of the Antarctic he distinguished two ecological races with local stocks:

– northern (Shag Rocks, South Georgia)

– southern (South Orkney Islands, Elephant Islands, South Shetland Islands).

Based on the growth of 0 age group of fish, North (1996) considered some variability in the stock of Shag Rocks and South Georgia. The variability of those two stocks was also emphasized by Zdzitowiecki (1992) based on differences in the infection levels of these fish with the endoparasite *Elytroptalloides oatesi* (Digenea).

Chaenocephalus aceratus

This species is present in all areas of the Atlantic sector of the Antarctic. The prevalence of fish infections with *Eubrachiella antarctica* was low in the area of South Georgia (2.0%), whereas it was relatively high (and quite similar) in two other areas South Shetland and Elephant Islands (29.7 and 34.0%) (Tab. 2).

Statistical analysis demonstrated the lack of prevalence variability between fishes from South Shetland and Elephant Islands areas, and at the same time it demonstrated a high level of variability of these two areas in relation to the South Georgia area (Tab. 3, Fig. 2). This result may suggest the homogeneity of stocks in the closely situated areas of South Shetland and Elephant Islands.

Siegel (1980a) believes that the stocks of *Chaenocephalus aceratus* from South Shetland and Elephant Islands are different and the depth between these islands can be a barrier for mixing of the adult schools and that only young fish, which lead a pelagic life, can migrate between the two areas. The analysis based on the material from the 1978/1979 season indicates that this is probably a single stock and the depth between the islands does not constitute a barrier for fish mixing. Based on differences in sexual maturity stages and the spawning season, Kock and Möller (1977) suggested the existence of several separate stocks. The present results of the statistical analysis indicate the existence of at least two separate stocks of *Chaenocephalus aceratus* in the areas of South Georgia and South Shetland/ Elephant Islands.

Pseudochaenichthys georgianus

The studies were carried out in three areas: South Georgia, South Orkney and South Shetland Islands. Statistical analysis revealed a lack of prevalence variability between the fish of those areas. The results obtained suggest therefore homogeneity of the stock of *Pseudochaenichthys georgianus* in the areas of South Georgia, South Orkney, and South Shetland Islands (Tab. 3, Fig. 2).

Chionodraco rastrospinosus

This is an endemic form occurring only in the southern areas of the Atlantic sector of the Antarctic. The study was carried out in the areas of South Orkney, Elephant and South Shetland Islands. The prevalence was distinctly lower in the area of the South Orkney Islands (2.1%) in relation to the areas of South Shetland and Elephant Islands (24.5 and 32.6%) (Tab. 2).

Statistical analysis demonstrated significant differences in the fish infection level between the stocks of South Orkney/Elephant Islands and South Orkney/ South Shetland Islands, whereas there were no significant differences between the fish from Elephant/South Shetland Islands. It may suggest that the fish inhabiting these two closely located areas constitute one stock.

Siegel (1980a) did not find statistically significant prevalence variability between specimens from those three areas. The above statement contradicts the results of the present study.

Chaenodraco wilsoni

This species is distributed on the shelf surrounding the Antarctic (Gon and Heemstra 1990). In the Atlantic sector of the Antarctic, it is present only in the southern areas 60°S. The fish were sampled in the areas of South Shetland (Joinville fishing ground) and South Orkney Islands (0.7 and 4.0% respectively) (Tab. 2).

Statistical analysis revealed significant differences in prevalence levels of the fish occurring in these areas, although differences between the u^o value and the values from the tables were low (Tab. 3, Fig. 2). The lack of the results from the Elephant Island area and substantial

differences in the quantities of materials collected from the South Shetland and South Orkney Islands do not allow for any further conclusions to be drawn.

Dissostichus eleginoides

This species also occurs outside the Antarctic north of the Antarctic Convergence. In the Antarctic sector of the Antarctic this fish inhabits the northern areas (South Georgia and Shag Rocks).

The prevalence values of *Dissostichus eleginoides* were the highest among all fish species studied in the 1978/79 season. They reached 79.0% in the area of Shag Rocks and 84% off South Georgia (Tab. 2).

Statistical analysis demonstrated a lack of significant differences in the infection levels of the fish (Tab. 3, Fig. 2) between the areas of Shag Rocks and South Georgia, which may be evidence of the homogeneity of the stock present in these areas.

The studies on the infection levels of *Dissostichus eleginoides* from Burdwood Bank (sub-Antarctic) and South Georgia conducted by Kock and Möller (1977) in the 1975/1976 season showed certain variability. The reasons for such variability could have been the existence of two separate populations or the change in prevalences caused by the separation of these areas by the Antarctic Convergence. The close relationship between parasite fauna and environmental conditions was emphasised by Polyanski (1961) who believed that the majority of the parasites were sensitive to changes in the salinity and temperature of the water. Waters on both sides of the Antarctic Convergence are characterised by the variability of these two parameters.

Notothenia (*Gobionotothen*) *gibberifrons*

This species is present in all areas of the Atlantic sector of the Antarctic. The study was conducted in four areas: South Georgia, South Orkney Islands, Elephant Islands and South Shetland Islands.

The materials collected allowed for statistical analysis which demonstrated the lack of significant differences in the prevalences of the fish from South Orkney – Elephant (Tab. 3, Fig. 2). Differences between the remaining areas proved significant, although with a relatively small difference between u^0 value and the value from the tables, particularly for the areas of South Shetland and Elephant Islands. Relatively, the highest variability in prevalences, compared to other areas, was demonstrated by fishes from the area of South Georgia.

Population variability, resulting from differences in prevalence values, was confirmed in an earlier study by Skóra (1985) based on the analysis of meristic features. He distinguishes six populations of *Notothenia* (*Gobionotothen*) *gibberifrons* in the Atlantic sector of the Antarctic, however, the most distinct was the population from South Georgia.

Notothenia (*Lepidonotothen*) *kempi*

The present study covered two areas: South Shetland and Elephant Islands Islands. Only a single specimen of the parasite was found in the area of the South Shetland Islands.

Statistical analysis, considering such a low infection level in the area of South Shetland Islands and the lack of infection in the area of Elephant Islands, did not show significant differences in the prevalence (Tab. 3, Fig. 2). The lack of the material from the remaining areas where this fish is present does not permit the drawing of more general conclusions.

In the case of three fish species [*Pseudochaenichthys georgianus*, *Chaenodraco wilsoni*, *Notothenia* (*Lepidonotothen*) *kempi*] no literature data was available to permit comparisons with the present data.

Summarising the results of the authors' own works and that of the published papers of other authors on *Eubrachiella antarctica* infection levels in fish in the Atlantic sector of the Antarctic, it can be concluded that:

- it is a parasite with a wide range of host species;
- depending on ecological conditions in a given area the composition of principal host species and auxiliary hosts changes;
- the parasites are distributed mainly on the fins or in the buccal cavity and the gills, and the site selection is typical for a given host species.

The study carried out based on the most representative data of the of 1978/79 season demonstrated that the prevalence levels in some fish species was variable in a significant way, depending on the area of the fish occurrence which indicated the existence of separate stocks within some species. The arrangement proposed above is consistent with the published data on the biological and meristic features of the fishes. Consequently, the ectoparasite *Eubrachiella antarctica* can be regarded as a biological tag useful in identifying separate stocks of fishes.

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The fecundity and reproduction of round goby *Neogobius melanostomus* (Pallas, 1811) in the Puck Bay (Baltic Sea)

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Abstract. This work presents results of fecundity and reproduction investigations of round goby, a species which is new to the Puck Bay. A total of 728 fish were analyzed. The material for initial fecundity analyses consisted of 135 chosen females with gonads in maturity stages IV and V on the Maier scale. The *longitudo totalis* (*l. t.*) of the females ranged from 7 cm to 22 cm. Analyses revealed that the majority of females and males, even at a *l. t.* of 80-90 mm, were mature. In 1999, round gobies began spawning in the Puck Bay in April and finished in late August and early September of the same year. The absolute fecundity of female round gobies ranging in length from 7.4 cm to 16.6 cm (average 12.4 cm) was 89 to 3,841 spawn grains. It was determined that the relationship between absolute fecundity F_a and *l. t.* (R = 0.888) is nonlinear, while that between absolute fecundity and female body mass GW (R = 0.877) is linear. The hypothetical, absolute fecundity of females 15 cm long was 3,036 eggs. The relative fecundity F_w of round goby females ranged from 17 to 109 eggs/g of female body mass.

Key words: round goby, *Neogobius melanostomus,* gobiidae, fecundity, reproduction, Puck Bay, invasive species, Baltic Sea

INTRODUCTION

The reproduction habits of the round goby *Neogobius melanostomus* (Pallas, 1811) is rather unusual in Polish waters. This fish is characterized by early maturity, a very long spawning period (during which females spawn in batches) and aggressive spawn protection, all of which contribute to high egg, larva and fry survival rates. Data on the fecundity and reproduction of this species comes mainly from Russian language and North American literature. The problems of reproduction biology, spawning, spawn and fry protection as well as the embryogeny are widely discussed in the works of Kalinina (1976) and Moskalkowa (1996). Information concerning round goby reproduction, fecundity and nuptial behavior can also be found in Swetowidow (1964), Miller (1986), Corkum *et al.* (1998) and Wickett and Corkum (1998). To date, all information concerning the subject published in Poland has appeared either in the form of reports or notices (Kuczyński 1995).

The aim of this work was to investigate the reproduction and fecundity of the round goby, a species new to the Puck Bay.

MATERIALS AND METHODS

The fish for investigations were collected from the beginning of March to September 1999. The material was collected during commercial catches, which were carried out in the Puck Bay, and during research catches from the R/V BALTICA (Fig. 1).

A total of 728 fish were analyzed (Table 1), with females constituting 44.5% (324 specimens) of the whole number investigated. The initial material for fecundity analyses consisted of 135 chosen females with gonads in maturity stages IV and V according to the Maier scale (Maier 1906). These females measured from 7.4 to 22.0 cm *l. t.*

The total fish length (*l. t*). and body length (*longitudo corporalis – l. c*.) were measured to the nearest millimeter. Additionally, total fish mass *W* and gutted fish mass *GW* were determined to the nearest 0.1 gram. In order to avoid errors resulting from, e.g. varying degrees of digestive tract content, gutted fish mass was used to report variables such as absolute fecundity and body mass. Gonad development stage was determined according to the eight point Maier

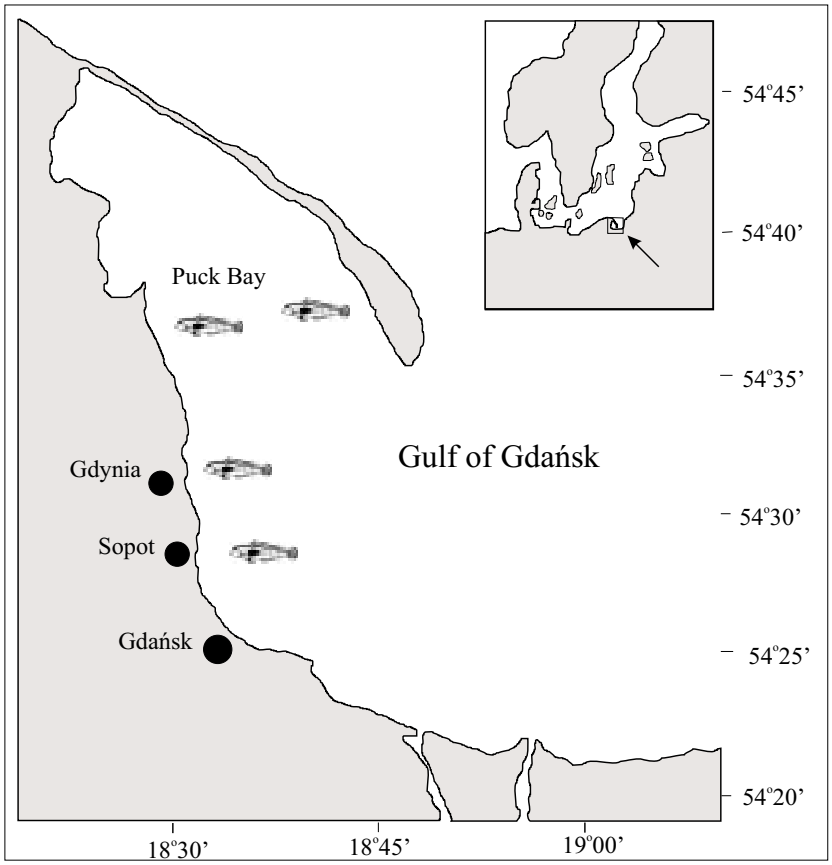

Fig. 1. Bay of Puck and the Gulf of Gdańsk – catch sites of fish used in investigations

Table 1. Gonad maturity stages of round goby used in investigations (according to Maier scale)

Catch date	Number in sample	Sex	Gonad maturity stages							
			II	III	IV	V	VI	VII	VIII	total
8.03.1999	184	F	5	9	62	2				78
		M	12	78	15	1				106
26.04.1999	80	F	4		38	2				44
		M	6	28	2					36
10.05.1999	68	F		2	34	20	4			60
		M		6	2					8
26.06.1999	102	F			1					1
		M		1	65	29	3	3		101
16.07.1999	60	F	6	18	6			3		33
		M	6	18	3					27
20.07.1999	90	F	18	32	14				2	66
		M	20	2					2	24
11.08.1999	64	F		12	12				2	26
		M	10	20	8					38
6.09.1999	80	F		16						16
		M	34	30						64
Total			121	272	262	54	7	6	6	728

scale. Fresh gonads were weighed to within 0.1g. The gonads which were collected for fecundity evaluation were preserved by freezing at a temperature of –20ºC, then defrosting and preserving them in 80% alcohol (Bleil and Oeberst 1993). The total number of spawn grains in a gonad was determined by collecting, depending on ovary size, one, two or three sub-samples of a defined mass from the dried, cleaned and weighed gonad. The number of eggs in each gonad was then counted and the total number of eggs in the ovary was calculated from the proportion.

The evaluation of the absolute and relative fecundity of females was carried out using the sample which was collected in the period before spawning (8 March 1999). The relationship between absolute fecundity and fish body length and mass was determined. The functions which describe the relationship between fecundity F_a and fish length *l. t.* as well as fecundity F_a and fish mass *GW* are as follows:

$$F_a = a * l.\ t.^b \qquad [1]$$

$$F_a = a * GW + b \qquad [2]$$

After calculating the parameters of the above formulae, the hypothetical total fecundity of a female 15 cm long was calculated using formula [1] (after Backiel and Zawisza 1988). This allows for future comparison of the expected fecundity of particular sized females from different populations. For the round gobies investigated, the relative fecundity F_w (number of eggs per 1 g of fish body mass) was determined.

In order to determine the percentage of gonad mass to fish mass, the gonadosomatic coefficient was employed. Gutted fish mass was also used to calculate this parameter.

RESULTS

Through a combination of numerous observations of round goby reproduction in the Puck Bay and the analyses of biological material, a wide variety of information and results concerning their spawning and fecundity were obtained.

In March 1999, the majority of females were characterized by gonads in maturity stage IV (79.5% of the sample) (Table 1). This figure was similar to that of April (86.4 %) and May (56.7%). During the investigations in June, only one female specimen was found, and from July females with ovaries in maturity stage III began to occur more often. In the September sample no fish with gonads in maturity stages IV and V were found; this leads to the conclusion that spawning in this area had finished. A small number of specimens from among the 324 females investigated had ovaries in the following maturity stages: V (24 specimens), VI (4 specimens), VII (3 specimens) and VIII (4 specimens).

The results of investigations reveal that both females and males in the Puck Bay begin spawning rather early. Ovaries and testes in the III and IV maturity stage were found among the smallest specimens (7-8 cm *l. t.*). Simultaneously, juvenile specimens not yet ready to begin spawning were still found among larger fish. In the 22 cm length class, 40% of all the males analyzed were immature fish with gonads in maturity stage II. This is evidence that individual specimens begin to reproduce at different times.

The investigations revealed that in 1999 round gobies begun spawning in the Puck Bay in April and finished in late August and early September.

The greatest values of gonadosomatic coefficients were found among females from the May sample (13.4). Later, along with the deposit of a greater batch of spawn, the average values of these coefficients gradually decreased to a level of 1.0 in September. This may indicate that spawning in the Puck Bay began in late April or at the beginning of May.

The total number of spawn grains in round goby ovaries varied throughout the year (Table 2). The greatest number of spawn grains was observed at the beginning of the investigations (March-May), but as spawning continued in batches the amount of spawn in female gonads gradually decreased. The average number of eggs in the ovaries of specimens from the March sample is relatively low, but this must be related to the small size of the females analysed. In order to better compare changes in the number of oocytes in particular months, specimens were chosen from particular samples and those similar in length were compared. The results are presented in Table 3.

Table 2. Number of oocytes found in ovaries in particular samples

Catch date	Number of eggs			*l .t.* [cm]			Number in sample
	average	min.	max.	average	min.	max.	
8.03.99	1,739	89	3,841	12.4	7.4	16.6	54
26.04.99	2,899	1,435	4,688	16.1	14.1	19.2	34
10.05.99	3,005	2,388	3,734	16.0	15.0	17.2	18
26.06.99	1,230	1,230	1,230	12.8	12.8	12.8	1
16.07.99	2,068	1,635	2,411	17.8	16.9	18.6	6
20.07.99	1,054	651	1,544	14.5	13.9	15.2	10
11.08.99	1,880	1,265	2,744	18.2	16.5	22.0	12

Table 3. Number of oocytes found in ovaries in particular samples (fish of similar length *l.t.*)

Date	Number of oocytes	*l. t* [cm]	Number in sample
8.03.99	3,310	16.6	1
26.04.99	3,048	16.7-16.8	4
10.05.99	3,205	16.5-17.2	4
16.07.99	1,784	16.9	3
11.08.99	2,049	16.5-17.2	6

Absolute fecundity

It was assumed that the absolute fecundity of females is determined by the number of mature eggs in the ovaries before spawning. Based on the 8 March 1999 sample, the absolute fecundity of round goby females ranging in length from 7.4 cm to 16.6 cm (average 12.4 cm) in the Puck Bay was from 89 to 3,841 (average 1,739) spawn grains. The distribution of empirical points in Fig. 2 indicates the nonlinear character of the dependence between absolute fecundity F_a and female length (*l. t.*). The derivation of parameters *a* and *b* from formulae [1] and [3] led to the following power function:

$$F_a = 0.1353 * l.t.^{3.6996} \qquad [3]$$

The data presented in Fig. 2 indicate the disproportionally high decrease of fecundity as body length increased. A strong correlation between female fecundity and length *l. t.* was observed in the investigated population of round gobies (correlation coefficient $R = 0.888$). Using formula [3] the hypothetical absolute fecundity of a female 15 cm long was estimated to be 3,036 eggs.

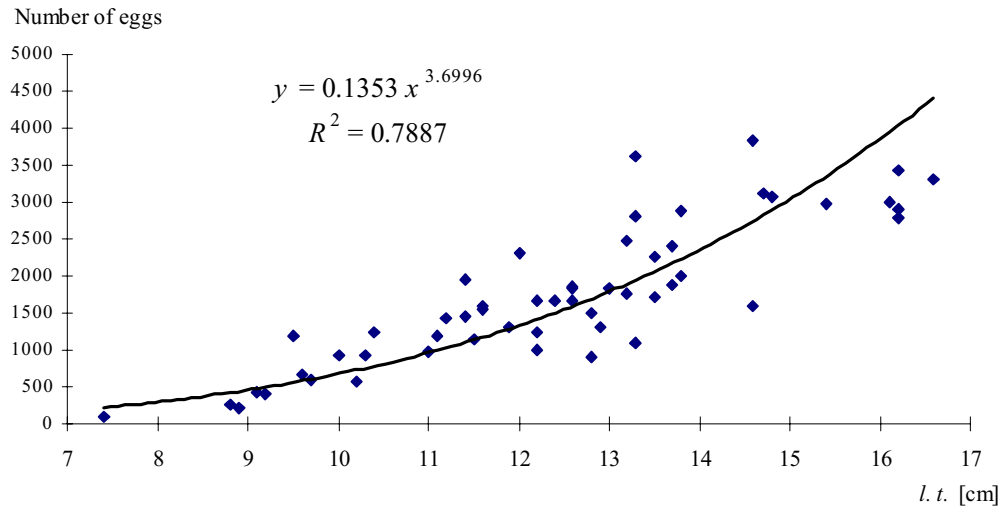

Fig. 2. Relationship between absolute fecundity F_a and total length *l. t.* in round goby females from the Bay of Puck

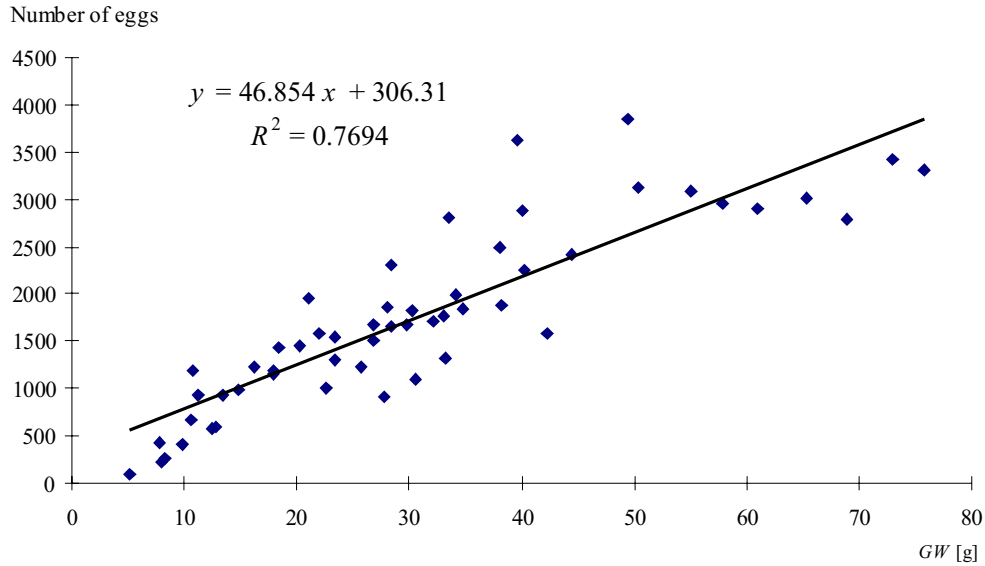

Fig. 3. Relationship between absolute fecundity F_w and body mass GW in round goby females from the Bay of Puck

Additionally, absolute fecundity and body mass GW demonstrate a good correlation (Fig. 3). The analysis of this equation revealed that it is linear and that the function describes this relationship best (correlation coefficient $R = 0.877$):

$$F_a = 46{,}854 * GW + 306.31 \qquad [4]$$

Relative fecundity

The relative fecundity F_w of round goby females varied from 17 to 109 eggs/g of body mass. With the exception of the smallest fish, data obtained during investigations indicate a decrease in the average values of relative fecundity as fish body length increases. The analysis of data presented in Table 4 revealed that the greatest value of F_w was characteristic of fish 11 cm long (75 eggs/g). The relative fecundity values gradually decreased with length to 44 eggs/g in the 17 cm class. The lowest fecundity F_w was determined for the only female investigated in the 7 cm length class (17 eggs/g of body mass). During the investigations, the greatest number of spawn grains per gram of female body mass was observed before spawning in March (58 on average) while the lowest was found in August (22 on average).

Table 4. Relative fecundity F_W of round goby females in particular length classes

l. t. [cm]	F_w [eggs/g]	Number in sample
7	17	1
9	39	4
10	71	7
11	75	5
12	61	9
13	58	12
14	57	6
15	57	5
16	45	4
17	44	1

DISCUSSION

Determining gonad maturity stages in fish which spawn in batches is more complicated than for species which spawn just once. This is due to the irregular maturation of oocytes in the ovaries. For example, in stage IV of gonad maturity, some eggs in the ovary are still in the developmental stage characteristic of stage III. These belong to a spawn batch which will be deposited later. After depositing consecutive spawn batches, the ovaries return to the pre-spawning stage unlike other species whose ovaries proceed to stage VIII (spent). The female gonad reaches stage VIII only after releasing the last spawn batch during the spawning season (Bieniarz and Epler 1991). During the investigations most of the round goby females from the Puck Bay had ovaries in maturity stage IV. Possibly, the gonads returned to this stage after releasing subsequent batches of spawn. Only in August and September did a large number of females have ovaries in maturity stage III, and no female with gonads in maturity stage II were observed. This may indicate that stage III is the winter resting stage for the females. After spawning, the gonads go to stage III without passing through stage II. This phenomenon has been observed in other fish species (Bieniarz and Epler 1991). Its possible that the ovaries go through stage II very rapidly, this would explain the absence of gonads in this stage from the analyzed material.

On the basis of these investigations, it seems to be necessary to replace the Maier scale, originally developed for herring, with one which better describes the batch spawning of round gobies. An appropriate six point gonad maturity scale, which takes into account species which spawn in batches, was developed by Sakun and Buckaja (1968).

Round goby spawning in the Puck Bay occurred from April to late August and the beginning of September in 1999. Miller (1986) states that round goby reproduction occurs between April and the end of September in Bulgaria and between May and August in Romania.

The round goby reaches reproductive maturity relatively early; however, information available in the literature concerning this issue varies significantly. The majority of the males and females investigated from the Puck Bay which had reached lengths of 80 - 90 mm were mature. Half of the gobies from the Sea of Azov and the Black Sea reached maturity at the age of 1+ when their average length ranged from 90 to 120 mm (Kostiuchenko 1964, cited in Kalinina 1976) or at the end of the first year of life when they reached a length of 55 to 60 mm (Trifonowow 1955, cited in Moskalkowa 1996). According to Miller (1986), round goby males reach maturity at the age of 3 to 4 years, while females mature at the age of 2 to 3 years.

The number of eggs in ovaries decreased over the course of the year as subsequent spawn batches were deposited in the water. According to Kalinina (1976), one batch of round goby spawn consists of 543 oocytes. The absolute fecundity of female round gobies ranging in length from 7.4 to 16.6 cm (average 12.4 cm) in the Puck Bay was from 89 to 3,841 (average 1,739) spawn grains. On the basis of data available in the literature, Swetowidow (1964) determined the total fecundity of round goby females from the Sea of Azov. It varied from 200 to 2,700 eggs – 1,400 on average (Ilin 1939)*, 300 to 2,300-1,079 on average (Rodionowa 1937)*, 325 to 3,323 (Moskwin 1940)*, 500 to 4,000 (Gudimowicz 1946)* and 360 to 2,742-1,330 on average (Trifonow 1955)*. For females from 12-17 cm in length this figure was from 1,116 to 1,774-1,403 oocytes on average (Winogradow and Tkaczewa, 1948)* and for those

*after Swetowidow (1964)

12 to 19.5 cm in length, it ranged from 1,112-2,724 grains (Winogradow and Tkaczewa 1949)*. Finally, the total fecundity of females 14.5 to 19.9 cm in length was from 1,098 to 6,200 oocytes in Winogradow and Tkaczewa (1950)*. Kuczyński (1995) determined the fecundity of two females from the Puck Bay at 2,700 to 3,000 eggs.

A clear relationship between absolute fecundity and *l. t.* was confirmed for round gobies from the Puck Bay (correlation coefficient $R = 0.888$). Its nonlinear character can be seen in the empirical points in Fig. 2. This dependence is typical for many fish species (Załachowski 1961, Brylińska 1963, 1967, Neja 1988, Wandzel and Neja 1998); however, for round gobies from the Detroit River the relationship was linear in character with correlation coefficient $R = 0.87$ (MacInnis 1997 cited in Corkum *et al.* 1998). Absolute fecundity and body mass also show a strong correlation. This relationship is linear and it is best described by function [4] (correlation coefficient $R = 0.877$). The linear character of this dependence has been described, among others, for perch (Linlokken *et al.* 1991, Wandzel and Neja 1998), yellow perch (*Perca flevescens*) (Jansen 1996), roach (Załachowski 1961), common bream (Brylińska 1963, 1967), ruffe (Neja 1988) and gobies from the Detroit River (MacInnis 1997 cited in Corkum *et al.* 1998).

The value of F_a, as cited by different authors, usually depends on the size of the fish under investigation; this is due to the strong positive correlation between female absolute fecundity and length. It is not easy to compare the absolute fecundity of different sized fish from different populations. Bagenal (1978) concluded that the only satisfactory method for the comparison of such data was to apply an appropriate equation which describes the relationship between fecundity and female length or mass. By applying this kind of equation, it is possible to estimate the hypothetical absolute fecundity of a female of a determined length. Using formula [3], the expected fecundity of a 15 cm round goby female from the Puck Bay was estimated. In this way, further comparisons of the fecundity of round goby females from different basins was made possible.

The analysis of the relative fecundity F_w of species appears to be a good means for describing fish fecundity. Bagenal (1973) states that F_w is also helpful when comparing the fecundity of different fish populations. However, this is possible when a constant number of eggs per female body mass unit, independent of fish size, is accepted. The relatively small amount of material collected on 8 March 1999 (54 specimens) does not confirm the dependence between body length and relative fecundity. Some authors report various changes in values of female F_w. Based on investigations of stream trout, Suworow (1954) confirmed that the relative fecundity of fish decreases as fish length increases. However, Załachowski (1961) did not confirm this relationship.

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