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PRELIMINARY RESULTS OF EUROPEAN GRAYLING (*THYMALLUS THYMALLUS* L.) FRY REARING TO THE AUTUMN FINGERLINGS STAGE

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Key words: fry growth, survival rate, stocking, breeding parameters, aquaculture.

Abstract

The aim of this study was to test the possibilities of rearing European grayling (*Thymallus thymallus* L.) under controlled conditions to the autumn fingerlings stage. Experimental fish originated from the farmed broodstock. Juvenile graylings were reared in plastic tanks with a bottom area of 1 m^2 and a depth of 0.5 m. Dan-Pel 1656 (Dana Feed) feed was used in the experiment. The mean body weight of an individual fish increased from 0.93 g to 14.60 g during the period of rearing (100 days), while the mean stock biomass increased from 0.37 to 5.15 kg m^{-3}. The highest values of the relative growth rate of an individual (RGR) and the relative biomass rate (RBR) were 4.36% and 3.85% a day, while the lowest values of this parameters were 0.74% and 0.61% a day, respectively. The mean value of the feed conversion ratio (FCR) increased steadily during consecutive stages of rearing, lying within the range from 0.61 to 4.11 for the initial and the last period of the experiment, respectively. The mean cumulative mortality rate was 13.23%, with the values in replications differing significantly ($P < 0.05$). The value of the length variability index (V_L) for the population lay within the range from 8.82% to 13.24%. The mean value of increase in total length index (ITL) were 0.15 and 0.11 for the growth period III and IV ($F = 9.886$; $P < 0.05$). The mean value of the protein efficiency ratio (PER) ranged from 0.39 to 2.82.

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WSTĘPNE WYNIKI PODCHOWU LIPIENIA EUROPEJSKIEGO (*THYMALLUS THYMALLUS* L.) DO STADIUM NARYBKU JESIENNEGO

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Słowa kluczowe: wzrost narybku, przeżywalność, materiał zarybieniowy, parametry hodowlane, akwakultura.

Abstrakt

Celem doświadczenia było sprawdzenie możliwości podchowu w warunkach kontrolowanych lipienia europejskiego (*Thymallus thymallus* L.) do stadium narybku jesiennego. Narybek pochodził od tarlaków ze stada hodowlanego. Ryby podchowywano w plastikowych basenach o powierzchni dna 1 m^2 i głębokości zalewu ok. 0,5 m. W doświadczeniu użyto paszy Dan-Pel 1656 (Dana Feed). W okresie trwania podchowu (100 dni) średnia masa osobnika wzrosła z 0,93 g do 14,60 g, nastąpił również wzrost średniej biomasy obsady z 0,37 do 5,15 kg m^{-3}. Najwyższe wartości relatywnego tempa przyrostów masy osobnika (RGR) oraz relatywnego tempa przyrostów biomasy (RBR) wyniosły odpowiednio 4,36 i 3,85% na dobę, natomiast najniższe wartości – odpowiednio 0,74% i 0,61% na dobę. Średnia wartość współczynnika pokarmowego (FCR) stale wzrastała w kolejnych etapach podchowu, wynosząc od 0,61 do 4,11 odpowiednio w pierwszym i ostatnim okresie doświadczenia. Średnia wartość śmiertelności skumulowanej wyniosła 13,23%, przy czym wartości w poszczególnych powtórzeniach istotnie się różniły (P < 0,05). Wartość wskaźnika zróżnicowania wielkościowego populacji V_L wynosiła od 8,82 do 13,24%. Średnie wartości wskaźnika przyrostu długości ITL wyniosły 0,15 i 0,11 odpowiednio dla III i IV okresu wzrostowego (F = 9,886; P < 0,05). Średnia wartość współczynnika PER mieściła się w zakresie od 0,39 do 2,82.

Introduction

Strengthening natural populations of endangered fish species with stocking material produced under controlled conditions is increasingly becoming the main tool used in their active protection (GORYCZKO et al. 2001). However, stocking open water bodies with farmed fish cannot be a goal in itself. In the case of the grayling (*Thymallus thymallus* L.), it should primarily make up for losses caused by intensive angling (AUGUSTYN and CIEŚLA 2000, HOLČIK 2004, WOŁOS et al. 2004, NÄSLUND et al. 2005), birds of prey (including great cormorant) and by poaching (BARTEL 2000). Ultimately, in the long term, stocking should result in the creation of stable populations, diversified both genetically and in terms of age, and – most importantly – capable of reproduction without any external stimulation. However, the ability to produce such effects depends on two fundamental factors. Firstly, the stocking practice should not violate the genetic uniqueness of local populations of the grayling (KOSKINEN et al. 2001, JANKUN et al. 2003). This is because regular stocking with foreign material, without the features of native populations, leaves a permanent genetic trace (SUŠNIK et al. 2004, DUFTNER et al. 2005) and it can considerably affect their condition (ŁUCZYŃSKI and BARTEL 1997). Secondly, constant improvement of the methods of the grayling breeding (ŁUCZYŃSKI et al. 1986, SZMYT et al. 2004, RANDÁK et al. 2009, SZMYT et al. 2012), as well as developing marking methods of juvenile stages of the species (NAGIĘĆ et al. 1995), provide an opportunity for much more successful accomplishment of stocking programmes (WITKOWSKI et al. 1994) and, consequently, improves the condition of wild fish populations supported with stocking.

Despite great scientific and technical progress, production of stocking material under controlled conditions is beset with problems. These involve – despite intensive efforts – the not-fully mastered breeding biotechniques of many species, the threats posed by pathogens (OCVIRK 1992, PYLKKÖ et al. 2005) and the fish inadvertently getting used to the presence of humans. It seems, however, that despite some reservations, the defined direction of protection of endangered fish species and modern fishery development should be pursued and expanded.

The aim of this study was to analyse the selected breeding parameters achieved in rearing of European grayling (*Thymallus thymallus* L.) to the autumn fingerlings stage.

Materials and Methods

Fish rearing

The experiment was conducted at the Inland Fisheries Institute, Department of Salmonid Research in Rutki. The experimental rearing was conducted for 100 days. Juvenile graylings (Table 1) obtained from the farmed broodstock were used as the study material (GORYCZKO et al. 1995); they were reared in plastic tanks with a bottom area of 1 m^2 and a depth of about 0.5 m.

The fish were fed every day, except on the days preceding the control weighing. The Dan-Pel 1656 feed (Dana Feed) – Table 2 was given by hand in four doses, between 7.30 a.m. and 5.00 p.m. The doses of the feed were calculated every day with the Djournal computer program (FROM and RASMUSSEN 1984). The entire experiment was performed in three replications.

Twice a week during the experiment, preventive baths in solutions of Chloramine T (9 g in 1 m^{-3} of water) were conducted. The tanks were cleaned

Table 1

Number, stock biomass [g] and mean body weight [g] of fish used in the experiment

Replication	Number of fish	Total stock biomass [g]	Mean body weight [g]
1	200	186	0.93
2	200	204	1.02
3	200	166	0.83

every other morning, before the feeding was started. Dead fish were removed from the tanks every day and their numbers were recorded. The temperature during the experiment ranged from 11.3 to 18.9°C (Figure 1), and the dissolved oxygen from 7.2 to 10.2 mg O_2 dm^{-1}.

Table 2

Chemical composition [%] and energy value [MJ kg^{-1}] of the feed (Dan-Pel 1656; Dana Feed) used in the experiment, as claimed by the producer

Component	Content in the feed [%]
Crude protein	56.0
Crude fat	16.0
N-free extractives	11.0
Crude fibre	1.1
Crude ash	10.4
Metabolizable energy [MJ kg^{-1}]	17.1

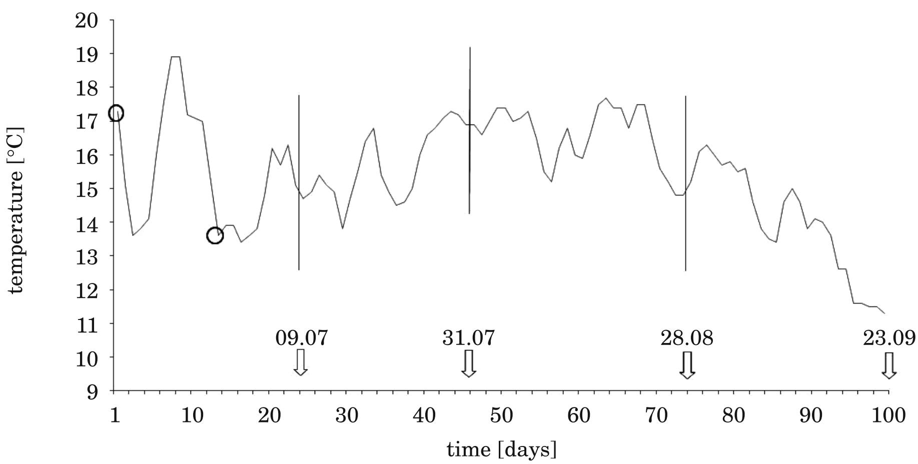

Fig. 1. Daily variability of the water temperature during the experiment. Arrows show the dates of the control measurements of the fish. The circles show the periods of rapid temperature increase.

The experiment was divided into four growth periods and control measurements of the fish were performed after each period. The period durations were: 24 (I), 22 (II), 28 (III) and 26 (IV) days. The measurements were performed after the fish were completely anaesthetised in a solution of Propiscin (IFI Olsztyn, Poland) at the concentration of 0.5 ml dm^{-3}. Each time the stock in the entire tank was weighed and all the fish were counted, which yielded the mean body weight per individual. Due to the small initial size of experimental fish and considerable sensitivity of graylings to all manipulations, especially at this stage, individual measurements of the body length were abandoned. Beginning with the second growth period, the *longitudo totalis* (*l.t.*) of a fish sample ($n = 60$) was measured to assess the variability of the total length. The fish were weighed at an accuracy of 0.1 g and their lengths were measured at an accuracy of 0.1 cm.

Data analysis

The following were calculated from the results: the SGR and SBR coefficients (BROWN 1957):

$$\mathrm{SGR} = 100 \cdot (\ln W_2 - \ln W_1) \cdot D^{-1}$$

$$\mathrm{SBR} = 100 \cdot (\ln n_2 W_2 - \ln n_1 W_1) \cdot D^{-1}$$

where:
SGR – specific growth rate; SBR – specific biomass rate; W_1 – average individual mass at the beginning of the rearing [g]; W_2 – average individual mass at the end of the rearing [g]; n_1 – the number of fish at the beginning of the rearing; n_2 – the number of fish at the end of the rearing; D – time of rearing [days].

Subsequently, the relative growth rate (RGR) and the relative biomass rate (RBR) were calculated (MYSZKOWSKI 1997):

$$\mathrm{RGR} = 100 \left(e^{\frac{\mathrm{SGR}}{100}} - 1\right),\ \mathrm{RGR} = 100 \left(e^{\frac{\mathrm{SBR}}{100}} - 1\right)$$

Feed conversion ratio, FCR:

$$\mathrm{FCR} = P \cdot B^{-1}$$

where:
P – amount of feed fed [g]; B – fish biomass growth [g]

Protein efficiency ratio, PER:

$$\text{PER} = (\text{FB} - \text{IB}) \cdot \text{FPS}^{-1}$$

where:
IB – initial fish biomass [g]; FB – final fish biomass [g]; FPS – amount of protein feed [g].

For the growth periods III and IV, the index of increase in total length – ITL – was also calculated (PEŇÁZ et al. 1989).

$$\text{ITL} = (L_2 - L_1) \cdot D^{-1}$$

where:
L_1 – mean length of an individual (*l.t.*) at the beginning of the rearing period [cm];
L_2 – mean length of an individual (*l.t.*) at the end of the rearing period [cm];
D – duration of the rearing period [days].

Moreover, the length variability index – V_L (RUSZCZYC 1981) of the fish was calculated:

$$V_L = 100 \cdot \text{SDL}^{-1}$$

where:
V_L – length variability index [%];
SD – standard deviation [cm];
L – mean length (*l.t.*) of an individual [cm].

Statistical Analysis

Statistical analysis was performed at the level of $P < 0.05$ ($\alpha = 0.05$). We tested the significance of differences between the values of the rearing coefficients. The following were used: the difference significance test (t-test), the Kruskal-Wallis test (non-parametric analysis of variance), analysis of variance (ANOVA) with Tukey's post hoc test (HSD). To assess the relationship between the examined parameters, we used the method of correlation analysis. The statistical analysis of the results was performed with STATISTICA 9.0 software (Stat Soft, Inc., USA).

Results

During the 100 days of rearing, the mean body weight of an individual fish increased from 0.93 g to 14.60 g (Table 3). A statistically significant relationship was found between the duration of rearing and the final fish body weight (r = 0.990; P < 0.05; Figure 2). The value of body weight was found to have increased during each of the four growth periods, with the maximum on the last day of rearing. The stock biomass was found to have increased during the experiment from 0.37 to 5.15 kg m^{-3}.

Table 3

Rearing parameters of European grayling (*Thymallus thymallus* L.)

Parameters	Growth periods			
	I	II	III	IV
Body weight [g $indiv.^{-1}$]	2.69 ± 0.16	5.99 ± 0.24	11.89 ± 0.43	14.60 ± 1.96
Relative growth rate RGR [% d^{-1}]	4.36 ± 0.22	3.71 ± 0.11	2.57 ± 0.01	0.74 ± 0.38
Relative biomass rate RBR [% d^{-1}]	3.85 ± 0.22	3.31 ± 0.25	2.46 ± 0.08	0.61 ± 0.42
Protein efficiency ratio PER	2.82 ± 0.08	2.22 ± 0.12	1.33 ± 0.04	0.39 ± 0.30
Feed conversion ratio FCR	0.61 ± 0.03	0.75 ± 0.03	1.11 ± 0.02	4.11 ± 1.82
Mortality [%]	5.83 ± 4.81	4.17 ± 2.92	1.49 ± 1.13	1.74 ± 1.15

Mean values ± SD, at the end of each growth period which lasted 24 (I), 22 (II), 28 (III) and 26 (IV) days, after which the control measurements were performed

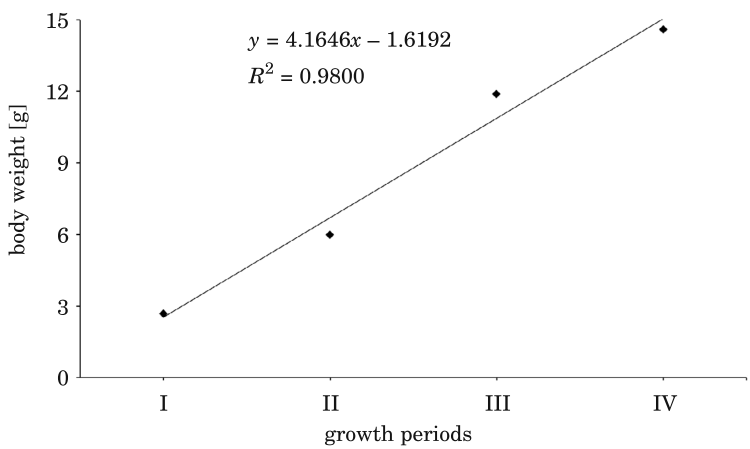

Fig. 2. Mean body weight [g] of European grayling (*Thymallus thymallus* L.) fry at the end of consecutive growth periods of the experiment. Particular growth period lasted 24 (I), 22 (II), 28 (III) and 26 (IV) days

The relative growth rates (RGR) and relative biomass rates (RBR) showed a decreasing tendency in all growth periods (Figure 3). The highest values of both parameters were recorded during the first stage of rearing and were 4.36% d^{-1} (RGR) and 3.85% d^{-1} (RBR), respectively. They were the lowest during the last period: 0.74% d^{-1} (RGR) and 0.61% d^{-1} (RBR) (Table 3; Fig. 3). Relationships between RGR and RBR, and the duration of rearing are expressed in both cases by a negative correlation coefficient, RGR (r = –0.999; $P < 0.05$; Figure 3) and RBR (r = –0.997; $P < 0.05$; Figure 3).

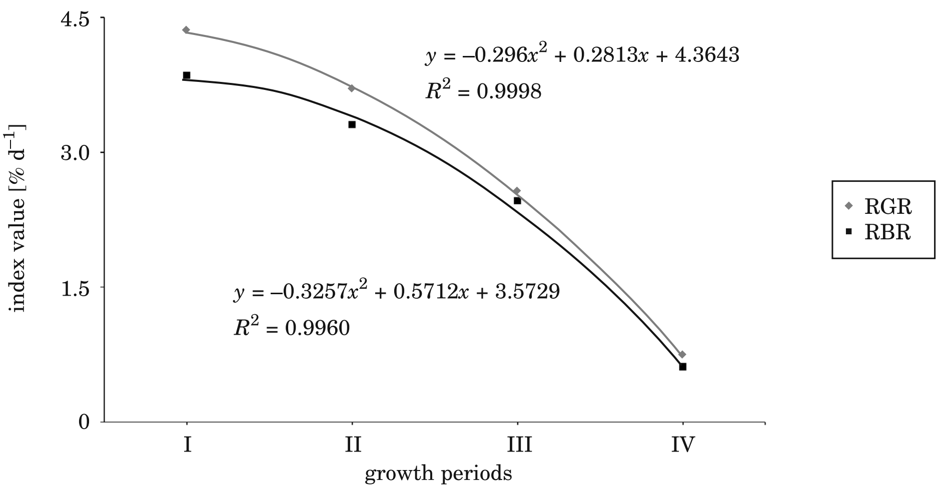

Fig. 3. Relative growth rate (RGR) and relative biomass rate (RBR) of European grayling (*Thymallus thymallus* L.) fry during the experiment. Particular growth period lasted 24 (I), 22 (II), 28 (III) and 26 (IV) days

The mean feed conversion ratio (FCR) increased during successive stages of the experiment (Table 3). There were no significant differences between the replications in growth periods I, II, and III ($P > 0.05$). The FCR values were highly varied during the last stages of the experiment. The values of this parameter during that time were 5.71, 2.12 and 4.51 in the three replications of the experiment, respectively (Figure 4). The variation of FCR was confirmed to be statistically significant with non-parametric analysis of variance (H = 24.789; $P < 0.05$).

The results were used as the basis for showing statistically significant differences in the mean value of protein efficiency ratio (PER) – Table 3 between subsequent growth periods ($P < 0.05$).

The mortality of the grayling observed during different rearing periods differed and ranged from 5.83% (stage I) to 1.49% (stage III) – Table 3.

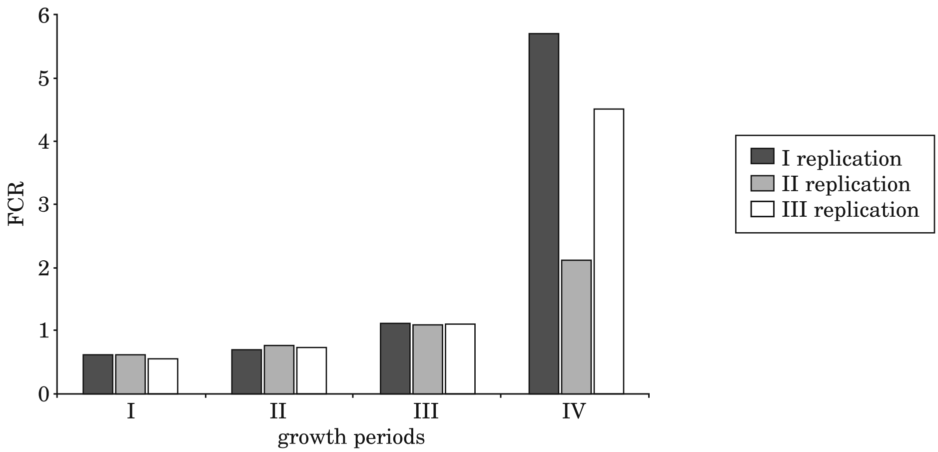

Fig. 4. Feed conversion ratio (FCR) values at the end of consecutive European grayling (*Thymallus thymallus* L.) fry growth periods with replications. Particular growth period lasted 24 (I), 22 (II), 28 (III) and 26 (IV) days. There were no significant differences between the replications in growth periods I, II, and III ($P > 0.05$). In IV growth period there are significant statistical differences between values from subsequent replications ($P < 0.05$)

The value of the cumulative mortality rate during the entire period of the experiment was 13.23%. However, statistically significant differences of 12.53%, 4.58% and 22.58% (test of significance of differences between structure indexes; $P < 0.05$) were recorded in individual replications.

The mean values of the total length increase index (ITL), calculated for the growth periods III and IV of the experiment were similar: 0.15 and 0.11. The analysis of variance showed them to be statistically significantly different ($F = 9.886$; $P < 0.05$). Tukey's test confirmed that the variability observed during the last period of rearing was responsible for the variability of ITL. The highest value of the length variability index (V_L) was recorded during the second growth period and was 13.24 (Table 4).

Table 4

Length of European grayling (*Thymallus thymallus* L.)

Parameter	Growth periods		
	II	III	IV
Mean fish length *l.t.*[cm]	5.2 ± 0.69 (3.8–7.4)	9.6 ± 0.85 (6.9–10.9)	12.7 ± 1.14 (10.2–15.8)
Length variability index V_L [%]	13.24	8.82	9.00

Mean ± SD, range; $n = 60$ as well as the mean value of the length variability index V_L , at the end of II (22 days) , III (28 days) and IV (26 days) growth period of the experiment

Discussion

The studies of the growth of the grayling conducted so far have dealt with fish caught in the natural environment (LUSK 1975, GERTYCHOWA 1976, WITKOWSKI et al. 1989, CARLSTEIN 1997, TUREK et al. 2010). Since the biotechnique of grayling breeding has not been fully mastered (VOVK 1984, OCVIRK and VOVK 1985, CARMIE and JONARD 1988, ŠUMER 1994, KOWALEWSKI 2002, SZMYT et al. 2006), much less data have been published on the growth of the fish under controlled conditions. However, constant aquaculture development aimed at optimising the methods of stocking material production for endangered or heavily exploited species provides ample opportunities in this area of research (ŁUCZYŃSKI et al. 1986, GRUDNIEWSKA et al. 2007, WUNDERLICH et al. 2011).

As the authors expected, steady growth of each individual was observed throughout the rearing period. A similar tendency in terms of the growth, but with a smaller body weight of the graylings during the initial period of rearing, has been observed in other studies (SZMYT and GRUDNIEWSKA 2005). After 9–10 weeks from hatching, they achieved the mean body weight of an individual of 3.8 g. The completion of rearing in the cited study, which took place on 23 July, corresponds to the period of approximately one week before the end of the second growth period in present study.

According to the authors, a distinct decrease in the value of RGR and RBR in the last growth period can be attributed to the decrease in the intensity of the fish feeding at the time. As a consequence, the total fish biomass decreased and the FCR index increased. The results may suggest that it is necessary to modify the feeding strategy during the rearing of grayling, which takes place in early autumn. This may be associated with the amount of feed, frequency of feeding or the energy value of the feed. A change of the feeding procedures is indicated by a rapid increase in the mean value of FCR during the final study period and its variability in individual replications during the period. Interesting results in the last rearing period can also be observed for the protein efficiency ratio (PER). Its mean value decreased steadily and it was variable during the last stage of the rearing. In the case of the rationed feeding strategy, a decrease in intensity of fish feeding is closely related to the increase in FCR and, consequently, a decrease in PER. Similar values to those discussed in present study have been reported by WUNDERLICH et al. (2011). The authors studied the whitefish *Coregonus lavaretus* (L.) and, after 42 days of rearing, they achieved, at different levels of feeding, mean PER ranging from 1.69 to 2.06. High values of the determination coefficient R^2 for the RGR and RBR parameters confirm the claim of the important role played by the duration of rearing in the case of the grayling. Since the stocking requires that the material used in the process should be suited to the local environmental conditions, in some cases it may be necessary to rear the grayling fry to the autumn fingerlings stage (GORYCZKO et al. 2004). Despite the difficulties, it is extremely important to continue the efforts aimed at optimising the breeding biotechnique of this species as it will make it possible to strengthen the natural populations of the grayling and to preserve the environmental biodiversity.

The results of this study allow one to conclude that the grayling is not too sensitive to slow and steady temperature increase or decrease. However, its rapid fluctuations can have an adverse effect on the fry mortality. This may have resulted in the high mortality rate during the first period of the grayling growth: 5.83% (0.23% per day). This value was the highest of all those recorded during the experiment. Similar observations have been reported by CARLSTEIN (1993), linking the highest mean daily mortality rate of the grayling fry (0.93÷2.69% per day) with the rearing period at the most rapid temperature increase and its maximum value (17.2°C).

The decrease in the length variability index V_L observed during the rearing period may have resulted from keeping the fish in controlled conditions and from their consequent gradual domestication. According to RUSZCZYC (1981), the index in excess of 10% should be regarded as significant. This may be of great importance in terms of the effect of stocking on the natural diversity of populations.

The high variability of the cumulative mortality rate in individual replications, as well as other parameters during the final phase of rearing, underlines the importance of verification of assumptions of future research projects involving the rearing of the grayling. Changes may include increasing the number of replications in the experiments, optimising the feeding parameters or the system of rearing. It may be an opportunity to solve at least some of the problems if fry could be reared in recirculating aquaculture systems.

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