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The Effect of the Live Diet Given to Hatchery-Reared Fry of the European Grayling (*Thymallus thymallus*) on Their Survival and Growth in the Wild

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Abstract

The survival rate and growth parameters of grayling fry were determined after stocking them in the wild. Before their release (7 July 2011) into the wild, the fry were reared for 7 weeks in the hatchery, in two groups: (i) fry fed on live daphnids and live chironomids larvae (LFG), (ii) fry fed on a pellet diet (PFG). On the 2 October 2011, the fish survival rate was checked; the fish were caught in the wild. The survival rate and specific growth rates were higher in the LFG than in the PFG. The most effective food for hatchery-reared fish to be used as stock was natural live food: zooplankton and chironomids larvae. In the wild the mean number of chironomids larvae and gammarids found in the stomachs of fish was significantly higher in the LFG than in the PFG. The prevalence of almost all taxa of macroinvertebrates in the stomachs of the LFG was higher than those from the PFG. The live diet supplied in the rearing period has a positively impact on fish foraging skills and their survival in the wild.

Keywords: European grayling, foraging skills, rheophilic fish, stocking.

Introduction

Recently, stocking with hatchery-reared fish is the main way to restore salmonids. Stocking with hatchery-reared fish ensures better survival and growth rates than trying to restore salmonids without rearing fry (Domagała and Bartel 1997; Domagała and Bartel 1999). But it is generally known that the survival of hatchery-reared salmonids in the wild is still low (Wiley *et al*., 1993; Sväsand *et al*., 2000; Weber and Faush 2003) in comparison to wild fish (Fjellheim *et al*., 1995). Therefore, the best way for the production of stocking material of salmonids is still needed. Many authors indicate that live prey used in the rearing of salmonids larvae or fry can lead to an increase of these fish in the wild (Stradmeyer and Thorpe, 1987; Maynard *et al*., 1996; Brown and Laland, 2002; Brown *et al*., 2003a, b), because of the shaping of their foraging skills (Brown and Laland, 2001; Warburton, 2003). Zooplankton, nekton and chironomids larvae as live prey were used in our previous studies to rear the best stocking material of Atlantic salmon, sea trout and brown trout (Czerniawski *et al*., 2010; Czerniawski *et. al*., 2011, Czerniawski *et al*., 2014). We showed that for these salmonids, rearing with live food is the most effective and best possible way for the production of stock-fry.

It is generally known that, the European grayling is the species that needs help of the man to maintain its populations among other salmonids (Koskinen *et al*., 2001; Harsányi and Aschenbrenner 2002; Vehanen *et al*., 2003; Szmyt and Grudniewska 2005). Despite of restitution programme in the last years in Poland, especially in the West Pomeranian Region, the European grayling from year to year in lower numbers has been observed (Witkowski *et al*. 2009). The main cause of this reduction are the river dam, pollutions, the lack of spawning places, and, in consequence, too low numbers of larvae (Vuorinen *et al*. 1998, Wiśniewolski 2002; Harsányi and Aschenbrenner 2002; Honkanen *et al*. 2005). Moreover, the hatchery-reared fry of the European grayling are similar to other salmonids which are also characterized by low survival rates in the wild (Harsányi and Aschenbrenner, 2002). Thus, the aim of the present study was to determine the effect of using live zooplankton and chironomids larvae in the hatchery for the survival and growth of hatchery-reared European grayling in the wild. Similar to the previous study (Czerniawski *et al*., 2011, Czerniawski *et al*. 2014), we assumed a hypothesis that the fish fed on live food during rearing would reach greater survival rates in the wild than pellet-fed fish.

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Material and Methods

The hatchery-reared fry of grayling (*Thymallus thymallus* L.) were used for the stocking experiment. Initial fork length and mass of fry was 22 mm and 0.07 g. The fry were reared for 7 weeks in 6 tanks (three replicates for each variant), and then released into the wild. The volume of water in the tanks was 55 L. The water temperature was kept between 12ºC (at the beginning of rearing) and 16ºC (at the end of rearing) by a cooling device. Every week the temperature in each stream (designed for stocking) was checked and any changes in temperature change were then changed in the hatchery. The density of fish in each tank was 70 fish. The rearing was performed in a closed recirculation system in two variants, in three replications: the live-fed group (LFG) – fry fed on live zooplankton and chironomidae larvae, and the pellet-fed group (PFG) – fry fed on prepared pellet food (Skretting, Perla Larva Proactive 4.0, contents: 62% protein and 11% fat, pellet size 0.3 – 0.8 mm). Live food was received from the culture of zooplankton (*Daphnia magna*) and chironomids larvae. The food for the LFG and the PFG was given *ad libitum* on the water surface, and then daphnids were drifted in the water column, while chironomidsae sank to the bottom. Chironomidae larvae were eaten by fish from the water column and from the bottom. Prior our rearing-experiment, the fish from the larvae stage were reared on a pellet diet in the hatchery of Polish Angling Association.

The fry from the LFG were marked. They had their adipose fin cut off (in the fourth week of rearing). Before the marking the fry was anesthetized with MS-222. The fry from the PFG were left untouched. After 7 weeks of rearing, (7^{th} July), the fish were released into the two similar streams: Trawna and Chojnówka (GPS coordinates: 53.360139, 14.637037). These streams are ca. 4-5 km long and end in the river Płonia. The bottom of these streams is predominantly covered with gravel, and their width varies from 1.0 to 2.0 m. The water temperature in summer does not exceed 20ºC. On the stocking day the water temperature was 16ºC. The fish were transported to the stocking place in plastic bags saturated with oxygen. The distance between the hatchery and the stocking place was 10 km and the transport lasted 15 min. The mortality of fish while being transported was not observed. To be able to perform the experiment, the fish were released to the closed section, using a net. The section was 2 km long. The mesh size of the net was 5.0 mm. It extended from the bottom of the stream to 1 metre above the water surface. In each section of each stream, 100 fish of each feeding group were released together (200 fish in each section). Each day the nets were cleaned.

Fish survival was checked on the 2^{th} October, when they were captured with the use of electric fish gear (Hans Grassl ELT60 II, Germany). On the capture day the water temperature was 12ºC. The fish were caught along the whole length of each section in each stream. To make sure that all the fish were caught, the procedure was performed three times on the same day. The fish were caught by three people: two people were collecting the fish, while the third person walked 50 metres behind them to check if the stunned fish which were left were not carried down the river. No other predatory species were caught because any other predatory fish had been caught with the use of an electric fish gear prior to stocking the stream with the hatchery-reared grayling. Although in the catchment area of these streams kingfisher, minks and otters occurred, they were not observed during the stocking or capture days. After capture, all the fish were measured and weighed. All the fish were killed by an overdose of MS-222 to check their stomach content in the laboratory.

The condition factor (K) was calculated by

$$K = 10^5 \, M \, L_F^{-3}.$$

The specific growth rate was found from the formula:

$$SGR = (\ln M_F - \ln M_I \, 10^2) \, t^{-1} \, (\% \, day^{-1})$$

where: $\ln M_F$ - the natural logarithm of the final mass; $\ln M_I$ - the natural logarithm of the initial mass; t - time (days) between $\ln M_F$ and $\ln M_I$. The statistical significance of the differences in the number of invertebrate taxa in the stomach of fish was tested by the Kruskal-Wallis test, whilst fork length, weight and the condition factor of the captured fish was tested by the variance analysis ANOVA, and the post–hoc Tukey test (Statistica 10 software).

Results

After rearing, both groups achieved the same, high survival rate (Table 1). The dead fish were not detected. In the last week of rearing, the PFG obtained significant higher values of the fork length and weight than the LFG ($P<0.05$) (Table 1). The condition factor values of reared groups differed insignificantly ($P>0.05$).

After the capture of the fish from the streams, the mean survival rate of the LFG was higher ca. 50% than those from the PFG (Table 2). In the wild, the LFG grew faster than the PFG, more inversely than during rearing. In both streams, the LFG obtained a higher rate of SGR than the PFG (Table 2). So, the results of the survival rate and the SGR in the wild could be affected by the way of rearing. The fork length and mass values of the captured LFG also were significant higher than the PFG ($P<0.05$).

Almost all the taxa of the macro-invertebrates that were found in the stomachs of the fish occurred more numerously in the LFG than in the PFG fish. The number of chironomidae larvae and gammarids

Table 1. Final results of rearing and basic characteristics (mean ± SD) of fry used in stocking experiment. LFG – live-fed group; PFG – pellet-fed group

Group	*n*	Survival (%)	Fork length (mm)	Mass (g)	Condition factor
PFG	*50*	100	33.24 ± 2.03 a	0.345 ± 0.046 a	0.94 ± 0.11 a
LFG	*50*	100	36.64 ± 3.45 b	0.498 ± 0.198 b	0.96 ± 0.13 a

Values in columns with different letters = significant differences (P < 0.05)

Table 2. Survival and mean values ± SD of fork length, mass, condition factor and specific growth (SGR) of grayling in streams

Stream	Group	*n*	Survival (%)	Fork length (mm)	Mass (g)	Condition factor	SGR (% day^{-1})
Trawna	PFG	*10*	10	92.68 ± 6.96 a	8.828 ± 1.413 a	1.11 ± 0.11a	2.46
	ZFG	*22*	22	97.70 ± 5.92 b	11.055 ± 2.089 b	1.17 ± 0.11 a	2.96
Chojnówka	PFG	*15*	15	93.16 ± 7.05 a	8.734 ± 1.290 a	0.81 ± 0.16 a	2.45
	ZFG	*28*	28	97.73 ± 6.96 b	11.275 ± 2.944 b	0.89 ± 0.18 a	2.98

Values in columns with different letters = significant differences (P < 0.05)

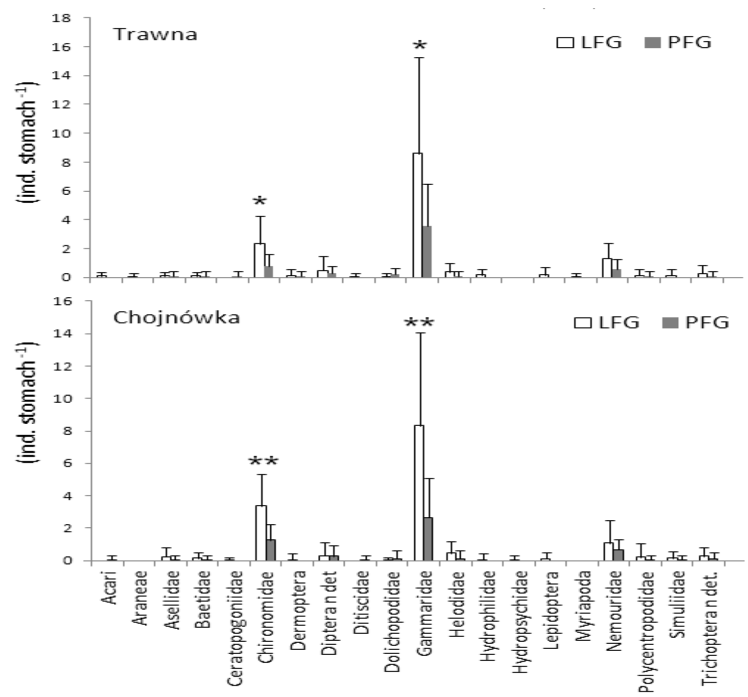

Figure 1. Mean + SD number of taxa individuals in stomach of two experimental groups (LFG and PFG) of grayling fry in the wild (Trawna and Chojnówka). Significant differences between two groups were marked with asterix: * P<0.05, **P<0.01.

was significantly higher in the stomachs of the LFG than the PFG fish (P < 0.05) (Figure 1). The taxa of macro-invertebrates were most common and most frequently encountered in the stomachs of the LFG and the PFG fish (Table 3). However, the prevalence of almost all taxa in the stomachs of the LFG was higher than those from the PFG fish (Table 3).

Discussion

Despite of the well enough-known knowledge of juvenile grayling rearing, new and better ways to produce good hatchery-stocking material are still wanted. Our results show that a much better feed for the commercial rearing of fish is the prepared diet. The faster growth of the reared PFG was caused by favourable nutrients in the fodder. The nutritive value of fodder for grayling fry, in determining the rearing success, was that the commercial pellet food is much better (Łuczyński *et al*., 1986; Carlstein, 1993; Carlstein, 1995), but the live food, although of a lower energetic value, has worth more in the foraging skills of salmonids fry. Additionally, the LFG obtained significantly lower values of fork length and mass than the PFG. A similar observation was made Czerniawski and Czerniejewski (2007), Czerniawski

Table 3. Prevalence of taxa macroinvertebrates in stomach of LFG and PFG (%)

	ZFG		PFG	
	Trawna	Chojnówka	Trawna	Chojnówka
Acari	9	-	-	7
Araneae	5	-	-	
Asellidae	9	18	10	7
Baetidae	9	14	10	7
Ceratopogoniidae		4	10	
Chironomidae	77	96	60	80
Dermoptera	9	4	10	-
Diptera n det	27	18	30	13
Ditiscidae	5	-	-	7
Dolichopodidae	5	4	20	7
Gammaridae	100	100	100	93
Helodidae	27	36	10	7
Hydrophilidae	18	4	-	-
Hydropsychidae	-	7	-	-
Lepidoptera	14	7	-	-
Myriapoda	5	-	-	-
Nemouridae	77	57	50	60
Polycentropodidae	9	7	10	7
Simuliidae	9	11	-	7
Trichoptera n det.	23	25	10	13

et al., (2009), Czerniawski *et al*. (2011), and Czerniawski *et al*. (2014), during the rearing of juvenile brown trout, sea trout and Atlantic salmon on living and prepared diets. However, we believe that prepared feed is not enough to shape the foraging skills of juvenile grayling and this feed could not have a positive impact on the survival of hatchery reared fish in the wild. In our previous studies we recorded that the fry of brown trout, Atlantic salmon and sea trout as hatchery-reared on live zooplankton and live chironomids larvae obtained, in the wild, significantly higher survival rates than fry reared on a pellet diet (Czerniawski *et al*., 2010; Czerniawski *et al*., 2011). The results of the present study show that the live food given during rearing could have contributed to increasing the survival in the wild of other rheophilic species such as grayling. The PFG achieved in the wild a significantly lower rate of survival than the LFG. The results of other authors who, prior to stocking into the wild, reared trout and salmon on pellet-fodder, are also low (Domagała and Bartel 1997; Domagała and Bartel 1999).

It seems that the rates of grayling survival in the streams examined could be lower if other fish lived in these environments. However, our experiment should be treated as semi-wild and semi-controlled conditions. In the streams selected by us, there were no fish predators. The absence of predators in the streams has a significant impact on the survival and growth of juvenile salmonids (Kennedy and Strange 1986)

Analysis of the stomach contents of grayling fry show that the majority of all the taxa of the macro-invertebrates that were found in the stomachs of the fish occurred more numerously in the LFG than in the PFG fish. The same results were observed in the studies of Czerniawski *et al*. (2014), where the LFG hatchery-reared trout, fed the same food as the grayling in the present study, had in their stomachs more organisms that the PFG fish. Moreover, the present study, similar to previous study, which concerned trout (Czerniawski *et al*., 2014), also show that chironomids larvae and gammarids were eaten by the LFG of grayling in larger amounts that by the PFG fish. Perhaps the LFG reared on live chironomidae larvae could catch these taxa better in the wild and the fish remembered this food from their time of rearing, or they preferred chironomids larvae as food in the wild. Chironomids are the main component of the food of fry and adult grayling and other salmonids (Witkowski *et al*., 1984, Meissner and Muotka, 2006). Moreover, during the larval phase the individuals will hunt for exactly the same or similar items they have been successful with in their first feeding trials (Kieffer and Colgan 1992; Brown *et al*., 2003a; Brown *et al*., 2003b, Nunn *et al*., 2012). The PFG also had in their stomach more individuals of chironomidsae and gammarids than other taxa, but this number was smaller than in the stomachs of the LFG. Salmons, trout and grayling also eat relatively abundantly crustaceans, particularly gammarids (Witkowski *et al*., 1984; Strandmeyer and Thorpe 1987; Amundsen and Gabler 2008,). The streams examined offer good nutritional conditions for the fry of salmonids, because of a high density of gammarids and crustaceans (Czerniawski *et al*., 2007). However, despite the selectivity of food by grayling, it seems that the way of rearing could have an influence on the higher number of chironomidae larvae eaten by the LFG than the PFG in the wild and on the higher rates of the survival and growth rates of the fish in the wild. The main cause of these positive rates of the LFG could be better to ready them to capture crustaceans and chironomidsae in the wild, as these foraging skills had been shaped in the period of rearing. According to Morrison (1983), live zooplankton is readily accepted as food because its continuous motion stimulates the interest of the fish and provokes them to attack. Similar conclusions were obtained during the rearing of trout fry on live chironomids larvae (Czerniawski *et al*., 2014). In what was shown in our previous study, we believe that the important factor in foraging skills was the motion not only of zooplankton, but also of the chironomids larvae, in the water column and on the bottom. The same pattern was observed in the present study on the fry of the grayling. In our other study we observed that hatchery-reared fish fed on chopped chironomidsae larvae achieved low rates of survival in the wild (Czerniawski *et al*., 2010). This result indicates that an important factor in the rearing of stocking material of salmonids is the movement of the prey.

On the basis of our previous experiments on salmon and trout fry (Czerniawski *et al*., 2010; Czerniawski *et al*., 2011; Czerniawski *et al*., 2014), and the present study on grayling fry, we indicate that live food such as zooplankton, fish larvae or chironomids larvae used in the rearing could positively affect the survival of hatchery-reared salmonids in the wild. As we said in our previous studies, the live food used during rearing can have a positive effect on the foraging skills, survival rates and the growth of salmonids fry in the wild, so we can say that the same positive effect of live food on the survival rates of hatchery-reared grayling fry in the wild has been observed.

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