Bulletin

2(162) 2004

of the Sea Fisheries Institute

Contents

Scientific papers

Nłodzimierz Grygiel
Analysis of variation in the fishing efficiency (CPUE) of the TV-3#930 bottom trawl
sing the alternate hauls method
Krzysztof W. Opaliński, Krystyna Maciejewska, Alina Krajewska-Sołtys nd Dariusz P. Fey
roduction and oxygen consumption in the early life stages of herring and smelt
n the Vistula Lagoon (Baltic Sea)
Barbara Piotrowska, Ilona Kołodziejska and Anna Wojtasz-Pająk
actors affecting the deacetylation of chitosans by chitin deacetylase from <i>Mucor rouxii</i>
 Production and oxygen consumption in the early life stages of herring and smelt n the Vistula Lagoon (Baltic Sea)

Short communications

Monika Harnisz and Izabella Zmysłowska	
Enumeration of selected potentially pathogenic bacteria	
in post-cooling waters used in fish fattening	1

Kordian Trella	
Some data on snoek (Thyrsites atun Euphrasen 1791)	
from the Hokitika region (Challenger Plateau, Tasman Sea)	41

Varia

Bohdan Draganik and Mirosław Wyszyński	
The European anchovy (Engraulis encrasicolus [L.]) in the Baltic Sea	53

Analysis of variation in the fishing efficiency (CPUE) of the TV-3#930 bottom trawl using the alternate hauls method

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Abstract. A statistically significant dependence was confirmed between variables – the CPUE of hauls repeated at the same places and times and the CPUE of first (prime) hauls, made with a TV-3#930 cod bottom trawl, using the alternate hauls method. In November 2002 and 2003 the average CPUE (except for sprat) in the southern Baltic Sea was higher in first hauls than in second (repeated) hauls by 39% for cod, 50% – herring, 17% – flounder, 59% – other species and 28% – all species.

Key words: bottom trawl calibration experiments, alternate hauls, CPUE, length distribution, southern Baltic fishes

INTRODUCTION

The calibration of different types of bottom trawls was conducted from several research vessels in the Baltic (Grygiel 1997, Oeberst and Grygiel 2004). The first international experiments were conducted in December 1983 and then in March 1986 within the scope of ICES (Study Group on Young Fish Surveys in the Baltic) (Schulz and Grygiel 1987, Grygiel 1997).

An international research project, EU Study Project No. 98/099 ISDBITS, was launched in January 1999 in response to the necessity of standardizing bottom trawls for research catches. Institutes from the Baltic countries began using the new TV-3 standard bottom trawl in November 1999 (Anon. 1998, Nielsen *et al.* 2001). Work was begun on estimating the data conversion factor (primarily regarding cod CPUE) for the old and new types of trawls (Oeberst *et al.* 2000, Oeberst and Grygiel 2004). Work on the calibration of these trawls continued in 2001-2003 under the auspices of the ICES (Baltic International Fish Survey Working Group [BIFSWG]).

A new stage of this work was undertaken in November 2002 and 2003 with the participation of the r/v BALTICA. This stage was based on the comparative analyses of fish CPUE obtained during type 3 calibration experiments in which a haul with the new gear (TV-3 bottom trawl) is followed by one with the same new gear and the alternate haul method is applied (Wileman *et al.* 1996, Anon. 2002, Oeberst 2003). The results of cali-

bration experiments are essential in order to comprehensively analyze the impact of the conversion coefficient of the CPUE obtained in 1977-2000 by particular research vessels using their own gear on the efficiency of the new TV-3 standard trawl (Oeberst *et al.* 2000, Oeberst and Grygiel 2004). The output from these studies is needed by ICES working groups as a basic component of evaluations of recruitment and the state of fish resources in the Baltic Sea.

The following hypothesis was tested in the current paper; the mechanical and acoustic disturbances of the operating vessel and the TV-3#930 bottom trawl had a negative impact on the CPUE (fish aggregations) of the second (alternate) haul made at the same place and time.

The aim of this work was to conduct a comparative analysis of the CPUE and the length distribution of cod, flounder, herring, and sprat, caught in the first and second sequential hauls from aboard the r/v BALTICA at the same geographical location and time using a TV-3#930 bottom trawl.

MATERIALS AND METHODS

The studies were conducted on 5-9 November 2002 and 12-21 November 2003. The randomly selected locations of the calibration hauls in the southern Baltic Sea are presented in Figure 1. The average catch depth during the first r/v BALTICA cruise was 33 m (from 20 to 65 m), and during the second it was 54 m (from 20 to 90 m).



Fig. 1. Location of bottom hauls (the southern Baltic) conducted by the r/v BALTICA in the framework of the TV-3#930 trawl calibration experiments.

In November 2002 ten pairs of calibration hauls were made with a TV-3#930 bottom trawl with a mesh bar length of 10 mm in the codend. Four pairs of hauls were made during the second cruise. The catches were conducted during daytime at a vessel speed of 3.0-3.2 knots. The standard fishing time was 30 minutes.

Each catch was sorted according to species. The fish were weighed, and the CPUE was calculated for each species in kg/h. The CPUE was accepted as the measure of fish concentration. The share of particular species to the weight of the catch was also determined. The materials were classified into two groups – shallow (20-40 m) and medium-deep waters (50-70 m).

A total of 2,686 cod, 2,746 flounder, 4,281 herring and 4,327 sprat were measured. The total length range was 4-102 cm, 8-44 cm, 8-34 cm, and 7-16 cm, respectively. The length distributions were estimated for the prime hauls (those which were conducted for the first time in a given location) and repeated hauls. The relative numbers of cod, flounder, herring and sprat specimens that were undersized (38, 25, 16, and 10 cm, respectively) were determined for each sample from each haul.

The type 3 calibration experiment (according to the BIFSWG; Anon. 2002, Oeberst 2003) was comprised of repeating catches using the same TV-3#930 bottom trawl at the same geographical location (depth) at short time intervals and then determining the CPUE for both types of hauls. The alternate haul method was applied (Wileman *et al.* 1996). The time it took the vessel to return to the location of the previous trawl deployment, including haul retrieval, was from 45 to 60 minutes. The catches were conducted along the same vessel routes at the same depths. In a few repeated hauls the trawling positions were not absolutely the same as in the first (prime) hauls; the differences reached up to 50-70 m.

Prime haul, one of the terms used in this study, refers to hauls made as the first in a sequence at a selected location, while the term repeated haul refers to hauls made as the second in a sequence (alternate haul) at the same location.

RESULTS

CPUE of cod, flounder, herring and sprat in prime and repeated hauls

Twenty-three fish species were recorded in November 2002. Flounder occurred in all the catches, while cod, herring, and sprat occurred in 96, 92, and 76% of the hauls, respectively. Twenty-nine species were recorded in 2003, although only herring was present in all the hauls. The respective frequency of occurrence of cod, sprat, and flounder was 97, 97 and 86% of the hauls. The species listed dominated in terms of CPUE in almost all of the study locations.

The average CPUE of all fish species in first hauls in the Bornholm and Gdańsk basins was 235 and 906 kg/h, respectively. These values were higher than in the second, alternated hauls by 10 and 33% (Fig. 2). A similar tendency was observed in the different depth layers; the average CPUE of all fish species in the first hauls at depths of 20-40 and 50-70 m was 649 and 482 kg/h, respectively. This was higher than in the second hauls by 27 and 19%, respectively.



Fig. 2. Mean CPUE of fishes in prime and repeated catches at different depth ranges in the Bornholm and Gdańsk basins; relative differences between the CPUE in the two analyzed types of hauls; total refers to commercial species.

6



Fig. 3. Mean CPUE of fishes in prime and repeated hauls conducted in all investigated areas during calibration experiments and relative differences between the CPUE of the two types of hauls analyzed (A) and results of regression analysis (B).

The average CPUE of prime hauls was higher in comparison with that of alternate hauls throughout the study region by 39% for cod, 17% – flounder, 50% – herring, 59% – other, 28% – all species (Fig. 3.A). Exceptionally, the sprat CPUE in first hauls was an average of 63% lower than in the alternate hauls. The average sprat share in all the prime catches was also lower by 3% than the average share of sprat in the repeated catches (Fig. 4.C).

The fraction of cod, sprat and by-catch in the weight of the repeated catches at depths of 20-40 m was similar to that in the first hauls (Fig. 4.A). The fraction of herring was 12% lower in the repeated hauls than in the prime hauls, while the fraction of flounder was 9% higher. The fraction of flounder and by-catch in the second hauls at depths of 50-70 m was



Fig. 4. Relative share (by weight) of particular fish species in prime and repeated catches conducted during calibration experiments at different depth range strata.

only 1% higher. It was 10 and 14% lower for cod and herring, respectively, than in the first hauls (Fig. 4.B).

The results of regression analysis (with the best fitted linear model) of the CPUE of cod, flounder, herring, sprat, and all species in repeated hauls versus the CPUE in prime hauls, indicate that there are statistically significant dependencies (r = 0.929, p < 0.00001; Fig. 3B) between variables. The statistical model applied explains 86% of the variance of the dependent variable. The CPUE in prime hauls was almost always higher than in repeated hauls.

Length distribution of cod, flounder, herring and sprat

The shapes of the length distribution curves for each of the analyzed species was similar in prime and repeated hauls (Fig. 5). Slight shifts of the frequency peaks towards the longer length classes were noted in samples from repeated hauls, especially in the group of specimens that was undersized (e.g., for clupeoids by 0.5 cm; for cod by 2 cm). Two groups of dominant individuals were observed in the length distributions of cod, herring, and sprat. The length distributions of flounder in both prime and repeated hauls were almost ideally single-peaked.

As was the case with the length distribution curves of the four analyzed fish species, the average length and weight, modal length and the percentage undersized fish were similar in samples from prime and repeated hauls (Table 1).

DISCUSSION

The results of type 3 calibration experiments confirmed that the mechanical and acoustic disturbance of the operating vessel and the TV-3#930 bottom trawl had a negative impact on the CPUE of the hauls repeated at the same place and time. The expected catch in the first haul depended on fish density and the gear catchability, while that in the second depended on the remaining fish density and the catchability of the fishing gear used as well as disturbance effect. These studies were coordinated by BIFSWG, and their results constituted a basic element of the evaluations of recruitment and the Baltic fish (principally cod) stock sizes.

		Ν	Iean	Modal	Percentage of under-	
		weight [g]	length [cm]	length [cm]	sized specimens	
	prime hauls	491.5	31.1	22.0	80.3	
Cod	repeated hauls	415.6	30.7	25.0	80.2	
	prime hauls	201.8	22.0	19.0	85.0	
Flounder	repeated hauls	204.9	21.4	19.0	88.4	
	prime hauls	36.0	17.0	11.5	44.5	
Herring	repeated hauls	39.4	17.2	12.0	41.8	
	prime hauls	8.2	10.6	9.0	52.6	
Sprat	repeated hauls	7.7	10.5	8.5	53.0	

Table 1. The mean length and weight, modal length and the percentage (by numbers) of undersized specimens of cod, flounder, herring, and sprat in prime and repeated hauls



Fig. 5. Length distribution of cod, flounder, herring, and sprat in prime and repeated hauls conducted in all investigated areas during the TV-3 bottom trawl calibration experiments; data for single specimens of cod from length classes 81, 82, 86, and 102 cm and of herring from length class 34 cm are omitted.

No references to the research presented in this paper were found in the available literature; however, preliminary evaluations of CPUE obtained using the TV-3 trawl in two types of hauls were presented at the last meeting of the previously mentioned working group in March 2003 (Oeberst 2003). The disturbance factor (β) can be estimated as the quotient of catch in the second haul and catch in the first haul. Oeberst (2003) drew attention to the following facts: the fraction of CPUEs below 10 kg/h was highly variable, which could have caused significant variation in the average value. Additionally, the disturbance to fish aggregations caused by different vessels was quite similar, although statistically insignificant. The effect of fish aggregation disturbances caused by TV-3#930 and TV-3#520 trawls was also similar and did not concur with the data obtained by Oeberst and Grygiel (2004).

The results of the authors' studies indicate disturbance from the vessel and trawl decreased the CPUE of all analyzed fish species, except sprat, by an average of 17 to 59% (Fig. 2 and 3A). The sprat CPUE in first hauls was, on average, 63% lower than in second, repeated hauls. The average weight share of this species was lower in first hauls in comparison with that in second hauls (Fig. 4). The ranges of the maximum and the minimum CPUE in both types of hauls was significant; *e.g.*, for cod in the first hauls the range was from 0 to 316 kg/h (SE = 23.27); herring – from 0 to 1921 kg/h (SE = 131.20); sprat – from 0 to 605 kg/h (SE = 43.39); flounder – from 0 to 780 kg/h (SE = 57.31).

The results of regression analysis indicate a statistically significant linear dependence between the CPUE of fish in repeated and prime hauls (Fig. 3B).

The possible cause of the differences in sprat CPUE in prime and repeated hauls might be related to the reaction of this species to the stress of the mechanical and acoustic disturbance caused by the vessel and trawl. Sprat, a pelagic school species, is characterized by greater mobility than cod, flounder, and mature herring, all of which feed near the bottom. Additionally, the natural defense mechanism of small school fish species is to reaggregate immediately after the disturbance factor clears (Zussier 1971). Disturbances generally did not impact the length and weight structure of the analyzed fish species in the second hauls (Fig. 5, Table 1). The length distributions, the average length and weight, modal length and the percentage of undersized fish were similar in both types of hauls studied.

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Abstract. The production and oxygen consumption in larval and juvenile Baltic herring (*Clupea harengus membras*) and European smelt (*Osmerus eperlanus*) collected in the Vistula Lagoon were measured in order to compare the energetic costs of growth for the two species. Production in herring and smelt are practically the same at 0.1200 mg Dw \cdot d⁻¹ for herring and 0.1145 mg Dw \cdot d⁻¹ for smelt. However, the specific metabolism of herring is approximately twofold higher in comparison with smelt (1.36 mm³ O₂ \cdot Ww^{0.79} \cdot h⁻¹ and 0.81 mm³ O₂ \cdot Ww^{0.74} \cdot h⁻¹, respectively). These results lead to the conclusion that the production of body mass in smelt is more effective than it is in herring.

Key words: herring, smelt, larvae, production, respiration, Vistula Lagoon

INTRODUCTION

The Vistula Lagoon is one of the main spawning grounds of Baltic herring (*Clupea harengus membras* L.) in the southern Baltic Sea (Krasovskaya 2002). During early spring, adults migrate from the southern Baltic Sea to the spawning area in the shallow, brackish waters of the lagoon, which is characterized in this period by high temperatures and high zooplankton abundance (Naumenko 1998). Herring larvae and juveniles spend up to four months in the lagoon. They migrate to the open waters of the Baltic Sea in June (Krasovskaya 1998). The other important fish species spawning in the Vistula Lagoon is smelt (*Osmerus eperlanus* L.), which is one of the constant elements of the fish assemblages in the Vistula Lagoon (Psuty-Lipska and Borowski 2003). Smelt spends its entire life in the brackish waters, but ascends small rivers to spawn (Hudd and Urho 1985). After hatching in rivers, smelt larvae are transported to the Vistula Lagoon and usually reach it several days after the herring larvae hatch (Margoński 2000).

The early developmental stages of smelt and herring are the most important elements of the Vistula Lagoon plankton community from April to June. The highest herring larvae

density recorded in the Vistula Lagoon was 495 ind. 100 m⁻³, while the highest abundance of smelt larvae reached 1 174 ind. 100 m⁻³ (Margoński 2000). It is typical for smelt to have only one cohort (Margoński 2000), while herring have three to four cohorts (Fey 2000). The food spectrum of early juvenile stages of herring and smelt is nearly identical (Tomasz Linkowski, Sea Fisheries Institute in Gdynia, unpublished data). These species probably compete for food. The growth rate of the fish can be a measure of the effect of this competition.

Begon (1984) suggests that size (or in this context, growth rate) can be understood as a measure of fitness. Translated into the herring-smelt system, this hypothesis is as follows: the competitor that has a higher growth rate (better ability to catch bigger prey and avoid predators – Hunter 1972) and lower maintenance costs (cost of attack and suction of prey – Drost *et al.* 1988) wins the competition for food in the Vistula Lagoon pelagic system.

The aim of this work was to verify which of these species living in the same environment, herring or smelt, wins the competition for food from an energetic point of view.

MATERIALS AND METHODS

Fish

The early life stages of herring and smelt were caught from a fishing boat at one station in the Vistula Lagoon (southern Baltic Sea) near Tolkmicko harbor on April 21 and May 13, 2004, (herring) and on May 18 and June 1, 2001 (smelt). The catches were carried out in the 1.5 m surface layer using a 5 m-long, neuston net with 500 μ m mesh and a 2 m² opening. Hauls varied from one minute for herring to five minutes for smelt. The fish were transferred to thermoses with aerated water and transported either to the laboratory in Gdynia (smelt) or to a field laboratory in the harbor of Tolkmicko (herring).

Production

Body mass production in early developmental stages of herring and smelt was determined as the average increase in standard fish length and wet and dry weights measured for individuals from April 21 to May 12, 2004, for herring, and from May 18 to June 1, 2001, for smelt. The mean water temperature was 11°C in 2004 and 15°C in 2001.

The fish were preserved in formaldehyde. Measurements of the standard body length to the nearest 0.01 mm and wet weight to the nearest 0.5 mg were taken immediately. The fish were then dried at 60° C to a constant weight and then the dry weight was measured to the nearest 0.01 mg.

Fish production was determined in mg dry weight per day (mg $Dw \cdot d^{-1}$) for 120 herring specimens (60 measurements in April and 60 in May) and for 60 smelt specimen (30 in May and 30 in June). Since the weight range in both species was nearly identical (see Table 1), production rate calculations (production per unit of weight) were not necessary.

			Time		Fish growth			Fish production	
Date	$T^{\circ}C$	2	interval,	IS	Ww [mg]	Dw [mg]	. .		
		-	mean	[mm]	mean range	mean range	$[mm \cdot ind^{-1} \cdot d^{-1}]$	$[mg Ww \cdot ind^{-1} \cdot d^{-1}]$	$[mg Dw \cdot ind^{-1} \cdot d^{-1}]$
			temp.	ſ'nmī	median	median			
					Herr	ing			
1011-004				00017101	1.23 ± 0.57	0.11 ± 0.29			
April 21, 2004	6	60		10.14±0.29	0.37-3.24	0.02-0.17			
2004			22 days		1.13	0.11		007 1	00010
Mor. 12			11°C		33.98 ± 2.14	2.75 ± 0.19	700.0	1.489	0.1200
, CI VIA)	12	60		24.49 ± 0.65	12.9-48.8	1.13-4.45			
2004					31.5	2.71			
					Sm	elt			
Mc. 10					2.59 ± 0.27	000.000			
May 10,	17	30		11.30 ± 0.48	0.90-4.00	0.20±0.04			
1007			14 days		2.70	0.00-0.40	101 0		
11			15°C		27.04 ± 1.75	2.70 ± 0.17	0.481	1./40	0.1729
Julie 1,	14	30		21.89 ± 0.58	16.0-49.0	1.61-4.90			
1002					30.0	2.70			
				Smelt, a	fter adjustment to	the temperature (of 11°C		
							0.318	1.156	0.1145

Table 1. Growth and production in Baltic herring and European smelt in the Vistula Lagoon T – temperature, n – number of measurements, SL – standard length, Ww – wet weight, Dw – dry weight. Mean values \pm Standard Error

15

Since production was measured under natural conditions at 11°C for herring and 15°C for smelt, the production of smelt was recalculated to a temperature of 11°C using the temperature coefficient $Q_{10} = 2.8$ according to Maciejewska *et al.* (2001). The following formula was used for this recalculation:

$$P_{t1} = P_{t2} / Q_{10} (t^2 - t^1)/10$$

where:

 P_{t1} – production at a temperature of 11°C,

 P_{t2} – production at a temperature of 15°C

(Duncan and Klekowski 1975, Maciejewska et al. 2001).

Oxygen consumption

Oxygen consumption of larval and juvenile herring and smelt was determined with the closed vessel method at the temperature of their natural environment. Respirometric vessels with a volume of 50 and 100 cm³ were used. Exposition time was 2 to 3 h. The oxygen concentration was measured using an OXI 3000 oxygen sensor by WTW. Unfiltered seawater with natural plankton as a food for fishes and four respirometers without fishes as a control were used (Maciejewska *et al.* 2001, Maciejewska and Opaliński 2002, 2004). The consumption of zooplankton by fishes in experimental respirometers can lower the results of fish oxygen consumption by approximately 4% (Krzysztof W. Opaliński, Center for Ecological Research PAS, Dziekanów, unpublished data).

Ninety oxygen consumption measurements were taken for the herring and thirty for smelt.

Fish oxygen consumption was expressed as mm^3 of oxygen per hour per individual (respiration $-mm^3 \cdot ind^{-1} \cdot d^{-1}$) or per mg of wet or dry weight (metabolic rate -mg Ww $\cdot ind^{-1} \cdot d^{-1}$). Since the fish can swim and eat in the respirometric vessels, the measured metabolism can be defined as the routine as in Fry (1947, 1957).

The herring and smelts used in the respiration measurements were caught on May 13, 2004, and June 25, 1999, respectively. The measurements were taken at the ambient temperature of 12°C for herring and 15°C for smelt. The oxygen consumption of the smelt was recalculated to a temperature of 12°C using the temperature coefficient $Q_{10} = 2.8$ according to Maciejewska *et al.* (2001).

Following the oxygen consumption measurements, the fish were preserved in formaldehyde and measurements of their standard body length to the nearest 0.01mm and wet weight to the nearest 0.5 mg were taken. The fish were then dried at 60°C to a constant weight (dry weight to the nearest 0.01 mg).

RESULTS

Production

The production of herring was determined directly as the average increase of body weight. This approach was possible because only one cohort was identified in this period in the



Fig. 1. Size distribution of larval and juvenile herring in the Vistula Lagoon on April 21, 2004, (n = 60, mean wet weight = 1.23 mg) and May 13, 2004, (n = 60, mean wet weight = 33.98 mg).

herring size frequency distribution (Fig. 1). The average wet weight of herring on April 21 was 1.23 mg. After 22 days, on May 13, it had increased to 33.98 mg (Table 1). Calculations done with the data in Table 1 revealed that the herring production rate under natural conditions (*i.e.*, at a temperature of 11°C) during the investigated period was 0.652 mm \cdot d⁻¹, 1.489 mg Ww \cdot d⁻¹, and 0.1200 mg Dw \cdot d⁻¹.

Since only one cohort was also determined in the smelt size frequency distribution (Fig. 2), the production of this species was determined using the same method. The average wet weight of smelt on May 18 was 2.59 mg. After 14 days, on June 1, it had increased to 27.04 mg (Table 1). Calculations done with the data in Table 1 re-



Fig. 2. Size distribution of larval and juvenile smelt in the Vistula Lagoon on May 18, 2001, (n = 30, mean wet weight = 2.59 mg) and June 1, 2001 (n = 30, mean wet weight = 27.04 mg).

vealed that the smelt production rate under natural conditions (*i.e.*, at a temperature of 15°C) during the investigated period was 0.481 mm \cdot d⁻¹, 1.746 mg Ww \cdot d⁻¹, and 1.1729 mg Dw \cdot d⁻¹.

The production rates presented in Table 1 are higher for smelt than herring by 17% in wet weight production and 44% in dry weight production. However, after corrections to compensate for the higher temperature (15°C) at which smelt grew, the results change dramatically. At the same temperature (11°C), the body mass production of smelt (0.1145 mg Dw) and herring (0.1200 mg Dw) are the same (Table 1). The difference of 2-3% should be considered insignificant. Only body length production in herring is approximately 50% higher.

Oxygen consumption

Herring oxygen consumption was 24.06 mm³ ind⁻¹ \cdot h⁻¹, and for smelt it was 25.59 mm³ \cdot ind⁻¹ \cdot d⁻¹ (Table 2). However, the wet weight of the smelt used for the measurements was about twice as high as that of the herring. In order to compare the respiration of the two species with different body weights, their specific metabolism (as in Brody 1945, see also Duncan and Klekowski 1975) was used. Specific metabolism is defined as:

where:

$$a = R/W^{\circ}$$

a – specific metabolism (or intercept of oxygen consumption versus wet weight regression), R – oxygen consumption,

 W^b – the metabolically effective body size (as in Brody 1945) or metabolic body size (as in Kleiber 1961), where b – the regression coefficient of the dependence between oxygen consumption and body wet weight.

The dependencies between herring and smelt body wet weight and their oxygen consumption were calculated; for herring it was $R = 1.36 \text{ Ww}^{0.79}$, and for smelt it was $R = 1.10 \text{ Ww}^{0.74}$ (Figs 3 and 4). Thus, the specific metabolism of herring



Fig. 3. Dependence between oxygen consumption (R) and body wet weight (Ww) in early developmental stages of herring from the Vistula Lagoon on May 13, 2004.



Fig. 4. Dependence between oxygen consumption (R) and body wet weight (Ww) in early developmental stages of smelt from the Vistula Lagoon on June 25, 1999.

 $(1.36~mm^3O_2\cdot Ww^{-0.79}\cdot h^{-1})$ is higher in comparison to that of smelt $(1.10~mm^3\,O_2\cdot Ww^{-0.74}\cdot h^{-1}).$

Smelt oxygen consumption was measured at a temperature 3°C higher than that at which herring consumption was measured. After adjustments for temperature were made (using the Q_{10} temperature quotient according to Maciejewska *et al.* 2001), the results change in the same direction. At the same temperature (12°C) the specific metabolism of smelt was 0.81 mm³ O₂ · Ww^{-0.74} · h⁻¹ and for herring it was 1.36 mm³ O₂ · Ww^{-0.79} · h⁻¹ (Table 2).

Table 2. Oxygen consumption, metabolic rate, and specific metabolism in the Baltic herring and European smelt in the Vistula Lagoon

n – number of measurements, T – temperature,	ww - fish wet weight, Dw - dry weight
R - oxygen consumption, MR - metabolic rate.	. Mean values \pm Standard Error

п	Т	Ww	Dw	R	MR/Ww	MR/Dw	Specific
	[°C]	[mg]	[mg]		$[mm^3 \cdot ind^{-1} \cdot d]$	-1]	metabolism
				Herring – May	2004		
90	12	37.23 <u>+</u> 0.80	3.91 <u>+</u> 0.10	24.06 <u>+</u> 0.50	0.652 <u>+</u> 0.012	6.303 <u>+</u> 0.153	1.36
				Smelt - June	1999		
30	15	74.48 <u>+</u> 3.80	6.63 <u>+</u> 0.38	25.59 <u>+</u> 1.10	0.352 <u>+</u> 0.009	3.992 <u>+</u> 0.213	1.10
Smelt, after adjustment to the temperature of 12°C							

DISCUSSION

Survival strategy in the early life stages of fish is based on an intense body growth rate. The primary aspect of the relation between fish growth rate and survival is the predator-prey size dependence; larger individuals are more successful in avoiding predator attacks and are simultaneously able to catch larger prey items (Blaxter 1969, Hartman 1986).

The growth of herring in the Vistula Lagoon in comparison with that of other areas (Table 3) is very high (0.50 to 0.57 mm). This means that the herring in the lagoon have good feeding conditions in comparison to other areas. Data in the literature concerning the growth of smelt provide similar results indicating that the growth of smelt from the Vistula Lagoon is fast (Table 3). These data suggest that in the early summer there is enough food in the Vistula Lagoon for herring and smelt and that they probably do not compete for food.

Growth	Author	Remarks
[mm]		
	Herring	
0.14 to 0.26	Munk et al. 1986	Buchan area, North Sea
0.23 to 0.64	Peltonen 1988	Baltic Sea
0.37 to 0.41	Moksness, Fossum 1992	North Sea (Norwegian herring)
0.50 to 0.57	Margoński 2000	April-May, Vistula Lagoon
0.65	Present paper	April-May, Vistula Lagoon
	Smelt	
0.098 - 0.101	Margoński (2000)	Vistula Lagoon, autumn
0.157 - 0.679	Sepulveda (1993)	Elbe River
0.243-0.432	Margoński (2000)	Vistula Lagoon, mid-June
0.481	Present paper	Vistula Lagoon, May

Table 3. Growth in early developmental stages of Baltic herring and European smelt

The production rates in both species are the same. However, what are the energetic costs of this production. Are they the same in both species? In early developmental stages of fishes the growth rate reflects their oxygen consumption rate, thus the oxygen consumption level can be used as an indicator of the energetic costs of growth (Kamler *et al.* 1987). The answer to the question above is that oxygen consumption, or the energetic cost of growth, is higher in herring and lower in smelt.

The general question regarding food competition between early developmental stages of herring and smelt in the Vistula Lagoon can be answered as follows: based on energetic criteria, smelt use less energy to produce the same effect (production) than herring do. The production of one milligram wet weight of body "costs" smelt 177 mm³ oxygen or 0.37 J, while it "costs" herring 348 mm³ oxygen or 0.81 J. From a purely energetic point of view, smelt is the winner.

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Factors affecting the deacetylation of chitosans by chitin deacetylase from *Mucor rouxii*

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Abstract. A two-stage, chemical and enzymatic process can be used to prepare chitosans with desired degrees of deacetylation. However, it is not possible to obtain products with a higher degree of deacetylation than 84% from chitosan with an initial degree of deacetylation of 68% when a 1.2% solution is used as a substrate for the pre-purified deacetylase from *Mucor rouxii* mycelium. The objective of this study was to investigate the reasons behind this phenomenon. The incomplete deacetylation of chitosan under the applied conditions of enzymatic reactions was not the result of the thermal denaturation of deacetylase during the relatively long enzymatic reaction. The amount of acetic acid released and the degree of deacetylation did not increase as a result of adding a fresh portion of the enzyme to the reaction mixture after 10, 16, and 24 h of the reaction. It was shown that enzymatic deacetylation could be partially inhibited by acetic acid released during deacetylation. The main product of deacetylation - chitosans deacetylated to about 82-84%, also decreases the rate of deacetylation. Only 35% of the total possible acetic acid released from chitosan with a degree of deacetylation of 82% was detected after 24 h of enzymatic deacetylation. The total inhibitory effect of both products of deacetylation on chitin deacetylase activity was investigated by conducting enzymatic deacetylation of chitosan with a degree of deacetylation of 82% in the presence of added acetic acid at a concentration of 12 µmoles/ml of the reaction mixture. After 24 h, the amount of acetic acid formed was so low that the degree of deacetylation of the product was almost unchanged.

Key words: chitin, chitosan, chitin deacetylase, deacetylation

INTRODUCTION

Crystalline chitin is resistant to enzymatic deacetylation (Davis and Bartnicki-Garcia 1984, Tsigos *et al.* 1996, Kołodziejska *et al.* 1997). This property limits the possibility of preparing chitosans directly from chitin using a mild, enzymatic procedure. In order to overcome this drawback in the preparation of chitosans, an alternative two-stage, chemical and enzymatic process was proposed (Kołodziejska *et al.* 2000). The first chemical stage in a hot NaOH solution ensures only the dissolution of chitosan in diluted acids or acidic buffers without

causing significant changes in the molecular mass of the polymer. Chitin that is partially deacetylated with a chemical treatment at 60°C after 90 min reaches a degree of deacetylation (DD) of about 68% and can be further modified by chitin deacetylase. Using the prepurified deacetylase or the crude extract containing deacetylase and chitosanolytic enzymes from Mucor rouxii mycelium and depending on temperature, pH, buffer, and inhibitors, it is possible to obtain chitosans of desired molecular weights (MW) and degrees of deacetylation (Kołodziejska et al. 2000, Rzodkiewicz et al. 2001). The properties of chitosans depend on the DD and MW of the polymer. Some chitosan applications require almost totally deacetylated products. Such chitosans show higher antibacterial and antifungal activity and are more efficient in recovering proteins and nutrients from food industry wastes than less deacetylated chitin derivatives (Johnson and Peniston 1982, Simpson et al. 1997). However, it was not possible to obtain products with a DD above 83-86% using chitosan with a DD of 68% as a substrate in a 1.2% solution (Rzodkiewicz et al. 2001). On the other hand, according to Martinou et al. (1995), almost all N-acetylglucosamine residues in chitosans with a DD of 58 and 72% in 0.5% solutions were deacetylated by the enzyme isolated from the same source.

The aim of this study was to explain why it is impossible to achieve complete enzymatic deacetylation of chitosan with a DD of 68%, when the substrate is used in a concentration of 1.2%.

MATERIALS AND METHODS

The culture conditions of *M. rouxii* ATCC 24905 and the preparation of the crude enzyme extract from mycelium were described previously by Kołodziejska *et al.* (2000). For prepurification, the extract was acidified to pH 4.0 using a 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 extracts was determined according to Lowry *et al.* (1951).

Chitosan with a DD of 73% (chitosan⁷³), used as the substrate for determining the deacetylase activity, chitosan with a DD of 84% (chitosan⁸⁴), and chitosan with a DD of 68% (chitosan⁶⁸) were obtained from krill chitin according to Kołodziejska *et al.* (2000). Chitosan with a DD of 82% (chitosan⁸²) was prepared by enzymatic deacetylation of chitosan⁶⁸ according to a procedure described by Rzodkiewicz *et al.* (2001).

The activity of chitin deacetylase was assayed by determining the acetic acid released from the substrate (Bergmeyer and Möllering 1974). The reaction mixture, containing 2.5 mg of chitosan⁷³ and 0.1 mg of the mycelium extract protein in 1 ml of 0.1 M glutamate-HCl solution, 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 the extract were incubated separately, and combined when the reaction was stopped. The samples were cooled to room temperature. The pH of each sample was adjusted to 8.0-9.0 using NaOH solution. Subsequently, the samples were centrifuged for 20 min at 15,000 × g, and the amount of acetic acid was determined. 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 deacetylation of chitosan⁶⁸ catalyzed by the pre-purified deacetylase extract was carried out in 0.1 M glutamate-HCl solution at pH 4.0 and 50 °C. The acetic acid released was determined as described above according to Bergmeyer and Möllering (1974). In the standard deacetylation procedure the pre-purified deacetylase at a concentration of 50 mU/ml of the reaction mixture was used. In another experiment, the reaction was started at a concentration of 50 mU of deacetylase/ml and after 10, 16, and 24 h a fresh portion of the enzyme, 50 mU/ml, was added. In some experiments, chitosan⁸⁴ obtained with the chemical process and chitosan⁸² prepared by the enzymatic deacetylation of chitosan⁶⁸ were used as substrates.

Deacetylation was also conducted in the presence of acetic acid added to the reaction mixture with chitosan⁶⁸, chitosan⁸⁴, and chitosan⁸² used as substrates.

Detailed conditions of all the experiments are presented in the table and figures illustrating the results. All of the experiments were carried out in triplicate and, unless otherwise stated, the standard deviation did not exceeded 5% of the mean values.

RESULTS AND DISCUSSION

A 95% DD of chitosan⁶⁸ was achieved after 24 h of enzymatic reaction at pH 4.0 and 50°C with the partially purified chitin deacetylase from *M. rouxii*, when the concentration of this substrate in the reaction mixture was 0.25% (Fig. 1). Almost complete deacetylation was also obtained after several hours of treating chitosans with 58 and 72% DD in 0.5% solutions by highly purified chitin deacetylase (Martinou *et al.* 1995). Nevertheless, in our previous experiments with a more concentrated chitosan⁶⁸ solution, 1.2%, the highest DD achieved after 24 of enzymatic reaction at pH 4.0 and 50°C was only about 84% (Rzodkiewicz *et al.* 2001).



Fig. 1. Effect of incubation time on the release of acetic acid from chitosan (a) and on chitosan DD (b) in concentrations 1.2% (▲) and 0.25% (●) by the enzyme extract. The reaction mixture, containing chitosan⁶⁸ and 50 mU of deacetylase in 1 ml of 0.1 M glutamate-HCl solution, was incubated for the appropriate time at pH 4.0 at 50°C.

According to Martinou *et al.* (1995), highly purified chitin deacetylase from *M. rouxii* was thermostable, and after 5 h of preincubation at 50°C it lost only 30% of its initial activity. One of the reasons for the incomplete deacetylation of chitosan⁶⁸ in a 1.2% solution could be the loss of the activity of partially purified deacetylase during the relatively long enzymatic reaction period. As shown in Figure 1, the amount of acetic acid released was directly proportional to the reaction time up to 8 h. Nevertheless, when the reaction time was prolonged the deacetylation rate decreased. This could be explained by the gradual loss of enzyme activity. Therefore, the enzymatic deacetylation of chitosan⁶⁸ in a 1.2% solution was carried out in two steps. Initially the reaction proceeded in the presence of 50 mU of deacetylase/ml of the reaction mixture. Subsequently, a fresh portion of the enzyme, 50 mU/ml, was added after 10, 16, and 24 h of reaction time (Fig. 2). However, the



Fig. 2. Effect of two-stage enzymatic deacetylation on the release of acetic acid from chitosan by the enzyme extract. (■)50 mU of deacetylase in 1 ml (■) 50 mU of deacetylase in 1 ml and 50 mU introduced to the mixture after 10 (a), 16 (b) and 24 h (c).
The reaction mixture, containing 12 mg of chitosan⁶⁸ in 1 ml of 0.1 M glutamate-HCl solution, was incubated for the appropriate time at pH 4.0 at 50°C.



Fig. 3. Effect of added acetic acid on the release of acetic acid from chitosan by the enzyme extract. The reaction mixture, containing 12 mg of chitosan⁶⁸ and 50 mU of deacetylase in 1 ml of 0.1 M glutamate-HCl solution, was incubated for the appropriate time at pH 4.0 at 50°C.
(◆) Control, (■) 2.5, (▲) 5, (□) 7.5, and (○) 10 µmoles of added acetic acid/ml of reaction mixture.

amount of acetic acid released was not higher than that obtained in the reaction carried out in one step at an enzyme concentration of 50 mU/ml. These results suggest that in the presence of the substrate the enzyme is not inactivated during prolonged incubation at 50°C and that incomplete deacetylation of chitosan⁶⁸ is not a result of the thermal denaturation of deacetylase.

Chitin deacetylase is totally inhibited by acetic acid at 200 μ moles/ml after 10 min of incubation at 30°C (Araki and Ito 1975). The amount of acetic acid released from the substrate during 6 to 54 h of incubation of the reaction mixture containing acetic acid added in a concentration of 5 μ moles/ml was about 10% lower than that in the control sample (Fig. 3). In the presence of acetic acid added in concentrations of 7.5 and 10 μ moles/ml of reaction mixtures, the amount of acetic acid released was 20% and 30% lower, respectively, than in the control sample (Fig. 3). After 6 h of deacetylation with added acetic acid was equal to the maximum amount of the acid formed during deacetylation without added acid. The results of this experiment suggest that acetic acid alone is not responsible for the incomplete enzymatic deacetylation of chitosan⁶⁸ in a 1.2% solution.

Following this, the inhibitory effect of the main product of the reaction, chitosan⁸⁴, on the activity of deacetylase was evaluated. According to Araki and Ito (1975), glycol chitosan, formed during the deacetylation of glycol chitin, exerted such an effect on the activity of deacetylase. Kołodziejska *et al.* (1997) also demonstrated that chitin deacetylase activity strongly depends on the DD of the chitosans used as substrates. That is why chemically produced chitosan, with a DD of 84% (similar to the DD of the product obtained from chitosan⁶⁸) was used as a substrate for enzymatic deacetylation. At a substrate concentration of 1.2%, chitosan was still deacetylated by the enzyme, and the amounts of

Concentration of chitosan [%]		µmoles of ac	etic acid /ml of reacti released	on mixture ^b	Degree of deacetylation [%]		
			0.5 h	24 h	0.5 h	24 h	
	0.25	0	0.4 ± 0.0	1.7 ± 0.0	86.7 ± 0.0	96.0 <u>+</u> 0.0	
Chitosan ⁸⁴	1.20	0	0.9 ± 0.0	4.3 ± 0.4	85.0 ± 0.0	90.2 ± 0.4	
	1.20	12	0.3 ± 0.3	1.6 ± 0.2	84.1 ± 0.3	86.0 ± 0.0	
C1::	1.20	0	0.4 ± 0.1	4.2 ± 0.2	82.3 ± 0.0	87.5 ± 0.2	
Cintosan	1.20	12	0.3 + 0.2	1.2 + 0.1	82.2 + 0.2	83.5 + 0.4	

Table 1. The released acetic acid and the degree of deacetylation of $chitosan^{84}$ and $chitosan^{82}$ deacetylated by the enzyme extract in the presence of added acetic $acid^a$

^{*a*} Mean value \pm the standard deviation from three separate samples

^bThe reaction mixture, containing chitosan and 50 mU of deacetylase in 1 ml of 0.1 M glutamate-HCl solution, was incubated for the appropriate time at pH 4.0 at 50°C. Chitosan⁸⁴ and chitosan⁸² were obtained in chemical and enzymatical processes, respectively.

acetic acid increased with the reaction time but the calculated DD reached only about 90%. Almost complete deacetylation was achieved when a 0.25% solution of chitosan⁸⁴ was used (Table 1). Since chitosans obtained by chemical treatment and enzymatic process can differ in the distribution of acetyl groups and susceptibility to further enzymatic deacetylation, a similar experiment was carried out with chitosan⁸² prepared with the enzymatic process. The amount of acetic acid released from this substrate after 24 h was on the same level as that of chitosans obtained chemically (Table 1). Furthermore, only 35-40% of the total acetic acid was released from the chitosans after 24 h of enzymatic deacetylation of the above substrates used at a concentration of 1.2% (Table 1). This implies that chitosans deacetylated enzymatically partially inhibit the activity of chitin deacetylase.

Chitosan⁸⁴ and chitosan⁸², i.e. polymers with a DD that corresponds to that of enzyme-catalyzed deacetylation products, were further deacetylated in the presence of acetic acid added at a concentration of 12 μ moles/ml to check the total effect of both products of deacetylation on chitin deacetylase activity. Such concentration of acid was the maximal amount of acetic acid released during deacetylation of chitosan⁶⁸. After 24 h, the amount of acetic acid formed was only 1.2-1.6 μ moles/ml (Table 1), and the calculated DD was almost unchanged. These results confirm that the enzymatic deacetylation of chitosan⁶⁸ in a 1.2% solution is inhibited by both products of deacetylation: chitosan with increasing DD and acetic acid.

In summary, it can be concluded that in order to obtain totally deacetylated chitosans with chitin deacetylase from partially purified extracts from *Mucor rouxii* diluted solutions of substrates should be used.

Acknowledgements

Financial support for this research was provided by the Faculty of Chemistry, Gdańsk University of Technology.

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Enumeration of selected potentially pathogenic bacteria in post-cooling waters used in fish fattening

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Abstract. The results of this study indicate that fish fattening did not influence the quantitative content of the *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Staphylococcus aureus* bacteria in cooling waters. However, higher average numbers of bacteria from the *Enterobacteriaceae* family were confirmed in the water flowing out of the fish farm in comparison with inflow water. It was noted that the numbers of the bacterial microflora in the cooling waters is probably impacted by the qualitative and quantitative composition of the bacterial microflora released from the digestive tracts of fish.

Key words: Aeromonas hydrophila, Pseudomonas fluorescens, Staphylococcus aureus, Enterobacteriaceae, fish farming, cooling waters

INTRODUCTION

The bacterial microflora of the digestive tracts of freshwater fish is dominated by members of the genera *Aeromonas*, *Plesiomonas*, *Pseudomonas*, representatives of the *Enterobacteriaceae* family and the obligatory anaerobes *Bacteroides*, *Fusobacterium*, *Eubacterium* (Esteve and Garay 1991, Feldhusen 2000, Zmysłowska *et al.* 2000a). These bacteria are excreted into the water. Since the bacterial concentration in l gram of fish feces varies from 10^5 to 10^{10} of bacteria (Sugita *et al.* 1985), they can influence the quantitative and qualitative parameters of the bacterial microflora of the water.

Bacteria of the genera *Pseudomonas*, *Aeromonas*, *Staphylococcus*, and from the *Enterobacteriaceae* family that occur in water used in fish farming can cause various diseases, the intensity of which depends largely on the virulence of the microbes and their pathogenic potential, as well as their numbers (Gołaś *et al.* 2002). If they come in contact with skin, mucus membranes, respiratory tracts and eyes, these bacteria are potential sources of disease in both humans and animals. Most fish farm wastewater is disposed of in running waters, which, in turn, facilitates the transfer of microbes to other surface waters, such as lakes. Thus it is essential to conduct microbiological studies of waters that are used in fisheries and to treat these waters prior to their release into reservoirs.



The aim of this work was to determine the impact of intensive fish fattening in cooling waters on the numbers of bacteria of the genera *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, and the family *Enterobacteriaceae*.

MATERIALS AND METHODS

The studies were conducted at the Fish Cultivation Center in Ostrołęka, where the fish, mainly *Cyprinus carpio* L. and *Silurus glanis* L., were cultivated in cages. The complex of 48 cages was located in the cooling water discharge canal of the Ostrołęka S.A. Power Plant. The average flow rate of the cooling waters in the discharge canal was 1.2 m³ s⁻¹. The fish farm wastewater was discharged to the Narew River.

During the study period, carp and European wels were cultivated intensively in cages. The fish were fed exclusively with Aller - Aqua - Safir, Kraft - Futter FM44/20 and Aller - Aqua granulated feed.

Water samples for bacteriological analyses were collected in 1999-2000 from spring to fall at monthly intervals, except in July and August 2000 when they were collected at approximately fortnightly intervals. The samples were collected at the four following stations:

- station l at the cooling water inflow;
- station 2 in the cage;
- station 3 beyond the 48-cage complex;
- station 4 in the Narew River at the wastewater outflow.

The tests performed on the water included determining the numbers of the following bacteria: *Aeromonas hydrophila* on mA medium by the membrane filter procedure after 48 h of incubation at 37°C (Rippey and Cabelli 1979); *Pseudomonas fluorescens* on Kinga B medium after 72 h of incubation at 25°C (Burbianka and Pliszka 1983); *Staphylococcus aureus* on Chapman agar after 48 h of incubation at 37°C (Burbianka and Pliszka 1983); family *Enterobacteriaceae* on Endo medium after 24 h of incubation at 37°C (Burbianka and Pliszka 1983).

The tenfold dilution method with a 0.85% NaCl solution was used during the tests. The bacteria were cultured in triplicate. Common microbiology practice was followed during the analyses. After the incubation period, the cultured colonies were enumerated, and the results were recalculated into colony-forming units (CFU) in l cm³ of water (Anon. 1985).

The number of *Aeromonas hydrophila* bacteria was determined by enumerating colonies that stained yellow on mA medium, and then the mannitol test was performed. *Pseudomonas fluorescens* was determined by enumerating the colonies on a selective substrate that produced fluoresceine, which was visible under a Wood's lamp. The *Staphylococcus aureus* bacteria on the Chapman medium were determined by enumerating the colonies that stained yellow. These colonies were then cultured on an agar substrate usually with the addition of 2% glucose and 5% sheep blood.

In addition to determining all the bacteria, the shapes of cells, Gram stain and motile ability in the microscopic preparations were checked. The ability to produce catalase and cytochrome oxidase and the oxidation or fermentation of glucose on Hugh-Leifson's substratum (O/F test) were also checked. The microbe species were identified with the API 20 NE, API 20 E, and API STAH biochemical tests by bioMerieux.

Single factor variance analysis (ANOVA) was applied to determine if the concentration of a given bacterial group was the same at the different sampling stations. This verified the hypothesis of the equality of average values ($H_0 : x_1 = x_2$) at a significance level of p = 0.05, assuming that the variances for the number of the investigated bacterial groups is uniform. The Leven test was used to verify the homogeneity of variance; when the Leven test was significant, the verified hypothesis was rejected. Then the Kruskal-Wallis test, the nonparametric equivalent of variance analysis (Stanisz 1998), was performed. The hypothesis was verified that the compared numbers of bacteria were collected from populations with the same distribution or with distributions with the same median.

Multiple reverse regression was applied to evaluate the dependence between the number of bacteria and the water temperature. The dependent variable was the abundance of the individual groups of bacteria, and the independent variable was water temperature. The results of statistical analyses in the regression equation remain only those variables which significantly (p = 0.05) influence the independent variable (Stanisz 1998).

RESULTS

The characteristics of the bacteria are presented in Table 1.

The average monthly cooling water temperature varied from 15.2°C in April 1999 to 29.3°C in July 1999. The temperature on the sampling day for the bacteriological analyses, varied from 16°C in April 1999 and October 2000 to 27°C in July 1999 and June 2000 (Table 2).

Aeromonas hydrophila numbers varied from 1 CFU cm⁻³ on 31 August 2000 at station 2 to 1,350 CFU cm⁻³ on 30 July 1999 at station 4 (Fig. 1). The lowest, average number of this bacteria (130 CFU cm⁻³) was confirmed at the water inflow at station 1, while the highest (330 CFU cm⁻³) was noted in the Narew River at station 4 (Table 3).

Pseudomonas fluorescens densities ranged from 1 CFU cm⁻³ (at least once during the study period at each station) to 590 CFU cm⁻³ on 30 July 1999 at station 3 (Fig. 2). Mean numbers *Pseudomonas fluorescens* were the lowest in the water flowing into the

	Shape of cells	Gram stain	Motility	Catalase	Cytochrome oxidase	O/F test
Aeromonas hydrophila	rods	_	+	+	+	+/+
Pseudomonas fluorescens	rods	-	+	+	+	+_/_
Staphylococcus aureus	cocci	+	-	+	_	+/+
Enterobacteriaceae	rods	-	+/-	+	_	+/+

Table 1	. Charac	teristics	of	groups	of	bacteria
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		Temperature	
Date	Monthly average [°C[Daily temperature range [°C]	On sampling day [°C]
04.1999	15.2	10-19	16
05.1999	19.9	14-26	20
06.1999	26.9	23-29	27
07.1999	29.3	27-32	27
00.1000 ^{<i>a</i>}	21.0	20.25	22
09.1999	21.0	20-25	22
05.2000	24.5	23.5-26	25
06.2000	27	26-28	27
07.2000	25	24-25.5	25
08.2000	26	25-26	25
09.2000	20	16-23	20
10.2000	15.5	13.5-17	16

Table 2. Temperature of cooling waters during intensive fish fattening in 1999-2000

^a samples taken twice



Fig. 1. Number of bacteria *Aeromonas hydrophila* in cooling waters and the Narew River during intensive fish fattening in 1999-2000 (CFU/cm³).



Fig. 2. Number of bacteria *Pseudomonas fluorescens* in cooling waters and the Narew River during intensive fish fattening in 1999-2000 (CFU/cm³).

Table 3. Average and standard deviation of the n	umber of bacterial	l groups in cooling v	water and Narew	River
during intensive fish fattening in 1999-2000 (CF	U cm ⁻³)			

	Sampling station	Aeromonas hydrophila	Pseudomonas fluorescens	Staphylococcus aureus	Enterobactericeae
1	average	130	40	11	490
1	standard deviation	245	61	14	343
2	average	210	150	13	550
2	standard deviation	304	221	18	387
2	average	180	100	12	902
5	standard deviation	221	179	13	1,090
4	average	330	50	8	5,870
4	standard deviation	418	82	10	11,598

facility (station 1) and the highest in the water in the wels cage (station 2) at 40 and 150 CFU cm⁻³, respectively (Table 3).

Densities *of Staphylococcus aureus* varied from 1 CFU cm⁻³ (at least once during the study period at each station) to 53 CFU cm⁻³ on 30 July 1999 at station 2 (Fig. 3). The average numbers of this bacteria species varied from 8 CFU cm⁻³ in the Narew River (station 4) to 13 CFU cm⁻³ in the cagewater (station 2) (Table 3).



Fig. 3. Number of bacteria *Staphylococcus aureus* in cooling waters and the Narew River during intensive fish fattening in 1999-2000 (CFU/cm³).



Fig. 4. Number of bacteria from the family *Enterobacteriaceae* in cooling waters and the Narew River during intensive fish fattening in 1999-2000 (CFU/cm³).

The numbers of bacteria from the family *Enterobacteriaceae* varied from 40 CFU cm⁻³ on 1 October 2000 in cagewater at station 2 to 39,200 CFU cm⁻³ in the Narew River on 17 May 2000 (Fig. 4). The average *Enterobacteriaceae* dentisities were at minimum values in the inflowing water (station 1) and at maximum values in the Narew River, 490 and 5,870 CFU cm⁻³, respectively (Table 3).

The results of the statistical analyses indicated that the test of the variance homogeneity was significant for bacteria *Pseudomonas fluorescsens* and those from the family *Enterobacteriaceae*. Thus, the Kruskal-Wallis test was applied to for these bacteria. Only with *Enterobacteriaceae* were statistically significant differences (p = 0.0158) observed between the various stations. Additionally, statistical analyses indicated the lack of a relation between water temperature and bacteria density.

DISCUSSION

Bacteria *Aeromonas hydrophila* are regarded as an indicator of organic pollution (Pianetti *et al.* 1998). The studied waters are rich in organic matter, which originates from unconsumed feed. The high numbers of these bacteria are also facilitated by the high temperatures (Rhodes and Kator 1994). The high temperature is characteristic of cooling waters. The presence of high numbers of these microorganisms in wastewater from fish farms might also result from the fact that the genera *Aeromonas*, *Plesiomonas*, *Pseudomonas* dominate the microflora in the digestive tracts of freshwater fish (Campell and Buswell 1983, Esteve and Garay 1991). This means that large amounts of these bacteria are discharged into the water with fish feces. The numbers of bacteria *Aeromonas hydrophila* in the studied water did not vary from values reported in the literature by other authors (Lewandowska *et al.* 2002, Niewolak and Opieka 2000, Zmysłowska *et al.* 2000c).

Pseudomonas fluorescens occurred in relatively high numbers in the studied waters (1-590 CFU cm⁻³). These microbes are common in surface waters, and their numbers indicate the content of organic substances (Zmysłowska *et al.* 2000b). They also readily adapt to variable environmental conditions, which may explain their common occurrence in natural environments (Lewandowska *et al.* 2002). The bacteria *Pseudomonas fluorescens* exhibit proteolytic and ammonification activity (Liao and McCallus 1998, Gennari *et al.* 1999), thus conditions for their development were advantageous in the waters of the discharge canal where there were remains of unconsumed feed. The high numbers of these bacteria in waters utilized in fish farms possibly was due to by the fact that they are the dominant microflora in the digestive tracts of fish (Gennari *et al.* 1988, Spanggard *et al.* 2000), and high numbers of them are released into the water with fish excrement.

The occurrence of *Staphylococcus aureus* was often noted in the present study. Niewolak and Opieka (2000) and Yoshe-Purer and Golderman (1987) regarded these bacteria as an indicator of water polluted by sewage. The numbers of these bacteria in the studied waters were low, and they did not vary substantially from the data reported by Niewolak and Opieka (2000) for the Czarna Hańcza River.

Bacteria from the family *Enterobacteriaceae*, similarly to those of the genera *Aeromonas* and *Pseudomonas*, are an important index of water pollution. In the current

study statistically significant higher average values of these bacteria were noted in the water flowing out of the fish farm than in that flowing into it. High numbers of these bacteria in the waters flowing out of the fish farm might be related to their occurrence in the digestive tracts of the fish. According to Spanggaard *et al.* (2000), up to 86% of all the bacteria isolated from rainbow trout digestive tracts were identified as *Enterobacteriaceae*.

In summary, this study indicates that fish fattening did not influence the quantitative composition of bacteria *Aeromonas hydrophila*, *Pseudomonas fluorescens* and *Staphylococcus aureus* in the post-cooling water of the canal. The only significant change was noted for *Enterobacteriaceae*; dentisities increased during the passage of the water through the canal. The numbers of the studied bacteria groups probably result from the numbers of these bacteria in the digestive tracts of the fish, from which they are deposited into the water along with excrement.

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Some data on snoek (*Thyrsites atun* Euphrasen 1791) from the Hokitika region (Challenger Plateau, Tasman Sea)

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Abstract. This paper presents the results of Polish studies of snoek from the Hokitika region (Challenger Plateau, Tasman Sea). The growth rate of males and females is described. The dependencies obtained were described with the von Bertalanffy equation. The coefficients of the equations were as follows:

- males: $L_{inf} = 84.55$; K = -0.338; $t_0 = 0.645$; - females: $L_{inf} = 83.60$; K = -0.481; $t_0 = 1.955$;

- the sexes combined: $L_{inf} = 84.07$; K = -0.380; $t_0 = 0.999$.

The studies indicated that the growth rate of male and female snoek from the Hokitika region were similar. The average back-calculated length did not differ much from the average derived from empirical data from the same age groups.

Key words: snoek, Thyrsites atun, New Zealand, growth

INTRODUCTION

Snoek Thyrsites atun (Euphrasen, 1791) from the family Gempylidae inhabits shelf waters in the temperate zone between the 35 and 55 parallels of the southern hemisphere. It is abundant around New Zealand and along the coasts of southern Australia, Africa, and South America (Blackburn and Gartner 1954, Nakamura and Parin 1993). These fish are pelagic predators and can weigh as much as 9 kg (Nepgen 1979). They achieve sexual maturity in the second or third year of life at a fork length of 50-60 cm (Mehl 1971). Juvenile individuals inhabit waters to depths of up to 100 m, while adults inhabit water layers deeper than 400 m (Grant et al. 1978, Jackowski 1994).

Snoek are an important commercial fish, and according to FAO statistics worldwide catches reach 42,000 tons annually. The average, annual catch in New Zealand was 25,000 tons over the last twenty years (Horn 2002). Although snoek plays an important role in commercial catches, there are relatively few publications on the biology, and especially the growth rate, of this species. Venidiktova (1988) estimated the growth rate of snoek from

the waters of the southwestern Atlantic using the back-calculation method. Grant *et al.* (1978) published results of investigations of the growth rate of snoek inhabiting the waters around Australia, while Hurst and Bagley (1987) and Horn (2002) addressed the growth rate of snoek from New Zealand waters.

According to Hurst and Bagley (1987, 1992, 1994), most of the commercial catches of this species in New Zealand waters are made in the Auckland East, Southland, and Chatham Rise regions (Figure 1), which are also the spawning grounds of this species. It migrates to the Auckland East in August and September and to the Southland and Chatham Rise region comprises a separate stock (Hurst and Bagley 1992). Juvenile specimens first migrate from the spawning grounds to shallower waters (< 100 m) surrounding the Southland and later move to deeper waters (below 400 m) in regions such as Hokitika (Stevenson 1996). The adult snoek which inhabit these waters can migrate to spawning grounds to wards the north, through the Cook Straits to the Auckland East region as well as to the south towards Snares Island, Southland (Horn 2002).

A thorough description of the biology of snoek that inhabit South African waters can be found in Griffiths (2002). It describes migrations, spawning grounds, the gonad maturation process, and the spatial distribution of juveniles in the ecosystem of Benguelea, South Africa. The snoek that inhabit this region attain 50% sexual maturity at a fork length of 73 cm in the third year of life. The spawning grounds of this fish are located at depths ranging from 150 to 400 m, and juvenile specimens attain a fork length ranging from 33 to 44 cm in their first year of life.

The aim of this work was to contribute to the knowledge of snoek using data from Polish studies conducted in New Zealand fishing grounds in the region of Hokitika in 1994.

MATERIALS AND METHODS

The materials for the studies were collected in July and August 1994 during commercial catches of hoki (*Macruronus novaezelandiae*) in the fishing grounds of Hokitika (Challenger Plateau, Tasman Sea) (Fig. 1). A total of 140 specimens were measured, of which 105 were subjected to ichthyological analyses. Each specimen was measured twice in order to determine the fork length (FL) and total length (TL). Determining the dependence between TL and FL in this way allowed the results of the current study to be compared to the earlier results of Venidiktova (1988). The fish measurements were rounded down to the nearest cm, and the fish were weighed to the nearest 5 g. Next, the sex, gonad maturity stage (Maier scale) and degree of stomach fullness were determined. Age was determined according to the commonly accepted principle for fish from the temperate zone, namely that the two morphologically different growth zones (hyaline and opaque) constitute the annual growth increment. Venidiktova (1988) applied a similar method to read age. The growth rate of snoek was determined empirically by comparing the average fork length of fish in various age groups. This characteristic was described with the help of the von Bertalanffy equation (Beverton and Holt 1957):



Fig. 1. Snoek study region in New Zealand waters (enlarged area shows the Hokotika region and the sites where this species was caught).

$$FL_t = L_{inf} [1 - e^{-K(t - to)}]$$

where: FL_t – fork length at fish age *t*;

- L_{inf} asymptote to which the growth rate approaches; *K* catabolism index;
- e natural log base;
- t_0 arbitrary origin of the growth curve.





Fig. 2. Snoek otolith. The drawing presents subsequent annual growth increments with the marked distances.

Since the youngest specimens in the sample were from the 4+ age group, the Pauly method (1984) with $L_{\text{max}} = 0.95L_{\text{inf}}$ was used to evaluate the parameters of the von Bertalanffy formula for the empirical data.

The dependence between the length and weight of fish was described using the following power equation: $W = a \cdot FL^b$, where W is fish weight, FL – fork length, a and b – equation coefficients.

Since the youngest specimens were classified to the IV age group, growth rate was estimated with the back-calculation method in addition to the analysis of empirical data. The results obtained using this method were compared to those of direct measurements.

The studies of the otoliths were comprised of determining their mass (to the nearest 0.001 g), length, and the width and length of the longest radius (further referred to as the radius) to the nearest 0.001 mm. Length measurements were conducted using a microscope-computer set-up running MULTISCAN software. This program permitted identifying the exact center of the otolith nucleus from which the radius extended (Fig. 2). Back calculations of snoek growth rates were done with the widely used Fraser-Lee formula (Fraser 1916, Lee 1920):

$$FL_n = a + \frac{R_n}{R_c}(FL_c - a)$$

where: FL_n – fork length of fish aged *n*;

 R_n – otolith radius length, which relates to the subsequent annual rings;

 R_{c} – total otolith radius length at the moment a given fish is caught;

 FL_{c} – fish fork length at the moment of catch;

a – equation coefficient.

RESULTS

No dense concentrations of snoek were noted in the waters of the Hokitika region. Specimens of this species were the by-catch of targeted catches of New Zealand hoki. This is also why snoek was noted only in 12 of 147 hauls. The specimens examined measured from 56 cm to 89 cm (*FL*) and ranged in age from 4 to 10 years. The small amount of material collected did not permit characterizing the length distribution of the snoek caught in the Hokitika region. Specimens ranging in age from 5 to 9 years dominated the sample and comprised 81.9% of it. The male specimens measured from 56 to 89 cm in length, while the females were from 62 to 88 cm long. Of the fish from the dominating age groups, 73.9% were males and 84.7% were females (Table 1). The lack of smaller (and younger) specimens was due to the use of a trawl with a 100 mm mesh bar length. The shape of the curves obtained based on the von Bertalanffy equation indicates that the growth of males and females in the different groups studied did not differ (Fig. 3). The equation coefficients were as follows: for males – $L_{inf} = 84.55$, K = -0.338, $t_0 = 0.645$; for females – $L_{inf} = 83.60$, K = -0.481, $t_0 = 1.955$; for the sexes combined – $L_{inf} = 84.07$, K = -0.380, $t_0 = 0.999$.

A 22 20010		F	ork length [cn	n]	Indiv	vidual weight	[g]
Age group	n	min	max	average	min	max	average
			Ma	les			
4	3	56	60	58.3	550	800	700.0
5	6	62	67	64.3	900	1450	1116.7
6	9	66	73	71.0	1100	1700	1472.2
7	13	68	76	72.6	1200	1950	1576.9
8	8	68	78	75.0	1200	2350	1893.8
9	6	75	82	78.2	1500	2750	2000.0
10	1			89.0			2750.0
Total	46						
			Fema	ales			
4	_	-	-	-	_	-	-
5	10	62	69	65.8	900	1350	1125.0
6	12	66	73	69.7	1050	2000	1462.5
7	11	71	79	74.3	1500	2050	1745.5
8	17	73	86	78.1	1500	2500	1998.8
9	8	78	85	81.9	1750	2500	2281.3
10	1			88.0			3500.0
Total	59						
			Males and	l females			
4	3	56	60	58.3	550	800	700.0
5	16	62	69	65.3	900	1450	1121.9
6	21	66	73	70.2	1050	2000	1466.7
7	24	68	79	73.4	1200	2050	1654.2
8	25	68	86	77.1	1200	2500	1965.2
9	14	75	85	80.3	1500	2750	2160.7
10	2	88	89	88.5	2750	3500	3125.0
Total	105	1					

Table 1. Individual weight and length of snoek from the Hokitika region by age group

The dependence between fork length and total length was linear and was described by the equation TL = 1.06 FL + 2.83 (r = 0.99). This equation indicates that the fork length of snoek comprised approximately 91.9% of the total length.

The individual weight of the fish ranged from 550 g to 3500 g and increased as the length and age of the fish increased (Table 1). The comparison of the length-weight dependence between males and females (Table 1) indicated that the larger males (longer than 75 cm) were heavier than females of the same length (Fig. 4). The degree of gonad maturity had a significant impact on differences in individual weight; mature specimens (55.6% in stages VI and VII) dominated among the males while developed specimens (77.6% in stage IV) dominated among the females.

Single factor analysis of variance ANOVA indicated that fish length was most strongly correlated to otolith weight, followed by radius length. The dependence between fish fork length and selected morphologic features of the otoliths are presented in Table 2. Variance analysis also indicated that the weight of the otolith is strongly correlated with fish age





Fig. 4. Length-weight dependence curves of male and female snoek.

Otolith morphological feature	Equation	r	R^2	F-ratio	Prob. level
Total length	v = 0.103 FL + 4.092	0.68	46.71%	96.44	n < 0.0000
Width	y = 0.038 FL + 3.481	0.61	36.65%	63.64	p < 0.0000
Radius length	y = 0.0698 FL + 1.85	0.73	53.27%	61.55	p < 0.0000
Weight	$y = 0.0012 \ FL - 0.03$	0.78	60.45%	168.12	p < 0.0000

Table 2. Equations of the dependence between fork length (FL) and chosen snoek otolith features

Table 3. Average otolith weight (mg) of snoek from the Hokitika region (Challenger Plateau, Tasman Sea)

Age group	п	min – max	Average otolith weight [mg]	δ
IV	2	40.5 - 41.5	41.0	0.71
V	16	35.0 - 61.5	45.8	6.58
VI	21	42.0 - 63.5	52.1	6.32
VII	24	43.5 - 68.5	55.8	5.77
VIII	25	47.0 - 72.5	61.2	6.16
IX	13	58.0 - 78.5	68.2	5.66
Х	2	63.5 - 72.5	68.0	6.36

(r = 0.79; F = 26.81; p < 0.000). Table 3 presents the average weight of otoliths from snoek from the Hokitika region by age groups.

The average length of fish in subsequent years of life was calculated and compared with the empirical data (Table 4). The difference between the empirical data and that obtained from back calculations ranged from 0.05 to 1.57 cm in respective age groups (x-y in Table 4).

DISCUSSION

According to New Zealand scientists, during spawning snoek form dense concentrations in the eastern part of the Cook Strait (Auckland East), the southwestern waters of Southland (Mehl 1971, Nepgen 1979), and in the Chatham Rise region (Hurst and Bagley 1992, 1994). It is fished commercially at this time. Upon the completion of spawning, this fish species migrates to the waters surrounding Southland, where the region of Hokitika is located.

Snoek as characterized by a relatively high length growth rate, particularly in the first three years of life. Mehl (1971) reported that the youngest snoek observed (aged four) measured from 56 to 60 cm. This author studied the snoek from New Zealand waters previously, and according to him, in the second or third year of life, or by the time they attain sexual maturity, these fish reach lengths of up to 60 cm. Similar conclusions were presented by Horn (2002).

A comparison of the average lengths in age groups indicated that the growth of snoek caught in the Hokitika region was similar to that observed by Hurst and Bagley (1987) in the Chatham region and by Horn (2002) in the Southland region. Differences that were apparent resulted from the fact that the studied material was collected in different months;

Table 4.	. Comparise	n of the ave	stage totk letigt	•									
			Males			Femal	les			Males and	l females		
Age	Empirica data	1 Back	k SD ted for hac	(" ") A	Empirical	Back	SD for back	(i)	Empirical data	Back	SD for back	(
5	(x)	(<i>y</i>)	calculati	$\frac{1}{2}$ ons $\frac{1}{2}$	(x)	(y)	calculations	(k - v)	(x)	(<i>y</i>)	calculation	$(\gamma - \gamma)$ si	
4	58.33	59.06	5 3.64	- 0.73		61.02	2.93		58.33	59.84	3.39	- 1.51	
5	64.33	65.41	1 3.25	- 1.07	65.80	65.71	2.70	0.09	65.25	66.05	3.00	-0.80	
9	71.00	6.92	4 2.81	1.06	69.67	70.03	2.56	-0.36	70.24	70.62	2.74	-0.38	
7	72.62	73.22	2 2.69	-0.61	74.27	74.22	2.76	0.05	73.38	74.41	2.99	- 1.04	
8	75.00	76.06	5 3.24	- 1.06	5 78.12	77.51	2.76	0.60	77.12	77.6	3.29	-0.48	
6	78.17	79.0(0 3.50	-0.84	81.88	80.31	2.41	1.57	80.29	80.23	3.43	0.06	
10	89.00	88.19	. 6	0.81	88.00	87.05		0.95	88.50	87.62		0.88	
	South	hland	Chathan	1 Rise	Victoria		Tasmania		Namibia ^a		Hokitika		
Ψυ	(Horn,	2002)	(Hurst and Ba	(gley, 1987)	(Grant et al. 1	978) (Gr:	ant <i>et al.</i> 1978)	(Veni	diktova 1988)		(current stud	ly)	
nge	males	females	males	females	males and female	s	males and females	aı	males nd females	males	females	males and females	
1	38.1	37.8	29.6	31.4	Ι		Ι		50.0	Ι	-	Ι	
0	51.8	52.1	I	I	59.3		62.5		66.2	Ι	I	I	
б	60.1	59.9	58.3	57.5	63.8		72.4		75.4	Ι	I	I	
4	63.9	65.4	65.7	67.2	68.7		80.3		88.5	58.33		58.33	
5	68.7	69.2	68.0	71.8	75.1		82.1		98.9	64.33	65.80	65.25	
9	71.1	72.8	72.9	75.8	80.7		85.1		104.2	71.00	69.67	70.24	
L	74.5	72.9	75.9	78.5	84.3		87.9		I	72.62	74.27	73.38	
8	79.0	81.8	I	80.3	87.3		89.2		106.3	75.00	78.12	77.12	
6	79.4	84.6	I	81.5	88.2		91.7		I	78.17	81.88	80.29	
10	81.3	86.3	I	I	I		I		I	89.00	88.00	88.50	
11	Ι	88.0	I	I	I		I		L	I	I	I	

^atotal length



Fig. 5. Length-weight dependence of male and female snoek from different regions of New Zealand waters.

the New Zealand scientists studied snoek caught in March, while the current study was conducted in July and August. Horn (2002) reported that in the majority of studied individuals the annual ring was not fully formed on the edge of the otolith, and this prompted him to describe the age groups as 0+, 1+, etc. He explained that snoek lay down the first annual ring between the ages of 0.5 and 1.5 years. Since the opaque and hyaline zones comprise one annual increment, the author of the current paper determined that the process of laying down rings was completed in July and August. The growth of the next opaque zone was just visible on the outer edge of the otolith. Due to this, the current author acknowledged that the studied individuals from the IV age group correspond to the 3+ group described by Horn (2002).

The results of the current author's studies indicate that the growth rate of snoek from the Hokitika region is lower that that observed by Grant *et al.* (1978) in Australian waters. Venidiktova (1988) attained similar results studying snoek from Atlantic waters (Namibia). Although Venidiktova (1988) used total length (TL) in the study, the results indicated that growth rate of snoek from Atlantic waters was higher than that of snoek from other regions. The largest specimen caught in Polish catches conducted in the Hokitika region had a FLof 89 cm and a TL of 97 cm; however, Venidiktova (1988) reported that the largest snoek specimen from Atlantic waters measured 118 cm.

The material collected by the current author indicates that male snoek achieve sexual maturity earlier than females do. It also indicates that males exceeding 75 cm in length were heavier than females of the same length. As the gonads matured the weight of the females increased and at the spawning grounds they were heavier than the males. Similar observations were made by Mehl (1971) and Nepgen (1979) who described the spawning of snoek from the eastern part of the Cook Strait (Auckland East) and the southwestern waters of Southland. However, Hurst and Bagley (1992, 1994) reported that the weight of males and females was similar in the Southland spawning grounds, while in the Chatham Rise region males were heavier than females (Fig. 5).

The average lengths estimated with back calculations differed only slightly from the average calculated from the empirical data in the same age groups. Horn (2002) made similar observations. He called attention to the fact that it is difficult to interpret the first annual ring due to the long period in which it is laid down. Horn also maintains that the annual rings are clearly visible even in older individuals. This means that the results of back calculations from empirical data correspond well, but only until the IV age group.

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The European anchovy (*Engraulis encrasicolus* [L.]) in the Baltic Sea

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The anchovy family (Engraulidae) numbers 139 species that are classified into sixteen genera. These fish occur in the coastal waters of the tropical and temperate zones in both hemispheres from the equator to 45°S and 60°N, as well as in the inland waters of North and South America, Australia and southern Asia. The characteristic features of the members of this family are the snout that protrudes markedly over the lower jaw, the articulation of the jaws behind the eye, and the lack of a lateral line (Whitehead *et al.* 1988). These fish are small and usually attain a body length of 10 to 20 cm. Specimens of the New Guinea thryssa (*Thryssa scratchleyi* (Ramsey & Ogilby, 1886)), a species that inhabits the rivers of New Guinea and those flowing into the Gulf of Carpenteria in Australia, attain a total length of 37 cm. In contrast, the Rio Negro pygmy anchovy (*Amazonsprattus scintilla*) from the Rio Negro and Rio Jufari rivers in the Amazon system attain a length of just 20 mm. (Roberts 1984).

The most commonly occurring anchovies belong to the genus *Engraulis sp.* Of all fish, these make the largest contribution to the annual production biomass and have a decisive impact on the state of the fisheries of many countries. For example, catches of the Peruvian anchovy (*Engraulis ringens* Jenyns, 1842) contributed between 8 and 12.5 million tons to the annual catch between 1993 and 1996. The members of this genus have a maxillia with a blunt tip that does not reach the pre-operculum. Of the eight species that belong to this genus, the European anchovy (*Engraulis encrasicolus* (Linneaus, 1758)) attains the largest size at 200 mm body length. The average annual catches of the European anchovy in the 1992-2001 period exceeded 542,000 tons. Only 33,000 tons were caught in European Atlantic coastal waters, 90% of which was caught by Spain and France. Nearly 70% of the catch of the European anchovy came from Mediterranean waters.

The European anchovy inhabits the coastal waters of European seas below southern Norway (60°N), the Mediterranean Sea, the Black and Azov seas, and the waters of the west coast of Africa down to Angola (Whitehead 1984). Although Whitehead stressed that the European anchovy does not occur in the Baltic, Popiel (1962) reported that single specimens were sighted frequently in the Bornholm Basin and Gulf of Gdansk, while in the north they were recorded in the waters near Stockholm.

Popiel (1962) emphasized that the anchovy never occurred in the Baltic in quantities that could be of commercial interest to fishermen. It is interesting that, with the exception of Popiel (1962), the majority of Polish authors of academic textbooks misspell the name

of the European anchovy as *E. encrasicholus*. Whitehead et al. (1988) reported that several other authors made the same mistake.

The Sea Fisheries Institute in Gdynia conducts surveys on the quantity and distribution of fish in the Polish EEZ twice a year in winter and autumn. The surveys are conducted from aboard the r/v BALTICA. The main source of information on the fish inhabiting the region is based on the results of hauls made with trawls fitted with a small mesh size codend. Although the grid of the haul distribution is not constant, each survey covers the entire area of the Polish EEZ. The European anchovy was first recorded in a haul made by the r/v BALTICA in 1996 (one specimen). Between ten to twenty individuals were recorded in four hauls made in the western and central areas of the investigated region in February 1998 (Fig. 1) (Grygiel et al. 1998). The European anchovy was also noted in r/v BALTICA catches in February 2003 (up to 270 g per 30 min. haul) (Grygiel et al. 2003). The largest quantity of European anchovy was noted in the catches of the r/v BALTICA in October of the same year (Wyszyński et al. 2003), and especially in November of the same year (Grygiel and Grelowski 2003). During the November cruise, fish belonging to this species were noted in 14 hauls (48%) occurring primarily in the western part of the investigated area. These fish were also confirmed in the waters of the Gulf of Gdańsk (to the east of $18^{\circ}20'$ E). The largest quantity of European anchovy registered in a thirty-minute haul was 3.5 kg. In February 2004 the European anchovy was noted in 29% (12) of the hauls (Grygiel et al. 2004) made in the same location as those conducted in November 2003. In comparison with hauls that were conducted during earlier cruises of the r/v BALTICA, the European



Fig. 1. Occurrence of European anchovy (*Engraulis encrasicolus* [L.]) in the southern Baltic in 1998, 2003, and 2004.



Fig. 2. Length frequency distribution of the European anchovy fished in the Bornholm Basin in November 2003 (n = 255).

anchovy occurred in significantly greater quantities (up to 48 kg per thirty-minute haul) in February 2004. They occurred most frequently in hauls conducted in the western part of the Polish Exclusive Economic Zone (EEZ) at depths ranging from 53 to 76 m. The water in these locations reached the highest temperature (7.5°-9.5°C) and salinity (13 PSU-17 PSU) during this period (Grygiel *et al.* 2004). In February 2004 fishermen deploying pelagic trawls in the Kołobrzeg region reported larger quantities of European anchovy by-catch in sprat catches than had been reported in previous years.

In November 2003 the length of the fish caught ranged from 8.5 cm to 18 cm (Fig. 2); individuals of a total length ranging from 11 to 14 cm and weighing from 7 to 15 g dominated. The value of the parameters describing the relationship between total length and weight ($W = a \cdot l^b$) were a = 0.00000169935 and b = 3.2389292. Sinovcic (2003) reported an identical relationship between body weight and total length for the European anchovy occurring in the coastal waters of Croatia (a = 0.0040; b = 3.1195 TL in cm). According to the same author, the condition of anchovies in the open waters of the Adriatic was decidedly better, and the average individual weight of the fish was much higher than it was in specimens caught in coastal waters. It was confirmed that by the time fish attain a length of 13 cm the individual weight of the European anchovy from the Aegean Sea (Loukmidou and Stergiou 2000) is nearly equal to that of those caught in the Baltic Sea in November 2003. This difference increases in favor of the Aegean sea anchovy as fish length increases.

A review of the structure of eighty pairs of otoliths from European anchovy specimens caught in the Baltic Sea in November 2003 did not provide a basis for identifying the age structure of these fish. This was primarily due to the fact that the growth increment in these otoliths is more difficult to identify than it is in those of Baltic herring or sprat. From zero to three seasonal increments were identified on the otoliths of the fish caught in November 2003. Pictures of the otoliths of the anchovies caught in the Baltic were magnified twelvefold. Following analysis, the authors observed that the majority of the otoliths came from age group I fish. According to Bellido *et al.* (2000), age group I anchovies in the Gulf of Cadiz reached a length range of 111.4 mm-113.0 mm. In the Black Sea the average length of fish from this age group was 100.2 mm (Unsal 1989). Ramos and Santos (1999) reported that the average length of anchovies from the coastal waters of Portugal were as follows: age group 0 - 94.6 mm; I - 122.6 mm; II - 133.7 mm; III - 139.8 mm; IV - 152.3 mm; V - 155 mm. The European anchovy population exploited off of the coast of Sicily was comprised of fish with a total length range of the fish classified as age group 0 was 7 cm - 11.5 cm, while that of age group I fish was 8.0 cm-13.5 cm. Fish from age groups I and II dominated. The range of the value of parameter L_{∞} estimated by various authors for the European anchovy inhabiting the waters of the Mediterranean Sea, coastal Africa, and the Iberian Peninsula ranged from 15.3 cm to 20.0 cm, while its growth rate is positively correlated to the chlorophyll concentration in the sea water (Basilone *et al.* 2004).

The rapid growth rate is related to the voracity of the fish from this family. Hartman *et al.* (2004) did not observe a difference in the food composition between juvenile and sexually mature specimens of bay anchovy *Anchoa mitchilli* that occurs on the other side of the Atlantic. Its primary food is comprised of copepods, mysid shrimp *Neomysis americana* and barnacle nauplii. However, the daily food ration of fish belonging to age group 0 was 2.7-fold larger that that of sexually mature specimens $(0.449 - 0.684 \text{ g} \cdot \text{g}^{-1} \text{ d}^{-1})$.

It is worthwhile noting that the Baltic Sea survey catches that included the European anchovy also contained other species typical of waters with a salinity level similar to that of the Atlantic Ocean; these included whiting *Merlangius marlangius* [L.], common mackerel Scomber scombrus [L.], and Atlantic horse mackerel *Trachurus trachurus* [L.]. This might be interpreted as evidence of a large inflow of North Sea waters containing typical representatives of ichthyofauna. Scientists must be patient as they wait for an answer to the question of whether the changes noted in the composition of the Baltic Sea ichthyofauna following the inflow of oceanic water at the beginning of the twenty-first century are temporary or if "Baltic" generations of the European anchovy, whiting or Atlantic horse mackerel will appear.

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Prace należy składać w 2 egzemplarzach maszynopisu pisanego jednostronnie, formatu A4, z podwójnym odstepem (konieczna jest dyskietka z całością materiału). Słowa, które powinny być złożone drukiem pochyłym (kursywą), tzn. łacińskie nazwy gatunków i rodzajów oraz symbole wielkości zmiennych należy podkreślić wężykiem (~~~~~). Innych podkreśleń nie należy stosować.

W pracach kategorii 1 i 2 obowiązuje następująca kolejność:

1. Tytuł: krótki (do 100 znaków).

2. Imię i nazwisko autora oraz nazwa i adres instytucji macierzystej.

3. Abstrakt musi poprzedzać każdy artykuł naukowy i notę; objętość – najwyżej 1 strona maszynopisu.

4. Słowa kluczowe: kilka pojęć pozwalających na odszukanie danej pracy w systemach komputerowych.

 Tekst. Objętość maszynopisu prac kategorii 1 nie powinna przekraczać 40 stron, a kategorii 2 – 15 stron. W pracach kategorii 1 i 2 stosuje się tradycyjny podział:
 1) wstęp, 2) materiał i metoda badań, 3) wyniki badań,
 4) dyskusja, 5) bibliografia. Wyniki pomiarów należy podawać w jednostkach miar przyjętych w systemie metrycznym, a ich skróty – zgodnie z Międzynarodowym Układem Jednostek Miar (SI).

6. Podziękowania należy ograniczyć do niezbędnego minimum (inicjały imienia i nazwisko osoby, do której są adresowane, bez wymieniania tytułów naukowych i nazw instytucji).

7. Bibliografię należy zestawiać w porządku alfabetycznym, podając bezpośrednio po nazwiskach autorów rok wydania i wymieniając tylko prace cytowane w tekście (np. Kowalski 1990). Tytuły czasopism – w pełnym brzmieniu. Tytuły prac – w językach oryginału (z wyjątkiem tytułów w języku rosyjskim wydrukowanych alfabetem niełacińskim, np. cyrylicą, które należy przetłumaczyć na język polski lub angielski). **8. Przypisy** oznacza się cyfrą arabską we frakcji górnej (...¹) i numeruje kolejno w całym tekście, z wyjątkiem tabel; treść przypisów – na osobnych stronach.

9. Tabele są dodatkowym źródłem informacji; nie należy powtarzać w nich danych występujących w tekście lub na rysunkach. Tabele numerowane, każda na osobnej stronie, muszą mieć tytuł; powołanie na nie należy umieścić w tekście. Każdą kolumnę w tabeli opatruje się tzw. "główką" wyjaśniającą zawartość kolumny. Przypisy w tabelach należy oznaczyć literami, kursywą, we frakcji górnej (np. Lata^a), a ich objaśnienie umieścić pod tabelą.

10. Ilustracje. Obowiązuje kolejna numeracja z przywołaniem każdego numeru w tekście. Podpisy pod ilustracjami – na osobnej kartce. Stosowane na rysunkach skróty, terminy i symbole muszą odpowiadać użytym w tekście. Każdy rysunek, umieszczony na osobnej kartce oraz opisany kolejnym numerem i nazwiskiem autora, po wyskalowaniu musi zmieścić sie w kolumnie; trzeba to uwzględnić stosując odpowiednią grubość linii i wielkość opisów na rysunkach. Redakcja przyjmuje wyłącznie rysunki wykonane techniką komputerową (konieczny wydruk i dyskietka). Prace można ilustrować fotografiami (mogą być kolorowe). Łączna objętość rysunków i zdjęć nie może przekraczać 30% objętości pracy.

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EGZEMPLARZE AUTORSKIE

Każdy autor opublikowanego artykułu otrzymuje 1 egzemplarz czasopisma, autorzy prac kategorii 1 otrzymują ponadto 10 nadbitek swej pracy; kategorii 2 – 5 nadbitek.

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The Bulletin of the Sea Fisheries Institute is indexed and abstracted in ASFA and FSTA.

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Sea Fisheries Institute. Center for Scientific Information and Publishing Kołłątaja 1, 81-332 Gdynia, POLAND http://www.mir.gdynia.pl e-mail: bulletin@mir.gdynia.pl Printed in Poland 2004 300 copies

THIS JOURNAL IS SUPPORTED FINANCIALLY by THE STATE COMMITTEE FOR SCIENTIFIC RESEARCH, POLAND