FACULTY OF **FISHERIES** AND **PROTECTION** OF **WATERS**
UNIVERSITY OF SOUTH BOHEMIA IN ČESKÉ BUDĚJOVICE

Adaptability of artificially reared brown trout (*Salmo trutta* m. *fario* L.) and European grayling (*Thymallus thymallus* L.) in free water condition

Adaptabilita uměle odchovaných násad pstruha obecného (*Salmo trutta* m. fario L.) **a lipana podhorního** (*Thymallus thymallus* L.) **v podmínkách volných vod**

Jan Turek

Vodňany, Czech Republic, 2010

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CHAPTER 1

GENERAL INTRODUCTION

1. REVIEW

Brown trout (*Salmo trutta* m. *fario* L.) and European grayling (*Thymallus thymallus* L.) are the most important native fish species in upper parts of running waters in the Czech Republic and in Europe. Both species are very popular for the recreational fishing. Population of both species has decreased in last years. Number of graylings and brown trout caught and appropriated by sport anglers in the Czech Republic is displayed in Fig. 1 and 2 (www.rybsvaz.cz) for 1993–2009.

The negative influences for salmonid populations in Europe are water pollution, regulations of river beds, destruction of spawning grounds, fish predators and overfishing (Magee, 1993; Northcote, 1995; Uiblein et al., 2001). Water pollution can evoke the changes in reproduction ability of wild salmonids population. Kolářová et al. (2005) observed biological responses of fish induced by anthropogenic pollution. In comparison with the control site locality at the source of pollution and on another locality downstream were found considerably polluted. This contamination was manifested also by repetitious findings of high vitellogenin concentrations in blood plasma, resulting in adverse effects on brown trout reproduction. The predation by otters, herons and cormorants is the main reason for destabilization of salmonid population in many localities (Lusk et al., 2003; Mareš et Habán, 2003; Harsanyi et Aschenbrenner, 2002). To re-establish threatened or extinct populations and increase the recreational fishery, hatchery-reared fish are commonly stocked as part of enhancement and restoration programmes (Cowx, 1994). The stocking of hatchery-reared salmonids in rivers has been common practice worldwide for several decades (Einum and Fleming, 2001). To evaluate the effect of these programmes, knowledge of post-stocking mortality, growth and movement of stocked fish is crucial. The success of stocking fish into natural waters depends on a variety of factors such as origin of fish, conditioning and acclimatization, handling and transportation of stock, stocking density, size or age of stock, timing of stocking and predation (Cowx, 1994). It is generally hypothesized that hatchery-reared salmonids are at a competitive disadvantage when introduced into waters with resident populations (Needham and Slater, 1944; Miller, 1958).

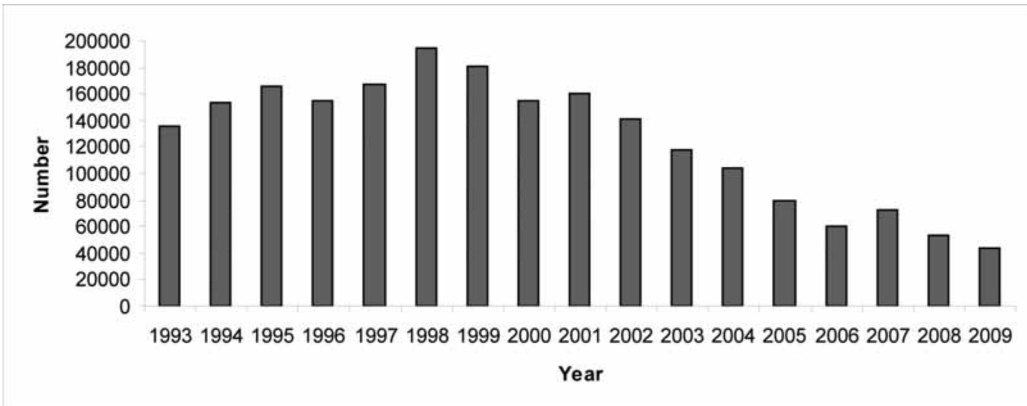

Figure 1. *Number of brown trout appropriated by anglers in fishing areas of Czech Anglers Union in years 1993–2009.*

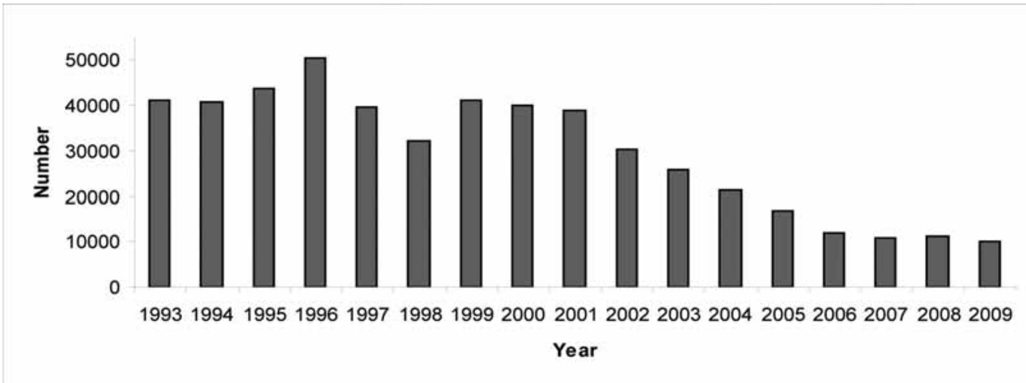

Figure 2. *Number of grayling appropriated by anglers in the fishing areas of Czech Anglers Union in years 1993–2009.*

SURVIVAL OF STOCKED SALMONIDS IN THE WILD

Most recent publications suggested that survival of stocked salmonids is lower in comparison with resident wild fish. Weiss and Schmutz (1999a) stocked two strains of individually marked hatchery-reared adult brown trout into a limestone stream. The estimated survival after one month was comparable to that for resident brown trout and rainbow trout, but declined to 14% after 8 months compared with 52% for resident trout. The high mortality of stocked juvenile brown trout was observed by Borgström et al. (2002), only 1% of stocked fish survived for 3 years in the natural conditions. A review by Cresswell (1981) compared several earlier studies from the U.S.A. and the U.K. of recapture rates and movements of hatchery-reared brown trout and found recapture rates of 3–35% for fish stocked into rivers. Mortality at level 99% in stocked artificially reared brown trout prey compared to 79% in wild conspecifics observed by Fjellheim et al. (1995).

Rearing technique of stocked fish is the important factor for post stocking behaviour and survival. Huet (1986) reported that fish reared in ponds were more suitable for stocking into natural waters because these fish were used to feeding on natural prey. Higher survival of pond-reared brown trout fed by natural prey versus in hatchery-reared ones fed by dry diets was observed by Näslund (1992) in a Swedish stream. Carlstein (1997) found that European grayling reared in a natural pond showed higher post-stocking survival in a lake than fish reared in a conventional hatchery. On the contrary, Johnsen and Hesthagen (1990) found great variations in the recapture rates of pond and hatchery brown trout in one stream between years, and between streams in different areas the same year. Their results indicate that the rearing method is not essential to the recapture rate.

POST-STOCKING FEEDING, GROWTH AND MOVEMENT OF STOCKED SALMONIDS

The survival and growth of stocked fish is dependent on their ability to adaptation for natural prey intake (Ersbak and Haase, 1983; Bachman, 1984; Johnsen and Ugedal, 1986). A number of studies, in which prey choice and prey handling ability in hatchery-reared fishes and in wild fishes from the same stock were compared. Behavioural deficits in cultured fishes were found. Munakata et al. (2000) observed several weeks after release into a natural river that hatchery-reared honmasu salmon had significantly emptier stomachs than did wild fish and were more likely to have eaten non-food material such as stones and leaves.

When a new prey item was offered to both groups (a cricket), the caught wild brown trout (which had an experience with a variety of live prey) ate 75% more items and handled them more efficiently than did hatchery-reared fish of the same stock (Sundström and Johnsson, 2001). On the other hand, no long-term differences were found in post stocking feeding between hatchery-reared and wild (Johnsen and Ugedal, 1986) or between pond- and hatchery-reared brown trout (Johnsen and Hesthagen, 1990; Johnsen and Ugedal, 1990). In the experiment of Thorfve and Carlstein (1998) 33.3% and 22.8% of the hatchery-reared juvenile grayling and brown trout, respectively, had eaten natural food items within 48 h of their release in a semi-natural steam. Hatchery-reared fishes can learn to feed on novel prey by seeing a trained conspecific 'demonstrator' feeding on such prey (social learning; Brown and Laland, 2001).

Hatchery-reared fish are commonly greater than wild fish of the same age. It may be caused by selection (intended or unintended) in hatcheries and by high energy content in used commercial feed (e.g. Fleming et al., 2002; Sundström et al., 2004; Tymchuk et al., 2006). Several studies found the differences between wild and cultured fishes in food intake quantity. For example, both Atlantic salmon and brown trout from strains selected for rapid growth show higher rates of food consumption than do the hatchery-reared offspring of wild fishes from the population of origin (Thodesen et al., 1999; Sanchez et al., 2001).

Inconsistent results were published about post-stocking growth rate of artificially reared fish. The slower growth of stocked brown trout in comparison with wild fish was observed by e.g. Näslund (1992), Fjellheim et al. (1995) and Hesthagen et al. (1999). On the contrary similar (Baer, 2004) or higher (L´Abee-Lund and Saegrov, 1991) growth rate was found in stocked than in wild brown trout. Weiss and Schmutz (1999b) showed that stocked hatchery-reared brown trout had lost 7–11% of their body mass three months after release in the limestone stream but gained weight 5–25% over the same period in the crystalline stream. This result indicated that abiotic factors in particular biotopes substantialy affected growth of stocked fish. The period between release and recapture might also be significant in post-stocking growth evaluation. Thorfve (2002) found a decrease of more than 10% from pre-release mass in stocked grayling after one week in the watercourse. Fish recaptured more than 2 weeks after release had lost a significantly smaller percentage of their initial weight compared with fish recaptured in the first 2 weeks.

Utility of stocking programs also depends on the migration of stocked fish out of stocking areas. Jorgensen and Berg (1991) reported that overall movement of wild brown trout was greater than a domestic strain, except, as in the present study, during the first few days after stocking when a domestic strain moved more than their wild counterparts. After flooding, higher residency of pond-reared fish than of wild fish was found by Weiss and Kummer (1999). On the contrary Bohlin et al. (2002) observed that stocked hatchery fish and introduced wild fish moved more than wild resident fish. Aarestrup et al. (2005) suggested a possible effect of movement on post stocking survival. After 5 weeks post-stocking, surviving hatchery-reared brown trout had a significantly lower mean movement per day than fish, which later either died or disappeared. This difference in behaviour was most pronounced 2 to 8 days after release.

Uniform reports are published about post-stocking movement of grayling. Some evidence from Magee and Byorth (1994) and Kaya and Jeanes (1995) showed a high degree of post-stocking downstream dispersal in juvenile Arctic grayling (*Thymallus arcticus*, Pallas), in the upper Big Hole River (Montana, USA). Studies conducted in a semi-natural experimental stream in Sweden did not demonstrate an influence of origin of the stocked grayling (Carlstein and Eriksson, 1996) or the presence of brown trout (Thorfve and Carlstein, 1998), on high downstream post-stocking dispersal.

EFFECT OF STOCKING PROGRAMS ON WILD POPULATION

The stocking programs are sometimes criticised for potential influences of stocked fish on the population of resident wild fish and even on water ecosystems. Stocked fish may influence wild fish negatively by predation, competition, earlier migration, genetic drift, higher attraction for predators and transmission of diseases (White et al., 1995). Several authors have demonstrated the competitive superiority of resident salmonids over introduced fish (Brännäs, 1995; Glova and Field-Dodgson, 1995), known as the 'prior residence effect' (Huntingford and De Leaniz, 1997; Rhodes and Quinn, 1998). In contrast, some authors suggested that hatchery-reared salmonids may have competitive advantages over wild conspecifics that may contribute to the displacement of wild fish (Nickelson et al., 1986).

For example Bohlin et al. (2002) found no effects of stocking wild or hatchery reared 1-year-old brown trout on the recapture rates of resident trout, which suggests that survival was not strongly affected by competition. Growth rate of resident brown trout was negatively affected by stocking of wild or hatchery conspecifics. Wild fish were also remarkably stationary, regardless of doubled density by stocking. Baer and Brinker (2008) showed that hatchery-reared trout grew more slowly and were more mobile than resident trout, and that their growth was inversely density dependent. In contrast, growth of the resident trout was not affected by stocking. The recapture of 1+ resident and hatchery-reared trout was inversely density dependent. The effect of stocking on dispersal of resident brown trout was significant in the limestone stream but not significant in the crystalline stream. Again, negatively affected growth of wild fish was discovered in the limestone stream only (Weiss and Schmutz, 1999b). It may suggest that interaction between resident and stocked fish is dependent on the local situation.

The behaviour patterns used during competition fights are often similar in cultured and wild fishes (Mork et al., 1999), the intensity of aggression may be higher or lower in cultured stock depending on feeding regimes, by means of domestication and careful selection (Ruzzante, 1994). Salonen and Peuhkuri (2004) observed in three populations of grayling the lower level of aggressive behaviour in the second generation hatchery strains than in the wild strains at the age of 0+ years. Differences still existed, when the strains had been reared under common hatchery conditions for a year. Due to similar rearing conditions, authors supposed genetic divergence of the strains.

Deverill et al. (1999) reported that in an artificial stream, established wild brown trout initiated 44% of the mean aggressive acts whilst hatchery-reared trout initiated 34% and introduced wild trout initiated 22%. Established fish stayed home stations and showed a positive mean specific growth rate during the experiments. Introduced hatchery reared fish were more aggressive and exhibited a lower mean specific growth rate (SGR) than simultaneously stocked wild fish (in both groups the SGR was negative), suggesting that excessive expenditure of energy for unnecessary aggression may contribute to the poor survival of hatchery-reared fish after they are stocked into streams.

Stocking with individuals from nonautochthonous populations might have severe effects on the genetic structure of local populations and seriously threaten their maintenance through the deleterious effects of inbreeding and outbreeding resulting in reduced fitness to local conditions (Avise and Hamrick, 1996). Duftner et al. (2005) found that the genetic composition of the Drau grayling population, situated in the southern Alps, has been seriously altered through the stocking of fish belonging to the northern alpine mtDNA lineage as only 62% of the fishes sampled carried haplotypes representing the native southern alpine lineage.

In contrast, Baer et al. (2007) considers genetic impact of stocked adult (legally sized) brown trout through introgression as limited by the short residence time of stocks in areas with sport fishing exploitation.

THE AIMS OF THE STUDY WERE TO:

- *Evaluate the overall performance of several age categories of brown trout and grayling, reared under different condition after their stocking into natural river.*
- *Asses the effects of stocking of artificially reared fish on wild salmonids population in river.*
- *Propose measures enabling to enhance the production of good quality and adaptable stockfish of the target species for restocking the open waters.*

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CHAPTER 2

POST-RELEASE GROWTH AND DISPERSAL OF POND AND HATCHERY-REARED EUROPEAN GRAYLING *THYMALLUS THYMALLUS* COMPARED WITH THEIR WILD CONSPECIFICS IN A SMALL STREAM

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POST-RELEASE GROWTH AND DISPERSAL OF POND AND HATCHERY-REARED EUROPEAN GRAYLING *THYMALLUS THYMALLUS* COMPARED WITH THEIR WILD CONSPECIFICS IN A SMALL STREAM

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ABSTRACT

The growth and dispersal of stocked European grayling, *Thymallus thymallus*, reared in a hatchery (fed dry food pellets) or in a pond (fed natural food) compared to their wild conspecifics was assessed from the recapture of individually tagged fish 168 days after their release into the Blanice River, Czech Republic. Recapture rates and site fidelity were higher for wild Grayling than for artificially reared fish. Specific growth rate and upstream/downstream dispersal did not significantly differ between any of the groups of fish. An influence of rearing conditions (pond v. hatchery) on the overall performance of stocked fish was not demonstrated. Initially lower condition factors of reared Grayling were equal to wild fish after recapture, suggesting adaptation of artificially reared fish that remained in the sections studied.

Keywords: stocking, river management, Salmonidae

1. INTRODUCTION

The stocking of hatchery-reared salmonids in rivers has been common practice worldwide for several decades (Einum and Fleming, 2001) to mitigate loss of stocks, to enhance recreational or commercial catches, and to restore or create new fisheries (Cowx, 1994). However, hatchery-reared salmonids are disadvantaged in several ways compared to wild fish. They show higher mortality rates (Cresswell, 1981; Weiss and Schmutz, 1999), reduced territoriality (Sundström et al., 2003), and lower feeding efficiency (Sundström and Johnsson, 2001). Furthermore, stocked fish are more vulnerable to predation (Milinski, 1993) due to rearing (Brown and Laland, 2001) or selection in hatcheries (Einum and Fleming, 1997; Fernoe and Järvi, 1998).

The European grayling, *Thymallus thymallus* L., as well as other salmonids, have been stocked in lakes and streams, but the efficacy of such operations has rarely been examined (Carlstein and Eriksson, 1996). Carlstein (1997) found that Grayling reared in a natural pond showed higher post-stocking survival in a lake than fish reared in a conventional hatchery. Some further evidence from Magee and Byorth (1994) and Kaya and Jeanes (1995) showed a high degree of post-stocking downstream dispersal in juvenile Arctic grayling (*Thymallus arcticus* Pallas), in the upper Big Hole River (Montana, USA). Studies conducted in a semi- natural experimental stream in Sweden did not demonstrate an influence of origin of the stocked Grayling (Carlstein and Eriksson, 1996) or the presence of brown trout, *Salmo trutta* L. (Thorfve and Carlstein, 1998), on such post-stocking dispersal.

The aims of this study were to test whether rearing technique (hatchery v. pond) has an effect on growth, post-stocking dispersal, and overall performance of Grayling released into a stream, when compared with similar sized wild fish. A mark-recapture technique was used in nine sections of the Blanice River, Czech Republic to investigate these questions.

2. MATERIAL AND METHODS

STUDY AREA

The Blanice River in South Bohemia is 93 km long, with a drainage area of 860 km^2. Nine sections, each 100–200 m long, were located within a 5 km length of the river (km 53–58). From these sections control (1, 2 and 9) and experimental (3–8) sections were randomly designated (Fig. 1 for details). All sections were situated in a protected area (with a ban on fishing), downstream from the Husinec water reservoir (37 ha; 2.5 x 10^6 m^3). There are no weirs in this part of the river, and the flow is characterized by large seasonal fluctuations, with an annual mean flow of 3.5 m^3 s^{-1}. During the experiment (May – October 2006), mean (± SD) flow was 1.6 ± 1.2 m^3 s^{-1}, mean (± SD) water temperature was 14.0 ± 3.3 °C, and the pH was 7–7.8. There were no big changes in flow or temperature regime during the study period in the river. The experimental sections, which are at an elevation of about 500 m above sea level, are located in the countryside with broad-leaved trees and meadows. These sections were similar with regard to bottom substrate (gravel and stones), banks (naturally formed), and water velocity. In each of the nine sections, the depth range was 10–80 cm. The dominant fish species were brown trout (*Salmo trutta* m. *fario* L.) and European grayling (*Thymallus thymallus*, L.). Common sculpin (*Cottus gobio*), stoneloach (*Barbatula barbatula* L.), roach (*Rutilus rutilus* L.), and gudgeon (*Gobio gobio* L.) were caught occasionally.

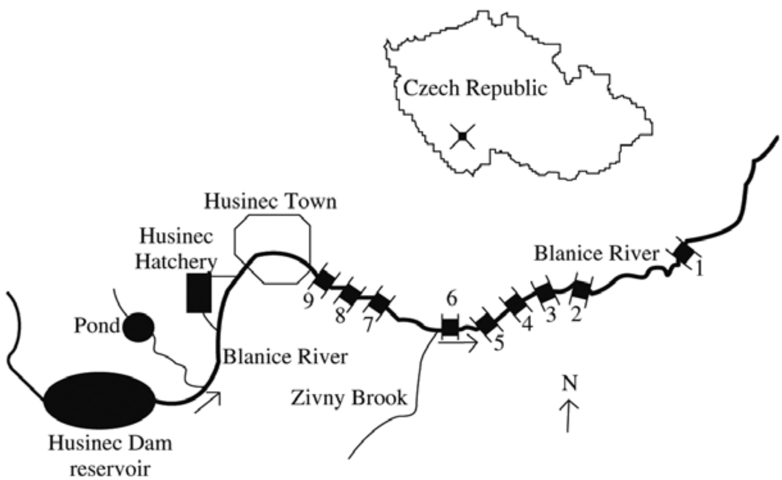

Figure 1. *Map showing the locations of control (1, 2 and 9) and experimental (3–8) sections in the study area.*

EXPERIMENTAL FISH

All the experimental fish were progeny of a resident wild broodstock in the Blanice River. Two year-old grayling originated from artificial spawning. They were reared from fingerling stage in ponds (pond fish) or in concrete tanks (hatchery fish). Pond fish ate naturally provided prey (zooplankton, benthos, terrestrial invertebrates) and were held in a natural pond of c. 1 ha supplied with water from a tributary of the Blanice River. Hatchery fish were fed on conventional dry food pellets and held in concrete tanks in the Husinec Hatchery (Czech Anglers´ Union), supplied with water from the Blanice River. Both rearing facilities were situated c. 5–7 km upstream from the study area. Wild fish of corresponding size originated from natural spawning and were caught by electrofishing in the sections studied. The total area of three sections with stocking pond/hatchery fish was similar (about 3,800 m^2), total area of three control sections was approximately half as large (2,000 m^2). The minimum distance between adjacent sections was 60 m. Initial density of wild fish was similar in all types of sections.

SAMPLING PROCEDURES

For evaluation of wild resident Grayling population in each section, electrofishing was conducted by wading upstream in the river. Sections were rigorously sampled twice using two back-pack pulsed-DC electrofishing units (FEG 1500, EFKO-Germany). Another two pulsed-DC electrofishing units (FEG 3000, EFKO-Germany) were situated at the upper border of the section to prevent fish escaping upstream. Every section was sampled once during the period 9–11 May 2007. Experimental wild grayling were anaesthetized with 2-phenoxy-ethanol (0.2 ml l^{-1}), measured (standard length L_S, mm), weighed (body weight M, g), and tagged (visual alphanumeric tags VIA, Northwest Marine Technology, Ltd., USA). Fish were released near the point of capture after they recovered their equilibrium and showed spontaneous swimming activity (c. 5 min after tagging). Artificially reared, grayling were stocked (in 2–3 small groups) in the middle part of experimental sections on 12 May 2007 (sections 3–5, pond fish; sections 6–8, hatchery fish). Water from the Blanice River was used for the transportation in a transport tank fitted with an oxygen injection system and the duration of the transport did not exceed 20 minutes. Those fish were similar as wild conspecifics individually tagged before release in the river. The number of stocked fish corresponded to the number of tagged wild grayling of a similar size occurring in a given section. Consequently, population of grayling of this size category was doubled in each experimental section. Control sections (1, 2 and 9) were left unstocked. For detailed information on tagged experimental fish, see Table 1.

Six months after release (24–26 October 2007), post-stocking performance of tagged grayling was evaluated. The fish were recaptured by the same method and with the same equipment described above. The experimental sections and the sections between them, including approx. 2 km of the river upstream and downstream from the experimental area, were electrofished thoroughly. All tagged fish were identified, measured, weighed, and released near the point of capture. Subsequently, condition factor (K) and specific growth rate (SGR) were determined for each recaptured fish ($K = (W L_S^{-3}).100$; $SGR = (\ln W2 - \ln W1)/(t2 - t1).100$, where W1 and W2 are initial and final weights on days t1 (stocking) and t2 (recapturing), respectively). Site fidelity was defined as recapture of fish in the sections where they were originally released. Otherwise, the direction of their post-stocking dispersal (upstream–downstream) was recorded.

Table 1. *Mean ± SD standard length (L_S), weight (W), condition factor (*K = ($W.L_S^{-3}$).100) of the tagged grayling.*

Group	Number of fish	L_S (cm)	W (g)	K*
Wild – C	43	17.0 ± 0.9	69.2 ± 10.0	1.41 ± 0.11
Wild – E	175	17.1 ± 0.9	70.4 ± 12.0	1.40 ± 0.13
Pond	88	18.0 ± 0.9	66.9 ± 8.6	1.15 ± 0.12
Hatchery	92	18.4 ± 1.5	75.9 ± 17.6	1.20 ± 0.13

Wild – C (wild fish in control sections) Wild – E (wild fish in experimental sections)

STATISTICAL ANALYSES

The data were analysed with SAS (version 9.1; SAS Institute Inc.). The data were tested for normality and transformed if necessary. Analysis of categorical, repeated measurements based on the generalized estimating equation (GEE) approach (Liang and Zeger, 1986) using the GENMOD procedure (SAS, version 9.1) with binomial distributions was applied. The GEE is an extension of generalized linear models that provides a semiparametric approach to longitudinal data analysis. In this study, the GENMOD procedure was designed to estimate the probability that i) fish would be recaptured; ii) would display site fidelity; iii) would disperse downstream from the release section; and iv) would disperse upstream from the release section. The explanatory variable 'origin of fish' was categorical, containing four classes (pond-reared fish, hatchery-reared fish, wild fish from experimental section, and wild fish from control section). The GEE analysis produced the score statistics and also parameter estimates with standard errors, confidence intervals, Z and Wald statistics, and P values. The Z and Wald statistics generally produce more liberal P values than the score statistic. It is recommended that the more conservative score statistics for Type 3 GEE analysis be reported, particularly for small sample sizes (Stokes et al., 2000). Therefore, we refer only to the score statistic in this study. Tests for multiple comparisons were constructed using the ESTIMATE statement.

Separate Linear Mixed Models (LMM) were applied for the following dependent variables: weight (LMM I), condition factor K (LMM II), and specific growth rate (LMM III). To randomize the effect of specimen and river section, all analyses were performed using mixed model analysis with individual fish and a river section as random factors using PROC MIXED. The fixed effects were, classes 'origin of fish' (pond-reared fish, hatchery-reared fish, wild fish from experimental section, and wild fish from control section); 'site fidelity' (yes, no); 'dispersal downstream' (yes, no); and 'dispersal upstream' (yes, no). The significance of each effect in the LMM was assessed by the F-test, on sequential dropping of the least significant effect, starting with a full model including all interaction terms. The significance of each fixed effect in the LMM models was assessed by the F-test. Least-squares-means (LSM, further referred to as 'adjusted means') were computed for each class, and differences among classes were tested by t test. For multiple comparisons, the Tukey-Kramer adjustment was used. The degrees of freedom were calculated using the Kenward-Roger method (Kenward & Roger, 1997).

3. RESULTS

A total of 398 tagged grayling were released and 93 were recaptured (Table I and II). Wild fish from control, as well as from experimental sections, showed a higher probability of being recaptured than pond or hatchery-reared fish (χ^2 = 15.45; d.f. = 3; P < 0.0015). The differences in recapture rates between the two groups of reared, and between the two groups of wild fish, were not significant [Fig. 1(a)]. Wild fish displayed site fidelity more often than artificially reared fish (χ^2 = 9.41; d.f. = 3; P < 0.0243) with non-significant differences between groups of wild and between groups of reared fish [Fig. 1 (b)]. The effect of the origin of fish on their upstream and/or downstream dispersal was not significant (Table 2).

Table 2. *Recapture and migration of released tagged graylings in percent. Numbers of fish are given in parentheses.*

Group	Total recapture (%)	Recapture in original section (%)	Recapture outside original section (%)	Migration upstream/ downstream (%)
Wild – C	40.(17)	21.(9)	19.(8)	12.(5)/7.(3)
Wild – E	28.(49)	16.(28)	12.(21)	8.(14)/4.(7)
Pond	16.(14)	6.(5)	10.(9)	7.(6)/3.(3)
Hatchery	14.(13)	10.(9)	4.(4)	2.(2)/2.(2)

Wild – C (wild fish in control sections) Wild – E (wild fish in experimental sections)

No significant differences in weight were found among the individual groups of fish (Table 3). Correspondingly, both overall and within-group differences between the initial weight and initial condition factors (within-group) of fish that were recaptured and fish that disappeared from studied sections (no-recaptured fish) were not significant. Wild fish showed significantly higher condition factors than reared fish at the beginning of the experiment ($F_{3, 98}$ = 18.14; P < 0.0001), with non-significant differences within groups of wild and reared fish (Fig. 2). No significant differences in condition factor among groups were found after recapture. The specific growth rate (SGR) of the recaptured fish did not vary significantly among the experimental groups (Table III) and was not influenced by migratory and/or sedentary behaviour (Table IV). Site fidelity and/or dispersal of recaptured fish did not influence their condition (Table IV).

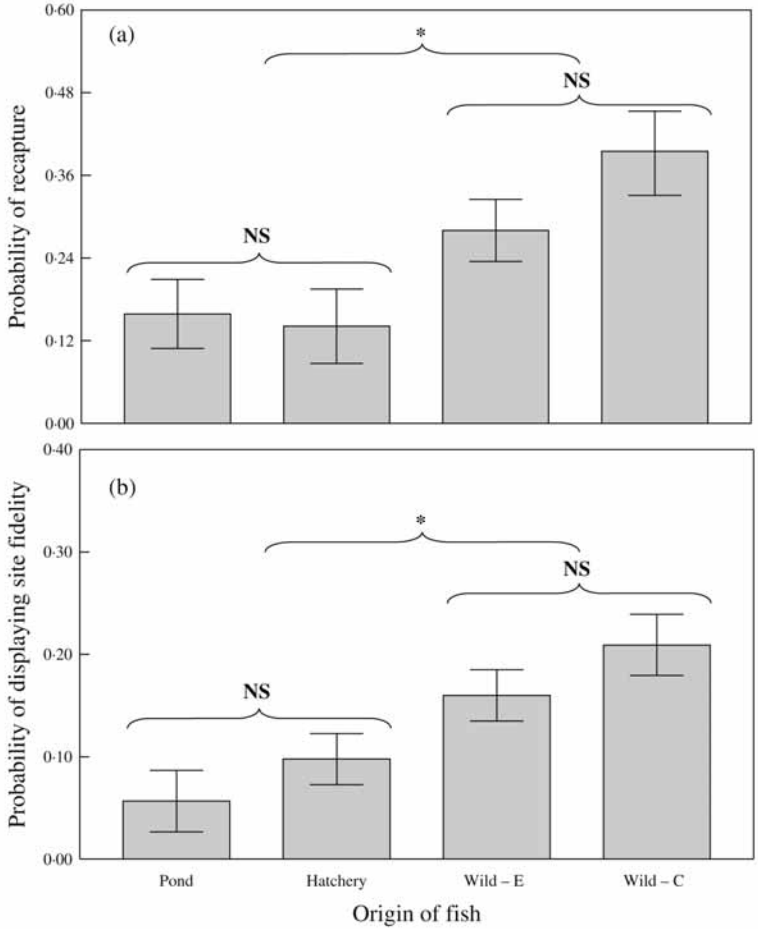

Figure 2. *Predicted values (with confidence intervals) resulting from logistic regression model testing (a) probability of recapture and (b) displaying site fidelity in relation to the origin of released fish (Wild E – wild fish from experimental sections; Wild C – wild fish from control sections; NS – non-significant differences among classes; 0.05 – significant differences among classes at P < 0.05).*

4. DISCUSSION

The study demonstrated a lower recapture of pond and hatchery-reared grayling released into a natural stream compared to similar-sized wild conspecifics. These results correspond with those of many authors (e.g. Ersbak and Haase, 1983; Arias et al., 1995; Weiss and Schmutz, 1999), who also found a lower post-stocking survival of hatchery-reared fish. Carlstein (1997) showed an advantage of rearing of grayling in natural ponds as opposed to a conventional hatchery because of their higher post-stocking survival in lakes. These results do not correspond to those of the present study, where no significant difference was found in recapture rates between pond and hatchery reared fish. The discrepancy could be the result of age difference as Carlstein (1997) released 1 year-old grayling, while those used in the present study were 2 years old. The release site could also play a role, since conditions in a pond are considerably closer to those in a lake than in a stream.

There are living otters and herons, in the study area. Predation of those predators may be one of the reasons of lower recapture of reared fish. Several authors have demonstrated the competitive superiority of resident salmonids over introduced fish (Brännäs, 1995; Glova and Field-Dodgson, 1995) known as the 'prior residence effect' (Huntingford and DeLeaniz, 1997; Rhodes and Quinn, 1998). This effect may be the other reasons of lower recapture of reared fish.

The period between release and recapture might also be significant. Thorfve (2002) found a decrease of more than 10% from pre-release weight in stocked grayling after one week in the watercourse. Weiss and Schmutz (1999) showed that stocked hatchery-reared *S. trutta* had lost 5–25% of their body weight three months after release in the stream. In the present study, no weight loss was recorded six months after release in any group of fish.

Sundström and Johnsson (2001) reported, when offered a prey item that was novel to both groups, wild-caught brown trout (which had a life-time's experience with a variety of live prey) ate 75% more items and handled them more efficiently than did hatchery reared fish of the same stock. In present study, the feeding by natural prey did not provide benefit for pond-reared fish in recapture rates and growth compared with hatchery fish (fed by pellets only), six month post stocking. This fact could be a result of social learning. Hatchery reared fish can learn to feed on novel prey by seeing a trained conspecific 'demonstrator' feeding on such prey (Brown and Laland, 2001).

Furthermore, while the condition factor of reared grayling was initially lower, it was equal to that of wild fish at recapture, suggesting adaptation of those artificially reared fish that remained in the experimental sections. No significantly differences in initial K values and weight between recaptured and no-recaptured fish within each group of fish suggest that recapture rate was independent on initial condition and/or weight of fish. Despite, lower initial condition factor could disadvantage both groups of reared fish compared to wild fish. Thus, even considering the lower recapture rate, the results cannot be interpreted as a general failure of artificially reared grayling in running waters, as was suggested by Kaya (1990).

Table 3. *Mean ± S.D. weight (W) and condition factor (K) of the recaptured graylings before and after the experiment and their specific growth rate (SGR).*

Group	n	W before $_{(g)}$	K before	W after $_{(g)}$	K after	SGR $_{(\%.day^{-1})}$
Wild – C	17	69.9 ± 0.13^a	1.43 ± 0.13^a	111.5 ± 17.1^a	1.21 ± 0.13^a	0.28 ± 0.08^a
Wild – E	49	71.1 ± 0.15^a	1.40 ± 0.15^a	115.9 ± 16.8^a	1.21 ± 0.13^a	0.29 ± 0.09^a
Pond	14	69.0 ± 0.09^a	1.16 ± 0.09^b	115.7 ± 19.8^a	1.25 ± 0.13^a	0.30 ± 0.09^a
Hatchery	13	79.2 ± 0.12^a	1.21 ± 0.12^b	118.8 ± 24.7^a	1.15 ± 0.15^a	0.25 ± 0.07^a

Wild – C (wild fish in control sections) Wild – E (wild fish in experimental sections)

The results also did not show a significant negative influence of artificially reared grayling on their wild conspecifics of a similar size, since the overall performance of wild fish in control sections did not differ from those in experimental sites. Several studies (Bohlin et al., 2002) have shown that growth of salmonids was negatively density – dependent, other authors (Weiss and Schmutz, 1999) observed no effects of stocking on the growth of resident salmonids. In the present study, it is clear that no differences in SGR values of wild resident fish in control and experimental sections are observed. This fact shows that growth of grayling was not influenced by duplication of fish density in experimental sections.

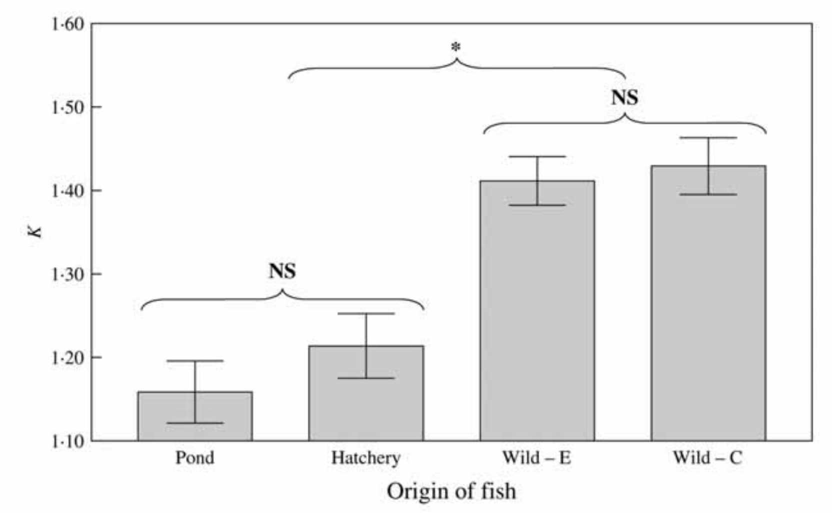

Figure 3. *Relationship between condition factor K (adjusted means +/- S. E.) and the origin of fish at the beginning of the experiment (Wild E – wild fish from experimental sections; Wild C – wild fish from control sections; NS – non-significant differences among classes; 0.05 – significant differences among classes at P < 0.05).*

Many authors refer to a post-release downstream dispersal of artificially reared grayling (e.g. Magee and Byorth, 1994; Kaya and Jeanes, 1995; Carlstein and Eriksson, 1996; Thorfve and Carlstein, 1998). However, the results of this study did not confirm this trend, since the differences in downstream and upstream dispersal between wild and reared fish were not significant. Fish that remained within the range of the survey dispersed outside the release section to a similar extent, irrespective of their origin. On the other hand, the higher site fidelity of wild fish in the present study suggested that previous experience and knowledge of a given environment is of importance. As was shown by Metcalfe et al. (2003), the wild fish dominated reared fish, demonstrating a prior residence effect; i.e. the first fish arriving in an area were likely to dominate those arriving later. Even the frequently mentioned greater aggressiveness of fish reared in hatcheries (e.g. Berejikian et al., 2001) may be ineffective in a stream environment. High densities in hatcheries induce aggressiveness (Berejikian et al., 2001) but, at the same time, preclude learning effective territory defence (Metcalfe et al., 2003).

Stocked grayling can be used to strengthen weakening populations of the species in natural waters, although it is necessary to take into account a lower survival rate compared to that of the wild fish present. Although the present study revealed no positive effect of rearing in a natural pond compared with rearing in concrete tanks, it was not possible to reject 'life skills training' of artificially reared fish in general. The positive effect of rearing fish in a more structured habitat with natural food has been demonstrated (Brown and Laland, 2003; Brown et al., 2003b), but this effect could disappear after six months in natural conditions, since fish are capable of complex learning in a wide range of contexts (Brown et al., 2003a).

Table 4. *Mean of condition factor (K) values of the migratory (M) and sedentary (S) recaptured graylings before and after the experiment and their specific growth rate (SGR).*

	K before		K after		SGR $_{(\%.day^{-1})}$	
Group	M	S	M	S	M	S
Wild – C	1.41	1.44	1.18	1.23	0.30	0.26
Wild – E	1.37	1.43	1.19	1.23	0.30	0.29
Pond	1.16	1.15	1.26	1.25	0.32	0.28
Hatchery	1.25	1.20	1.17	1.13	0.26	0.24

Wild – C (wild fish in control sections); Wild – E (wild fish in experimental sections)

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CHAPTER 3

RECAPTURE RATE AND GROWTH OF HATCHERY-REARED BROWN TROUT (*SALMO TRUTTA* V. *FARIO*, L.) IN BLANICE RIVER AND THE EFFECT OF STOCKING ON WILD BROWN TROUT AND GRAYLING (*THYMALLUS THYMALLUS*, L.)

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ABSTRACT

Hatchery-reared adult brown trout, *Salmo trutta* v. *fario* L., [215–335 mm standard length (L_S), n = 82] were individually tagged and released into three sections of the Blanice River in May 2007. Wild populations of brown trout and grayling, *Thymallus thymallus*, L., in these sections and in three unstocked control sections were also tagged. The recapture rate of hatchery-reared adult brown trout after six months (18%, n = 15) was comparable to that of wild adult brown trout in stocked (15%, n = 14) and control (14%, n = 11) sections. The recapture rates of wild brown trout and grayling after six months were higher in control sections than in stocked sections, but differences were not significant. The movement of recaptured large juvenile wild brown trout from stocked sections was significantly higher (36%) than from control sections (9%). The growth of wild brown trout and grayling was unaffected by stocking with adult hatchery-reared brown trout.

Keywords: stocking, river management, Salmonidae

1. INTRODUCTION

Stocking of hatchery-reared salmonids in rivers has been common practice for several decades (Einum and Fleming, 2001). Widespread controversy exists over the efficacy of river stocking programmes in supporting sustainable fisheries and the potentially deleterious effects that stocked fish may have on dwindling native stocks (Hansen and Loeschcke, 1994; Maynard et al., 1995). A review by Cresswell (1981) compared several early studies from the U.S.A. and the U.K. of recapture rates and movements of hatchery-reared brown trout and found recapture rates of 3–35% for fish stocked into rivers. Several studies have shown higher long-term mortality rates of hatchery-sourced fish compared to wild fish (Weiss and Schmutz, 1999a, b), which may have been caused by behaviour deficits in hatchery fish. Compared to wild brown trout, hatchery fish are reported to display reduced territorial efficiency (Sundström et al., 2003) and reduced feeding efficiency (Sundström and Johnsson, 2001). Furthermore, increased density after stocking may reduce growth for all fish in the watercourse (Bohlin et al., 2002).

Baer et al. (2007) reported the recapture rate (through sport fishing) of adult, hatchery-reared, brown trout to vary between 17% and 29% and the time between stocking and capture (referred to as residence time) to range from 1 to 160 days. It was suggested that, due to the short residence time of stocked trout, long-term impact through competition for space and food, or genetic impact through introgression, are limited.

The present study was designed to evaluate the growth, recapture, and movement of hatchery-reared adult brown trout for a six month period following stocking in the Blanice River (without exposure to fishing pressure) and to determine if these hatchery fish had an impact on resident brown trout and grayling (*Thymallus thymallus*, L.).

2. MATERIALS AND METHODS

STUDY AREA AND MONITORING OF POPULATION

The Blanice River in South Bohemia (Fig. 1) is 93 km long with a drainage area of 860 km^2. Six sections, each about 130 m (area 650–930 m^2), located along a 5 km length of the river (km 53–58), were randomly designated as control (1, 4, and 6) and stocked sections (2, 3, and 5). These were divided by separation sections of min. 70 m (Fig. 1). All sections were situated in a protected area (with a ban on fishing), downstream from the Husinec water reservoir (37 ha, 2.5 x 106 m^3). There are no weirs in this part of the river, and the annual mean flow rate is 3.5 m^3 s^{-1}. During the experimental period (May – October 2007), mean ± SD flow was 1.6 ± 1.2 m^3 s^{-1}, mean ± SD water temperature was 14.0 ± 3.3 °C, and pH was 7–7.8. The course of water temperatures in the study period is displayed in Fig. 2. There were no major changes in flow or temperature regime during the study period. The experimental area, which is at an elevation of about 500 m, was located in countryside comprising broad-leaf trees and meadows. The river sections were similar with regard to substrate (gravel and stones), banks (naturally formed), and water velocity. Depth range in the sections was 10–80 cm, and width ranged from 5–9 m. The total area of stocked sections was similar (about 2,400 m^2) to the total area of control sections (about 2,100 m^2). The minimum distance between adjacent sections was 70 m. Initial density of wild adult brown trout was similar in all sections. The dominant fish species were brown trout (*Salmo trutta* v. *fario* L.) and grayling (*Thymallus thymallus* L.). Common sculpin (*Cottus gobio* L.), stoneloach (*Barbatula barbatula* L.), roach (*Rutilus rutilus* L.), and gudgeon (*Gobio gobio* L.) were caught occasionally.

From 9 to 11 May 2007 all sections were thoroughly fished twice using two backpack pulsed-DC electrofishing units (FEG 1500, EFKO-Germany). Two further pulsed-DC electrofishing units (FEG 3000, EFKO-Germany) stationed at the upper border of the fished section prevented the fish escaping upstream during fishing.

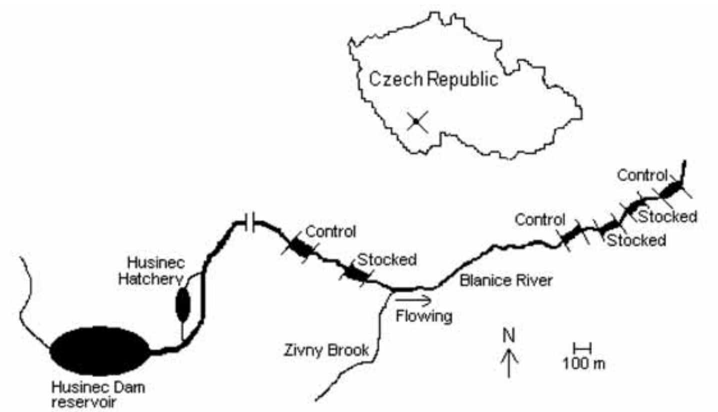

Figure 1. *Schematic map showing the locations of control and stocked sections of the Blanice River.*

All captured wild brown trout and grayling were counted, and total length (L_T), standard length (L_S), and weight (W) were recorded. Fish were individually or group (one-year old brown trout) tagged (Table I) and returned to the section from which they were taken.

The wild population of brown trout was divided into three groups: < 150 mm L_S [juvenile small (one year old)], L_S 150–199 mm (juvenile large), and > 199 mm L_S (adult). The wild population of grayling was divided into two groups: < 200 mm L_S (juvenile) and > 199 mm L_S (adult).

EXPERIMENTAL FISH

Experimental hatchery-reared brown trouts (n = 82) were randomly chosen from three year old brown trout, reared from fingerlings in concrete tanks in the Husinec Hatchery (Czech Anglers´ Union) and fed conventional dry food pellets. These fish were progeny of resident wild broodstock from the Blanice River (hand spawning). The hatchery is supplied with water from the Blanice River and is at a distance from the experimental sections of 5–7 km.

MARKING AND STOCKING

All fish (wild and hatchery-reared) > 150 mm L_S were anaesthetized with 2-Phenoxyethanol (0.2 ml l^{-1}) and tagged with an individually coded visible implant alphanumeric tag (VIA-tags) [North-west Marine Technology (NMT), Seattle, WA, USA] in the membrane posterior to the eye. Wild brown trout < 150 mm L_S were tagged with a visible implant elastomer tag (VIE-tags) which enabled differentiation by group (tag colour and location – left or right side).

In the afternoon of 12 May 2007 the individually tagged hatchery-reared brown trout were transported from Husinec in a tank fitted with an oxygen injection system and released into the selected (stocked) sections (number 2, 3, and 5). Water from the Blanice River was used for the transportation of the fish, and the duration of transport did not exceed 20 min. The number of reared fish introduced into individual sections corresponded to the number of tagged wild adult brown trout (> 199 mm L_S) in the given section. Three sections (1, 4, and 6) were left as control sites.

RECAPTURE

Six months after release (24–26 Oct. 2007), the post-stocking performance of the stocked fish was evaluated. Fish were recaptured by the same method and equipment used for the catch in spring. All the sections and the separation sections, including approximately 2 km of the river upstream and downstream from the experimental area, were thoroughly fished. All tagged fish caught were identified, measured, and weighed. Using the data gathered, it was possible to determine the growth in weight and length for each recaptured fish. The specific growth rate (G) was calculated for all recaptured fish [$G = (\ln W2 - \ln W1)/(t2 - t1).100$] where W1 and W2 are initial and final weights on days t1 (stocking) and t2 (recapture), respectively. In small juvenile brown trout, G was calculated from mean initial weight of all tagged fish and mean weight of all recaptured fish.

The site where each fish was recaptured was also recorded. This made it possible to determine whether the fish had remained in the sections where they were originally released and, if not, the direction of their migration.

Table 1. *Standard length (L_S) and weight (W) of the tagged brown trout and grayling in the Blanice River on beginning of the experiment (May 2007). Values with identical superscripts within a column did not differ significantly (P < 0.05) in this category.*

Species (category)	River section	n	L_S (mm) Mean ± SD	W (g) Mean ± SD
Brown trout				
Adult – wild	Control	77	221 ± 22^a	172 ± 64^a
Adult – wild	Stocked	82	224 ± 22^a	179 ± 57^a
Adult – hatchery	Stocked	82	269 ± 26^b	287 ± 87^b
Brown trout				
Juvenile – wild (large)	Control	99	183 ± 10^a	98 ± 17^a
Juvenile – wild (large)	Stocked	78	186 ± 9^a	105 ± 16^b
Brown trout				
Juvenile – wild (small)	Control	144	134 ± 14^a	37 ± 11^a
Juvenile – wild (small)	Stocked	111	135 ± 15^a	37 ± 14^a
Grayling				
Adult – wild	Control	45	246 ± 19^a	224 ± 56^a
Adult – wild	Stocked	29	236 ± 14^b	196 ± 33^b
Grayling				
Juvenile – wild	Control	43	170 ± 9^a	69 ± 10^a
Juvenile – wild	Stocked	25	168 ± 12^a	69 ± 15^a

STATISTICAL ANALYSES

A one-way analysis of variance (ANOVA) and was used to test for weight, length, and G differences among adult wild brown trout in stocked and control sections and stocked hatchery-reared brown trout. Post hoc comparisons were made by Tukey's Honestly significant difference (HSD) test. Student´s t-test was used to test for weight, length, and SGR differences among other categories of wild brown trout and grayling tagged in stocked and control sections. Within-group differences of the initial weight of fish that were recaptured, as well as fish that disappeared from sections (non-recaptured fish) were compared with Student´s t-test. Recapture rates and movement of tagged fish were compared with the Pearson and maximum likelihood χ^2 test. Significance was accepted for values of $P < 0.05$.

Movement was defined as recapture outside the release site for stocked fish or outside of the tagging section for resident fish. The percentage of moved fish was calculated from the number of recaptured fish in each group.

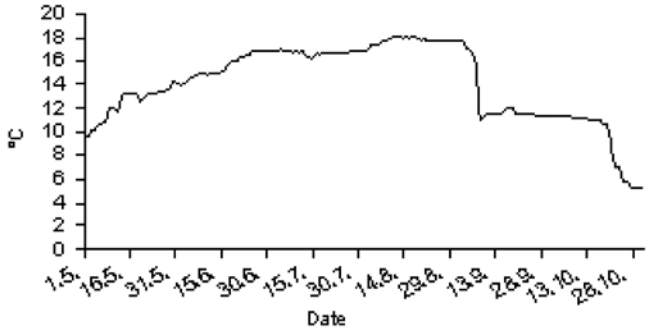

Figure 2. *The course of water temperatures in the Blanice River (1. 5. – 31. 10. 2007). Data of the daily report of outflow from the Husinec Dam reservoir (n = 184).*

3. RESULTS

RECAPTURE

Six months after stocking, no significant difference was found among the total recapture rates in three groups of adult brown trout (χ^2 = 0.48; d.f. = 2; P = 0.787). The recapture of hatchery-reared adult brown trout was 18% (n = 15), of wild adult brown trout tagged in stocked sections was 15% (n = 13), and of wild adult brown trout tagged in control sections 14% (n = 11).

The recapture rate of large juvenile brown trout tagged in control sections (24%, n = 24) was higher than in experimental sections (14%, n = 11), (χ^2 = 2.83; d.f. = 1; P = 0.093).

In both groups of small juvenile brown trout the recapture rate was similar (χ^2 = 0.25; d.f. = 1; P = 0.618). In this group, 50% of tagged fish in control sections were recaptured (n = 72) and, in stocked sections, 47% of tagged fish (n = 52) were recaptured.

The recapture rate of adult grayling tagged in control sections (42%, n = 19) was higher than in experimental sections (24%, n = 7), (χ^2 = 2.53; d.f. = 1; P = 0.112).

No significant difference was found among the total recapture rates of juvenile grayling (χ^2 = 2.76; d.f. = 1; P = 0.097). Recapture rate of tagged juvenile grayling was 40% (n = 17) in control sections and 20% in stocked sections (n = 5).

MOVEMENT

Movement of adult brown trout did not differ significantly (χ^2 = 1.81; d.f. = 2; P = 0.406) among the three groups. About 47% (n = 7) of the recaptured hatchery-reared adult brown trout had moved from their section of stocking. Emigration of adult recaptured wild brown trout tagged in stocked sections (54%, n = 7) was higher than in control sections (27%, n = 3), but this difference was not significant (P = 0.188).

Movement of large juvenile brown trout from control sections was significantly different from that of stocked sections (χ^2 = 4.17; d.f. = 1; P = 0.041). Only two (9%) brown trout tagged in control sections were recaptured outside their original section.

Four (36%) large juvenile brown trout tagged in stocked sections had moved. Thirteen (18%) small juvenile brown trout tagged in control sections were recaptured outside their section of origin. This is not significantly (P = 0.238) less than the number of small juvenile brown trout tagged in stocked sections that were recaptured outside of their origin section (27%, n = 14).

In both categories of grayling, higher movement was found in those tagged in control sections [adult 26% (n = 5); juvenile 47% (n = 8)] than in stocked sections (no movement in either category). These differences were not significant (P = 0.131 adults and P = 0.055 juveniles).

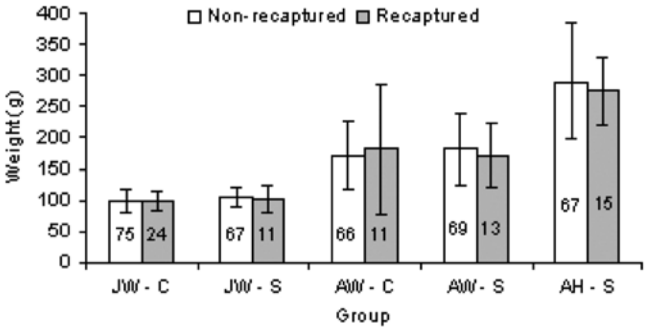

Figure 3. *Initial weight (mean ± SD) of non-recaptured and recaptured brown trout in each group which were at the begining of the experiment. JW: wild juvenile (large) fish marked in control (-C) and stocked (-S) sections; AW: wild adult fish marked in control (-C) and stocked (-S) sections; AH – S: hatchery-reared adult fish in stocked sections. Sample sizes are given inside each column of the graph.*

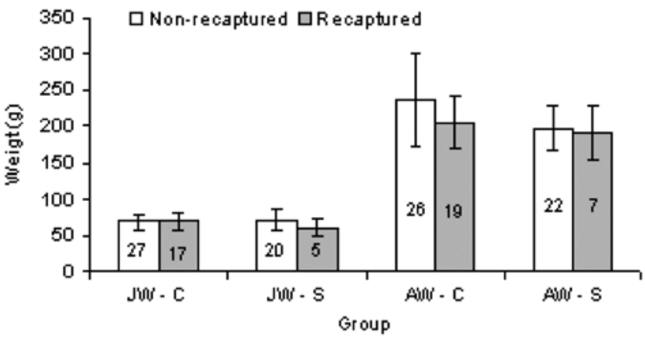

Figure 4. *Initial weight (mean ± SD) of non-recaptured and recaptured grayling in each group which were at the begining of the experiment. JW: wild juvenile fish marked in control (-C) and stocked (-S) sections; AW: wild adult fish marked in control (-C) and stocked (-S) sections. Sample sizes are given inside each column of the graph.*

GROWTH

At the beginning of the experiment, there were significant differences between the tagged adult hatchery-reared brown trout and the wild brown trout in both control and stocked sections with respect to weight (Table 1) (F = 8.24; d.f. = 2; P = 0.001) and L_S (F = 15.12; d.f. = 2; P < 0.001). These differences were also significant in later recaptured fish (Table 2). Significant differences were found in initial weight (t = 2.40; d.f. = 72; P = 0.019) and L_S (t = 2.61; d.f. = 72; P = 0.011) of tagged adult grayling in control and stocked sections. These differences were not statistically significant in later recaptured adult grayling. The initial weight of large juvenile brown trout tagged in stocked sections was significantly higher (t = 2.55; d.f = 175; P = 0.012) than in control sections, but was not significantly different in later recaptured fish (Table 1 and 2). In other categories, no significant differences were found in intial W and L_S of all tagged fish with those of the fish later recaptured. Within-group differences between the initial mass of fish that were recaptured and fish that disappeared from studied sections (non-recaptured fish) were not significant in the any groups of individually marked fish (Figs. 3 and 4). G values of recaptured fish in each category were not different between groups tagged in control and stocked sections (Table 2).

Significant differences were found between adult hatchery-reared brown trout and wild brown trout in both control and stocked sections with respect to final weight (F = 6.99; d.f. = 2; P = 0.003) and final L_S (F = 16.84; d.f. = 2; P < 0.001). Differences in final weight and L_S of fish in control and stocked sections were not significant in other categories (Table 2).

4. DISCUSSION

In the present study, the lack of difference observed in the recapture rates of three groups of adult brown trout contrasts with many reports (e.g. Ersbak and Haase, 1983; Arias et al., 1995; Weiss and Schmutz 1999a,b; Weber and Fausch, 2003), which also reported a lower post-stocking survival of hatchery-reared fish compared with wild fish. The doubled density of adult brown trout in stocked sections did not show an impact on the recapture of fish as reported in Bohlin et al. (2002) and Jenkins et al. (1999). Conversely, Baer and Brinker (2008) observed that recapture rates fell with increasing density, regardless of the origin of brown trout.

Several authors have demonstrated the competitive superiority of resident salmonids over introduced fish (Brännäs, 1995; Glova and Field-Dodgson, 1995), known as the 'prior residence effect' (Huntingford and De Leaniz, 1997; Rhodes and Quinn, 1998). In the present study no significantly greater movement of recaptured hatchery-reared fish compared to wild resident fish was observed. This could be explained by the higher initial weight of hatchery-reared fish than resident fish, which is consistent with Johnsson et al. (1999), who demonstrated this among brown trout fry.

No significant differences in the specific growth rates of three groups of adult brown trout were observed. This implies that, as long as the hatchery-reared fish adapt to the conditions of the natural watercourse, they are able to make full use of the food available in the environment. Johnsen and Ugedal (1986) observed rapid feeding adaptability, when hatchery- reared brown trout, yearlings, appeared to be feeding on wild prey nearly as well as did wild fish by about a week after release,. On the contrary, many authors (e.g. Weber and Fausch, 2003; Sundström et al. 2004; Baer and Brinker, 2008) have reported that stocked hatchery-reared salmonids have slower growth in the wild than resident wild fish.

However our results showed that growth of resident wild adult brown trout was not influenced by doubling of fish density in stocked sections, similarly as reported by Weiss and Schmutz (1999b). Also biotic and abiotic factors in particular biotopes substantialy affected growth of stocked fish (Weiss and Schmutz, 1999a).

The higher initial size of stocked fish may have been an influence in the lower (not significantly) recapture rate and significantly higher movement of large juvenile brown trout tagged in stocked sections compared with control sections. The high site fidelity of large juvenile brown trout tagged in control sections is consistent with the findings of Knouft and Spotila (2002). The specific growth rate between large juvenile brown trout tagged in stocked and control sections did not differ. It is possible that food availability was sufficient for all fish, and the shelter capacity of the stream was a limiting factor in this study.

No effect of stocking adult brown trout on recapture rates, movement, and growth of small juvenile brown trout was observed. The territorial requirements of small brown trout may be different from those of adult fish. Behaviour of small brown trout was unaffected by higher density of adult fish. However, Nordwall et al. (2001) showed that reduced densities of brown trout older than 1 year resulted in increased apparent survival of age 0, 1 year, and 2 year fish in the subsequent year.

Behaviour of both categories of grayling (juvenile and adult) was not significantly affected by stocking adult brown trout. Nevertheless, recapture of grayling tagged in control sections was nearly twofold that of graylings tagged in stocked sections in both categories, although the movement of grayling tagged in control sections was higher than in stocked sections. There is little available information on competition between brown trout and grayling in a natural stream. Thorfve and Carlstein (1998) reported that the presence of stocked grayling at the time that brown trout were stocked resulted in significantly fewer brown trout remaining in the upper reaches of a semi-natural stream. This implied the possibility of habitat competition between brown trout and grayling. The growth of grayling was not affected by brown trout stocking in the current experiment. Evidently, food availability was sufficient for increased density of fish in the experimental sections.

Table 2. *Mean ± S.D. of weight (W) and standard length (L_S) of the recaptured fish before release, after recapture and their specific growth rate (G). Values with identical superscripts within a column did not differ significantly (P < 0.05) in this category.*

Species group/ category	Section of river	n	L_S (mm) before	L_S (mm) after	W (g) before	W (g) after	G (% day^{-1})
Brown trout							
Adult – wild	Control	11	225 ± 30^a	237 ± 30^a	182 ± 105^a	205 ± 117^a	0.072 ± 0.052^a
Adult – wild	Stocked	13	222 ± 20^a	235 ± 17^a	172 ± 52^a	187 ± 46^a	0.060 ± 0.073^a
Adult – hatchery	Stocked	15	267 ± 19^b	284 ± 24^b	276 ± 55^b	295 ± 65^b	0.038 ± 0.067^a
Brown trout							
Juvenile – wild (large)	Control	24	184 ± 9^a	202 ± 12^a	98 ± 15^a	119 ± 18^a	0.116 ± 0.054^a
Juvenile – wild (large)	Stocked	11	182 ± 13^a	210 ± 15^a	102 ± 22^a	130 ± 29^a	0.145 ± 0.080^a
Brown trout							
Juvenile – wild (small)	Control	72	134 ± 14^a	170 ± 13^a	37 ± 11^a	67 ± 14^a	0.353
Juvenile – wild (small)	Stocked	52	135 ± 15^a	167 ± 14^a	37 ± 14^a	65 ± 19^a	0.332
Grayling							
Adult – wild	Control	19	239 ± 15^a	256 ± 14^a	206 ± 36^a	228 ± 34^a	0.063 ± 0.054^a
Adult – wild	Stocked	7	235 ± 14^a	256 ± 15^a	192 ± 39^a	223 ± 43^a	0.091 ± 0.044^a
Grayling							
Juvenile – wild	Control	17	170 ± 10^a	210 ± 14^a	70 ± 10^a	112 ± 17^a	0.278 ± 0.081^a
Juvenile – wild	Stocked	5	161 ± 6^a	211 ± 15^a	62 ± 12^a	106 ± 8^a	0.331 ± 0.081^a

The lack of difference in initial weight of recaptured and not-recaptured fish within each group suggested that the recapture rate was independent of initial weight of stocked/tagged fish in both control and stocked areas.

In contrast with results of earlier research, this study showed post-stocking performance of hatchery-reared salmonids to be similar to that of their wild resident conspecifics. Generally, the effects of stocking on wild population were not significant. The greater samples of used fishes would probably bring more significant differnces.

ACKNOWLEDGEMENTS

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CHAPTER 4

TECHNOLOGY OF BROWN TROUT CULTURE IN CONTROL CONDITIONS FOR RUNNING WATERS STOCKS PRODUCTION

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TECHNOLOGY OF BROWN TROUT CULTURE IN CONTROL CONDITIONS FOR RUNNING WATERS STOCKS PRODUCTION

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1. AIM OF THE NEW TECHNOLOGY

The purpose of the described technique is to provide fisheries management with information about the method of breeding and management of local brown trout (*Salmo trutta* m. *fario* L.) populations in controlled conditions. Implementation of this technique should increase and stabilize the production of high quality indigenous brown trout stock genetic lines for the stocking of natural waters, and also result in the subsequent reduction in transport of stock between different regions and countries. Furthermore, stocking of indigenous genetic lines will help to support original wild trout populations, preserve the intraspecies genetic variability and will prevent genetic contamination by allochthonous material. The proposed technique must comply with the standards of sustainable production of stock with preserved wild population characteristics.

2. TECHNOLOGY DESCRIPTION

INTRODUCTION

In the Czech Republic, brown trout (*Salmo trutta* m. *fario* L.) is one of the most commercially important fish species in running waters. However, in recent years there has been a significant decline in population numbers due to many factors. The reasons and possible solutions of trout population decline have been discussed in both scientific and non-professional communities. The Czech Fishing Union, an organisation responsible for the management of open waters, is often criticised for insufficient stocking of rivers. However, the situation is more complicated, and satisfactory solutions can be achieved only by adopting complex measures. Such measures have to be based on a thorough analysis of the situation in every particular river. It is important to know that successful natural reproduction is crucial for population development, and under optimal conditions, it ensures the preservation of genetic diversity and purity and thus ensures the stability of the population. Successful natural reproduction requires sufficient amount of brood stock. Numbers of parent fish (and fish in general) are affected mainly by the river morphology (Harsányi a Aschenbrenner, 2002; Turek et al., 2009), hydrological conditions (Rogers et al., 2005), intensity of the predation pressure of fish-eating predators (Mareš and Habán, 2003; Spurný, 2000, 2003a, b), water contamination (Kolářová et al., 2005), fishery management, and finally by fishing pressure on the river (Lusk et al., 2003).

In order to prevent further weakening of trout populations, it is necessary to implement enhanced protection measures, such as the prevention of unreasonable amendments of river beds, improvement of river morphology, reduction of predatory activity, and change in angling rules and fishery management of the river etc.

Brown trout stocking is a relatively easy and practicable way of supporting wild populations. There has been an increased tendency among fisheries to intensify the support of native populations by stocking. However, numbers of stocked fish is limited by the numbers of stripped brood fish. Brood stock is usually caught in rivers during the pre-spawn period, but the numbers of available brood fish are declining. In order to obtain sufficient numbers of brood stock, it is necessary to fish in longer and more valuable stretches of Czech rivers. In some parts of the country the numbers of fry for stocking are still insufficient and stock is imported from other regions or even from abroad. Stocking of different genetic breed lines coming from an intensive breeding process has become common practice (e.g. Ital, Kolowrat).

Photo 1. *Brown trout.*

Intensive farming practice is increasingly used for the production of stock due to the need for higher production efficiency. Stocking of fish from hatcheries is often criticized because of its negative effect on wild fish populations (L´Abee-Lund, 1991; Einum and Fleming, 2001). Stocked fish can affect local wild populations by higher aggressiveness, predation, food competition and the spreading of disease etc. (Hedenskog et al., 2002; Petersson and Järvi, 2003; Huntingford, 2004). There is also a potentional risk of genetic contamination in cases where stocked fish crossbreed with the native population (Weber and Fausch, 2003). On the other hand, there is reduced adaptability in stocked fish resulting in higher mortality rates after stocking. Farmed fish often become preferred prey for piscivorous predators after stocking due to the lack of experience, and reduced anti-predatory behaviour (White et al., 1995; Weiss and Schmutz, 1999). There are also difficulties with the adaptation to the natural food source. Searching for food and defending already used food territories poses high energetic expenditure for fish which have been fed in hatcheries. This often leads to exhaustion and in combination with other factors (such as predators, diseases, fishing) it can lead to eradication of stocked fish in the river (Ersbak and Haase, 1983; Johnson, 1983; Bachman, 1984; Nicholls, 1985; Lachance and Magnan, 1990).

Uncontrolled stocking of fish of a different origin can have a negative effect on genetic characteristics of local wild populations and can lead to a significant decline or even extinction of genes and genotypes which are best adapted to the local conditions. Uncontrolled stocking can thus weaken the local fish populations, and in such cases restoration is very difficult.

Stocking still remains an effective way of supporting wild fish populations, especially in the localities where the natural reproduction is limited. However, genetic characteristics of stocked fish must be similar to those of wild fish populations living in stocked water and stocked fish must have high adaptability to the local conditions. In order to produce such stock, it is necessary to have high quality brood fish with the required genetic characteristics.

Successful technology of brood fish rearing in controlled conditions can be a suitable solution for stabilizing, or even increasing brown trout stock production. This can be done without the elimination of natural reproduction as there is less need for catching parent fish for stripping. A breeding technique must involve methods that do not significantly affect genetic characteristics of fish even from the long term perspective. For the preservation of the original gene pool of local fish populations and their intra species diversity it is therefore necessary to work with these native fish populations from the particular region.

This technology describes veterinary aspects, principles and methods of brown trout brood stock production in controlled conditions and rearing of the particular age classes (fry, fingerling, juveniles, brood fish).

METHODS OF BROOD STOCK ESTABLISHMENT AND REARING OF DIFFERENT BROWN TROUT AGE CLASSES

Fisheries designed for rearing brown trout should have a strong all year round supply of high quality water, the temperature of which does not exceed 18 °C, even on hot summer days. The rearing system should be designed as a flow-through system.

BROOD STOCK ESTABLISHMENT

Rearing of brood fish in controlled conditions should be carried out from the fry stage. A wild trout population from the local area is the best source of parent fish to use for brood stock establishment. Ideally it is best to obtain fish egg of brood fish from as many local rivers in the same catchment area as possible. Such rivers should have limited influence of fishery management (mainly brown trout stocking) and fish should naturally spawn (e.g. fish protected areas or parts of rivers with no fishery management).

Stripping must ensure maximum genetic variability of fry. Common methods where large amounts of eggs (from tens of females) are fertilised by the sperm of many males (polysperm fertilization) has proved to be inadequate for genetic variability preservation. Several studies have shown that polysperm fertilization causes a competition in spermatozoa, with most eggs being fertilised by only a small number of males originally used for stripping. Different methods are thus recommended for preservation of genetic variability. (Kašpar et al., 2008). The following recommended technology can be used in conditions under which most of the hatcheries in the Czech Republic operate.

The most suitable method is dry fertilization, where eggs are stripped straight into the dry bowl together with ovarian fluid. Eggs can also be stripped to the sieve where the ovarian fluid is left to drip off and eggs are than carefully moved into a dry plastic bowl.

Each female should be stripped separately, i.e. eggs from each female are stripped into one dry plastic container or sieve. Quality of eggs should than be visually checked for the presence of blood, clumps of eggs, white eggs or evidently damaged or low quality eggs. A defined volume of eggs (approximately an average number of eggs from one female) is then transferred by a gauge (ladle) into a bigger plastic container (container A). The aim of this procedure is to put similar numbers of eggs from each female into the container. The rest of eggs (if there is any) are put into another container (container B). If the quality of eggs is evidently poor, it is better to put these eggs straight into container B, or discard immediately.

The eggs are thus collected into containers A and B during the entire stripping process. It is necessary to cover both containers with a damp cloth. The eggs should never be exposed to sun radiation during the entire process, as the temperature can rise well above the incubation temperature. It is also important to prevent eggs from getting into contact with water, as the presence of water in eggs before fertilization considerably decreases their fertilization success rate.

When all the females are stripped, eggs from container A are carefully mixed and then separated (if possible) into as many smaller parts as possible. Each part is then fertilized by milt from different males. The number of males used for fertilization of one partial part of eggs should be equal to the proportion of available males and to the proportion of containers with eggs. It means that every male is used for the fertilization of 1 partial group of eggs only. The optimum number of males used for fertilization of one group of eggs should be 1–6. The fewer males are used for the fertilization of one partial group of eggs, the higher are the chances of achieving maximum genetic variability. But it is recommended to use at least two males for every part of eggs as it eliminates the loss of eggs if the sperm of one male is not fertile. Milt is added straight onto the eggs, which should be protected from any contact with water by drying off the urogenital area of stripped fish, including the anal fin, with a damp cloth. The egg is then mixed and water is added to activate gametes and initiate the process of fertilization. The eggs are then carefully mixed again using a suitable tool, e.g. clean spatula or trowel (plastic, rubber or wooden). The source of water must be the same throughout the whole process. To prevent excessive diluting of milt which could result in lower fertility, the water level in the bowl should not be higher than 1–2 cm above the eggs. Containers are left to stand for about 2–3 minutes for the process of fertilization to finish. The eggs are then carefully rinsed with water, and all groups of eggs which were originally in container A are poured together again into one container and mixed with caution. This step of mixing all the eggs together is important for homogenisation of eggs originating from many different parent fish before placing them into individual incubation apparatus. When the rinsing with clear water is finished, as much water as possible is added to the container(s) and eggs are left to rest for about 2–3 hours. During this stage the eggs swell and are very sensitive to shaking. Once the eggs have swollen, it is time to place them into the incubation apparatus.

The same procedure is repeated with the eggs in container B. The same males used for the container A are used for insemination. It is necessary to mark individual incubation apparatus to distinguish eggs from containers A and B. If the stripping takes several days, groups of eggs (A and B) are produced by the method described above for every stripping day separately. Approximately the same volume of eggs from each female will be transferred into container A throughout the whole stripping season (i.e. the same gauge will be used throughout the season). If the stripping in one day takes more than several hours and the number of stripped fish is high, the procedure of stripping and fertilization is repeated several times during the day.

Brood fish which have been stripped are put into a potash bath for a short period of time (Kolářová and Svobodová, 2009) before being released back into the river as soon as possible.

Brood stock should always be preferably established from offspring originating from container A. It is necessary to ensure maximum genetic variability of the future brood stock. If stripping was carried out over different time periods, then the same amount of fry has to be taken from every stripping process (i.e. from each container A in a given season). Fish from the container B are used only when there is not enough fish produced from the container(s) A.

In order to produce one brood female about 100 fertilized eggs are needed. The optimum number of brood stock is about 100–200 females. Thus for brood stock of 100 females some 10,000 fertilized eggs are needed. These eggs should come from as many parents as possible (minimum of 20–30 pairs).

To prevent major phenotype and genetic changes of reared brood stock and subsequently of their offspring (Fleming and Einum, 1997; Einum and Fleming, 2001; Verspoor, 1988; Hanák, 2008), it is always necessary to raise brood stock from offspring of wild fish acquired by the method described above.

Using only the first generations of artificially raised fish ensures maximum preservation of the original characteristics of parent populations. For the same reason it is advisable to keep only females (or only a small number of males as their presence may positively affect maturation of females) and their eggs fertilized by the milt of wild males taken from a suitable river (for example a fish protected area).

Photo 2. *Catching of brown trout brood stock in natural habitat.*

Optimally, every brood stock rearing fish farm should have its own fish protected area as a source of native brood fish and wild males for the fertilization of eggs from reared females. Any Fish Protected Area (FPA) must comply with the following requirements:

- *The character of the river section chosen as a fish protected area should be as close to natural conditions as possible and unpolluted by industry and communal waters.*
- *The whole section of FPA should allow migration for fish and migration barriers should not be in the connecting parts of the river.*
- *The size of the area should allow the occurrence of several hundreds of brood fish.*
- *No selection measures are recommended within FPA and its tributaries. No stocking should take place is such areas (especially reared stock from hatcheries) and restocking of fish should only be based on natural spawning.*
- *To ensure successful natural reproduction, it is necessary to leave part of the brood stock (c. 1/3) in the river when catching brood fish. It is therefore advised to put young fish at their first spawning season back to the river.*

INCUBATION OF EGGS

The most suitable incubation device is the classic Rückel-Vacek apparatus. Its construction enables the setting of circular (sideways), or bottom water flow by the easy shifting of the inside plate with eggs. The apparatus is usually set to the circular flow during the incubation (but bottom flow is also possible) and to the bottom flow after hatching to prevent damage to the yolk sac. The eggs in the apparatus should be placed in one row only. The capacity of one incubation apparatus is thus around 8–10,000 eggs.

The incubation apparatus used for hatching has to be clearly marked. After the fertilization and placement of eggs into the apparatus, it is necessary to carefully remove dead (white) eggs. Incubating eggs are very sensitive to shaking, manipulation and light until they reach the stage of eyed eggs (220–230 days degree /D/ from fertilization). The length of incubation period depends on the water temperature, usually between 350–500°D.

Photo 3. *Incubation of brown trout eggs in Rückel-Vacek apparatuses.*

Preventive desinfection bath treatment of the eggs during incubation is possible (Kolářová and Svobodová, 2009). The fertilization success rate usually exceeds 95% and the mortality during incubation is up to 10% provided that good quality water is available and rules of hygiene and careful manipulation are kept. High losses can be caused especially by improper manipulation with the eggs before the stage of eyed eggs. Hatching takes place in the apparatus. It is necessary to remove egg shells and set the apparatus to the bottom flow during the hatching because of higher oxygen demand of fry.

REARING OF FRY AND YEARLING

After hatching, yolk sac fry stay on the bottom of the apparatus absorbing nutrients from its yolk sac (so called quiescent phase). This stage ends when the fry has absorbed about 1/2–2/3 of its yolk sac and starts to swim-up. This stage usually lasts for about 150–200 °D (c. 3 weeks). At the end of this stage it is possible to start with initial feeding directly in the apparatus.

At the beginning of the next (so called active) phase of rearing, when the fry is remarkably active and changes its diet from endogenic to exogenic, the fry must be transferred into shallow troughs (usual size 4 x 0,4 x 0,2 m) where it is necessary to start early feeding. The fry from one apparatus should be placed into one trough. Raising troughs must be shaded and protected from direct sunlight. It is recommended to feed fry only with compound feed. The feed for rainbow trout with lower fat content from reputable producers have proved to be suitable for trout fry. The size of granules used at the early feeding should be about 0.5–0.6 mm and should not be floating on the surface but slowly sinking. Ideally, the trout should be fed small portions at a higher frequency (6–10 times per day) by hand over the whole area of the trough. Later, when fish readily take the food, it is possible to use automatic feeders (for example operated by a clock), preferably two feeders per trough. The feed rations should be at the bottom bounds of the rations recommended for the rainbow trout by the manufacturers of the compound feeds. The size of granules should be adequate to the size of raised fish according to the manufacturer catalogue. At the beginning the water level in the troughs should be kept low (c. 10 cm). Feeding of plankton increases the risk of contamination and slows the process of becoming accustomed to compound feed. Plankton can be used only temporarily if there is a problem with compound feed intake at the stage of early feeding. In such cases it is advisable to feed both plankton and compound feed together. The mortality at the first stages of rearing is usually up to 10%.

After 4–6 weeks the fry are transported to larger tanks, usually rectangular troughs or circular basins, where rearing generally continues to the stage of yearling. The stocking rate depends on the size of tank and the water oxygen content, the usual stock rate is between 1,000–2,000 fry per 1 m^3 of water. The oxygen saturation should not fall below 60% in the tank outlet. It is possible to use oxygenators or oxygen apparatus, which enable an adequate increase in the stocking rate. During the rearing the fish are separated into more tanks according to the growth rate. The stocking rate at the end of yearling rearing is usually between 300–600 individuals per m^{-3}. The mortality during the rearing of yearling is usually about 20%.

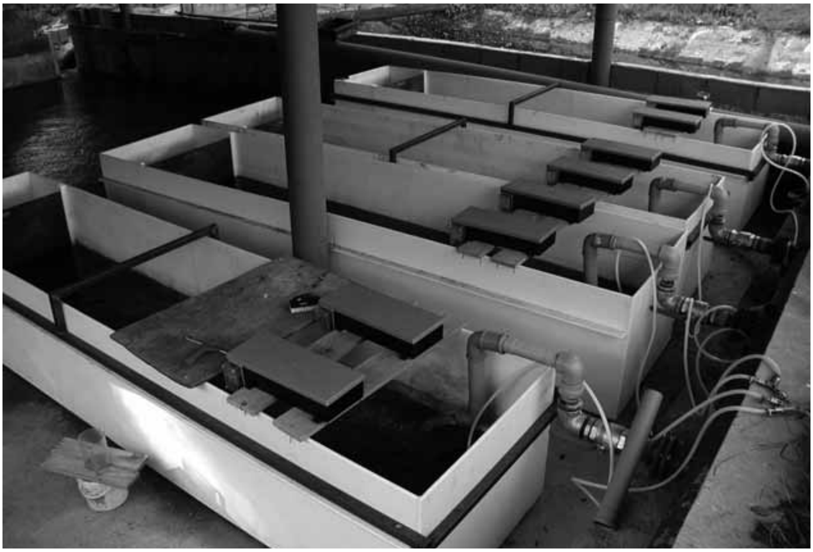

Photo 4. *Trough room for rearing brown trout fry.*

It is important to carry out regular preventive inspections of the fry for the presence of parasitic infections (in minimum intervals of two weeks, in case of higher mortality immediately) and the rearing facility exceptionally tidy (i.e. remove excessive food remains, excrement and dead individuals). When there is a high risk of parasitic infections (summer months) it is advisable to use preventative baths (Kolářová and Svobodová, 2009).

The habituation of brown trout fry to granulated feeds at an early stage gives the opportunity for continuation of rearing in controlled conditions and to successfully raise required brood stock. This technique also solves the problem of obtaining plankton for feeding, allows regular food supply and eliminates the risk of parasitic infections.

REARING OF JUVENILES AND BROOD FISH

The technology of rearing brood fish juveniles (1–3 years old) has to be adapted to the quality of water supply. Ground ponds are not recommended if the water is supplied from a river as there is a potential risk of pathogen transmition. Flow-through tanks with a high flow and a hard bottom (concrete store-ponds, trench ponds, channels, flumes etc.) have been proved suitable in such cases. If there is a source of good quality water with no fish stock available, it is possible to use ground ponds. It is optimal to keep particular age categories (1–2 year, 2–3year, parent fish) in the separated tanks. Three year old fish can be placed together with the brood stock. The oxygen saturation should not fall below 60% in the outlets.

Juveniles of brood fish prosper well in flow-through tanks with the water volume up to 10 m^3. The stocking rate of yearling trout is about 100–300 fish per m3. After one year (two year old fish) it is appropriate to reduce the stocking rate to 30–50 individuals per m-3. At the same time the fish should be re-sorted and smaller individuals placed among younger fish categories. It is not recommended to do any selection, except for removing ill or deformed individuals. Fish which appear to be "outsiders" in fish farm conditions can bear important genetic characteristic for survival in natural conditions. The mortality during this stage of rearing is usually about 10%.

Photo 5. *Example of suitable rearing tank for juveniles and brood trout.*

The brood stock can be reared in the flow-through tanks holding several tens of cubic metres of water. The stocking rate is about 10 fish per m^{-3}. The mortality during the brood stock rearing is between 10–30% per year, the highest being in the post spawning period.

If a source of brood male fish from the wild is available (e.g. from FPA) for fertilizing stripped eggs, it is advisable to remove most of the males before placing juveniles to the brood stock. This selection should be done in the autumn, when males are easily distinguishable (tab. 1). Only a few (usually 10–20) males are left in each tank with the brood stock. The presence of males in the tank probably improves maturation of females. In the spawning period, males tend to fight each other if there are more of them in the tank. Injured fish are a source of bacterial and fungal infections which can spread to other fish weakened by the stripping.

Photo 6. *Trout brood stock raised in controlled conditions.*

If there is no source of wild males available it is necessary to rear them in controlled conditions in sufficient numbers. In this instance the selection is avoided and males are kept in the tanks together with females. The old (big) males can become very aggressive so they should be removed from the rear. It is also necessary to regularly check the fish health condition (especially in the post spawning period), remove all individuals with high levels of fungal infection and treat them accordingly in a bath or with antibiotics. It is not recommended to keep males separately from females as they tend to fight more and injuries can cause high and very often total losses of the stock.

Table 1. *Overview of external morphologic characteristics with significant sexual dimorphism, i.e. typical for particular gender of mature fish.*

Characteristic	Male	Female
Enlarged abdomen	indistinctive	distinct
Stimulation of abdomen releases	sperm (milt) of white colour	Eggs – just before spawning
Colour of abdomen	dark	pale
Urogenital orifice	slit shaped	oval, swollen
Maxilla extends	beyond eye	up to the eye
Lower jaw	hooked (older males)	straight
Front part of upper jaw (rostrum)	straight (sharp)	rounded
Body colour (spawning)	distinct	less distinct

It is recommended to use only high quality rainbow trout compound feed. Younger categories are fed with less intensive feed (less fat content) and mature fish with specially designed feed for this category. The daily ration should be at the bottom bound of the ration recommended for the rainbow trout by the manufacturer of the feed. The size of granules should be appropriate to the size of fish. The granules should not be floating on the surface but should be slowly sinking. It is possible to use either hand feeding or the automatic feeding apparatus.

Photo 7. *Sexual dimorphism of reared brown trout brood (top – male, down – female).*

RESTOCKING OF BROOD STOCK

The spawning season does not significantly differ from wild populations, which in the territory of the Czech Republic usually begins in September and lasts until mid October. The manipulation of fish from wild and reared populations must be done with caution to prevent their shuffling. Shortly (c. 1 week) before stripping, the brood fish are transported from the rearing tanks to smaller manipulation tanks with males and females separated. If there is a river with a fish protected area or other suitable source of wild brood stock, the required numbers of males are caught and placed into a separate manipulation tank. Before spawning, females are sorted and only mature fish (abdomen cranilally from urogenital orifice soft, urogenital orifice swolen, pressure to abdomen releases eggs) are used for stripping. Immature fish are put back to the manipulation tank and are checked at weekly intervals.

The hormonal stimulation is not necessary for the stripping. It is suitable to use anaesthetics to prevent damage of bigger brood fish during manipulation (Kolářová et al., 2007). Immediately after stripping it is advisable to put fish into a potash bath (Kolářová and Svobodová, 2009) and release them back to the rearing tanks or the river, depending on where the fish came from. The eggs from artificially reared fish should be inseminated by the sperm of wild males from the ancestral population. For the maximum preservation of genetic variability, the process of stripping for stocking of natural waters must be done by the same way as described in previous part. If however, all offspring are going to be sold to other fisheries, it is not necessary to separate fertilized eggs to parts A and B. If females from open waters are also stripped, the eggs of these fish have to be incubated separately from the eggs of reared females and the incubation apparatus should be carefully marked. The parameters for estimating future egg production from reared brood stock are as follows:

- *absolute fertility (number of stripped eggs per female) about 1,000 eggs;*
- *relative fertility (number of eggs per kg of female weight) 1,500–2,000 eggs;*
- *40–60% of females mature in their third year, the rest of fish mature later. Males usually mature one year earlier than females, so they can be taken out from the rear in their 2nd or 3rd year.*

Farmed fish will usually live longer (about 5–8 years) than fish in the wild. Reared fish thus grow bigger and have more eggs than in natural conditions. Weight of females used for stripping is in most cases between 300–1,000 g. Fish usually undertake 3–5 strippings during its life. The post-spawning mortality of the young brood fish (1st and 2nd stripping) is minimal. The fish reared for longer periods can develop morphological (reduced fins) or behavioural (loss of shyness, reactions to feeding) changes as an adaptation of the organism to the new environment and impact of unnatural conditions. However, if the above described method is used, these changes are not likely to be transferred to offspring as they are not usually genetically fixed.

One very useful tool for fish identification can be tagging. This can be done either for the group of fish (e.g. VIE system based on implantation of coloured visible implant elastomers under the upper layer of skin) or individuals (microchips, VIA systems based on implantation of visible coloured alphanumeric plates under the skin). Tagging can be done at any time provided that the size of fish matches the requirements of the method used (more information on tagging can be found at www.nmt.us). It is recommended to use anaesthetics during tagging (Kolářová et al., 2007). It is not advisable to tag fish at the juvenile stage as tagging of small sized fish can result in high losses of applied tags. Chip systems ensure clear individual identification and minimum losses.

Offspring of females artificially reared by the above described method have a potential for successful adaptability in natural conditions. The best method is to plant yolk sac fry or fed fry (4–6 weeks) into the brood streams or straight into the salmonid fishing grounds. In order to support native populations it is not recommended to plant older stock (more than 1 year old).

HEALTH ASPECTS OF REARING

A good health condition is essential for successful rearing of the brown trout brood stock. The health condition depends on many factors, such as the observance of hygienic rules, good quality of food and effective prevention against importing infection into the hatchery. Nevertheless caution must be taken and the fish farmer must be ready to tackle an unexpected infection outbreak. The following overview describes some of the most significant diseases of the brown trout. Detailed description, treatment and prevention of individual diseases have been published in Kolářová and Svobodová (2009) and Kouřil et al. (2008).

VIROSES

There are basically 4 viroses which can occur in salmonids and which are listed as Notifiable (serious) diseases in the Czech Republic and the EU: **Viral Hemorrhagic Septicaemia (VHS), Infectious Pancreatic Necrosis (IPN), Infectious Haematopoietic Necrosis (IHN) Infectious Salmon Anaemia (ISA)**. ISA has not been recorded so far in brown trout populations either in the salmonid fisheries in the Czech Republic or world-wide. IPN affects young fish (up to 5 cm) and it has been diagnosed in brown trout, as well as VHS. Brown trout is more immune to IHN than rainbow trout, but can act as a disease vector.

The symptoms of all described diseases are very similar in the first stage of illness – darkening of the body, exophtalmus (bulging eyes), torpidity, swimming malfunction, loss of reflexes. Veterinary care should therefore be sought everytime suspicion arises. Exact diagnosis of salmonid viruses is only feasible by laboratory tests which are carried out by the National Reference Laboratories of The State Veterinary Administration (SVS ČR) for fish viruses: The Veterinary Research Institute (VÚVeL Brno) for Moravia and Silesia area and The Reference Laboratory of the SVS ČR for fish diseases, The State Veterinary Institution (SVÚ) České Budějovice for the Czech area. About 10–15 live fish with symptoms should be sent to the laboratory for testing. The water temperature should not exceed 10 °C during transportation.

Treatment of viral diseases is not carried out.

The liquidation of serious disease is carried out according to the public notice No. 299/2003 Coll. The breeder whose animals have symptoms of dangerous disease is obliged to ensure that suspicious animals do not leave until the arrival of an official veterinary surgeon.

Prevention. Veterinary Act No. 286/2003 Coll. imposes obligations for breeders who supply salmonids to the market to examine salmonid fish for dangerous diseases IHN, IPN, VHS and ISA. The legislation classifies brown trout as receptive species to all the above named diseases. Fisheries with production of stock for open waters provide ovarial liquid taken from individual species of stripped fish for virological examination. During the sampling it is advisable to cooperate with the responsible veterinary surgeons.

BACTERIAL DISEASES

All three undermentioned bacterioses are listed as fish diseases on list III. of the European legislative (public notice No. 299/2003 – list III. and n. 381/2003 Coll. in the Czech Republic). If such infection occurs, an epicentre of infection and protection zone is declared by the veterinary authority. If after disinfection and re-stocking of the fishery with the sensitive species and age category the disease or suspicion does not re-occur during the observation time determined by the veterinary authority, the disease is considered to be defeated.

Bacterial diseases are treated with antibiotics (Kolářová and Svobodová, 2009). Suitable antibiotics are chosen according to the results of the bacteriological test and the test of sensitivity of the causative agent. Fish are tested at the State Veterinary Institutions and The University of Veterinary and Pharmaceutical Sciences Brno. The fish must be alive when sent for examination. The most frequent bacterial diseases are considered to be Furunculosis, Enteric Redmouth Disease and Bacterial Kidney Disease.

Furunculosis

Furunculosis is a globally widespread disease including the Czech Republic. All salmonids are sensitive to this disease, with brown trout being more sensitive than rainbow trout. The causative agent is the bacterium *Aeromonas salmonicida* subsp. *salmonicida*. The disease develops mainly during higher water temperature (15–21 °C). In temperatures below 7 °C the disease develops in a latent form. The disease can also develop in peracute form without the specific symptoms (it causes rapid mortality). An acute form is characterised by neurotic symptoms and anal prolapse. Typical changes of skin (abscesses – furuncules) are developed only in a subacute and chronic form.

Enteric Redmouth Disease (ERM)

A causative agent is the bacterium *Yersinia ruckeri*. Brown trout are among the most sensitive species to this infection. The disease develops in fish in their first year when water temperature is between 13–15 °C. Infected fish are dark, lethargic, and blood stains develop at the base of fins, jaws, upper roof of the mouth and operculum.

Bacterial Kidney Disease (BKD)

A causative agent of the disease is the bacterium *Renibacterium salmoninarum*. Brown trout are more sensitive than rainbow trout. The disease usually develops at spring when the water temperature rises to 13–18 °C. Fish generally get infected in their second year of life, soft and alkaline water worsens the disease progress. Infected fish are dark and have neurotic symptoms.

PARASITICAL DISEASES

Parasitoses are treated with an antiparasitic bath and antiparasitic medications added to the food (Kolářová and Svobodová, 2009). Parasitical diseases can be endoparasitic or ectoparasitic.

Ectoparasitic diseases

A causative agent of parasitosis is located on the skin and gills and causes necrosis of the affected area. Parasitoses develop especially during high concentrations of fish and a lack of light. Mature trout are most commonly infected by the protozoan *Chilodonella piscicola*. It survives without the host fish for several days at water temperatures between 3–5 °C, but dies without a host after 1 hour in 20 °C temperature. Sunlight and light negatively affects the reproduction of the parasite. Another common parasitic protozoan is *Ichthyophthirius multifiliis* (white spot disease). The developmental cycle of this protozoan is 35 days in water temperatures off 10 °C and 3–4 days in 21–24 °C. The developmental stage released from the cyst (theront) dies within 2–4 hours without a host. Theronts die in light about 2–3 times faster than in the dark and do not survive in pH 5.5. Contamination is caused by infected fish or by the water containing theronts. Skin and gills are also often invaded by flukes *Monogena*, family *Gyrodactylus and Dactylogyrus*.

Endoparasitic diseases

Salmonids are threatened by the parasite *Hexamita salmonis*, especially in their first year of life. This protozoan does not attach itself to the organs surface, but floats in liquids and affects the caudal part of the intestine, cholecyst and bile duct. It causes digestion disorders followed by a loss of weight and death. Intestines of salmonids are also attacked by flukes (class *Trematoda*), thread worms (*Nematoda*) and tapeworms (*Cestoda*), such as *Proteocephalus neglectus* reaching lengths of 100–150 mm. Intermediate hosts are copepods. Adult tapeworms attach themselves to the mucous membrane of pyloric appendixes with only the body protruding to the intestine. The mucous membrane is mechanically damaged and this causes an inflammation and necrosis along with malfunction of physiologic function of the intestines. Affected fish lose weight and can die, with massive invasions causing intestinal obstruction.

3. COMPARISON OF „TECHNOLOGY NOVELTY“

Traditional and widely used methods of obtaining brown trout stock for open waters consist of fishing for the brood fish from native rivers, their stripping and rearing of fry. Rearing can be done in extensive conditions (brooks, ponds) or in controlled conditions to the stage of 1 or 2 year old fish which are then stocked into the fishing grounds. However, numbers of brood fish in open waters are declining for a number of reasons. To obtain sufficient numbers of brood stock, it is necessary to fish in longer and most valuable stretches of rivers. This of course has a negative effect on the natural reproduction which is the basis for the survival of the species and conservation of its genetic variability. There is a lack of brood stock in some parts of the country, and this situation is often dealt with by importing and stocking of fish originating from different regions or even abroad. A relatively common practice is stocking of intensively farmed fish and breed lines of a different genetic origin (e.g. Ital, Kolowrat). Such methods can threaten the stability of local wild populations. Fry production from traditional methods varies considerably from year to year because its dependence on seasonal conditions. Because of this instability, purchasers secure the stock with supplies from other sources as they cannot rely on one fishery production. Expenses related to fishing for brood fish, transport and rearing of fish often exceed the profit from sale of the produced stock. On many rivers the negative impact of brood stock fishing and their stripping on a native population is evident.

Implementation of new techniques will help to increase and stabilise production of brown trout stock on the level needed by organisations responsible for management of rivers in the Czech Republic. The creation of the system of regional fry production facilities working with local populations of trout will help to move back from stocking of allochthonous fish from other regions or abroad. Self-breeding of brood fish and abidance of the above mentioned method will fulfil the requirement of long term sustainability and stability of production of high quality stock with a characteristic maximally similar to wild populations. At the same time this technology will enable us to abandon massive fishing for brood fish from natural waters, which will support their natural reproduction.

4. APPLICATION OF THE TECHNOLOGY

This technology is designed especially for the brown trout stock production facilities, such as angling unions or small private hatcheries. The aim of the implementation of this technique should be to increase and stabilise the production of high quality brown trout stock of native genetic origin for natural waters, stocking and reduction of stock transport between different regions and from abroad. Stocking of indigenous genetic lines will increase the support of the wild population, preservation of the gene pool and intraspecies variability and will prevent genetic contamination from stocking of allochtonous stock.

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CHAPTER 5

TECHNOLOGY OF EUROPEAN GRAYLING BROODFISH CULTURE IN CONTROL CONDITIONS FOR SUSTAINABLE RUNNING WATERS STOCKS PRODUCTION

Randák, T., Turek, J., Kolářová, J., Kocour, M., Kouřil, J., Hanák, R., Velíšek, J., Žlábek, V., 2009. Technology of European grayling broodfish culture in control conditions for sustainable running waters stocks production. Metodology edition (in Czech), FFPW USB Vodňany, no. 97, 24 pp.

TECHNOLOGY OF EUROPEAN GRAYLING BROODFISH CULTURE IN CONTROL CONDITIONS FOR SUSTAINABLE RUNNING WATERS STOCKS PRODUCTION

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1. AIM OF TECHNOLOGY

The aim of this technology is to provide fish farming practice with information about procedures and management in farming of brood European grayling (*Thymallus thymallus* L.) under controlled conditions. The purpose for application of this technology in practice is to improve and stabilise the production of quality genuine European grayling fry to be planted in open waters with subsequent restriction of fry transport between regions including purchases from abroad. Planting of genetically genuine fry will contribute towards enhancement of support for the naturally living populations, preservation of the intra-specific variability and prevention of genetic contamination due to planting of non-genuine fry. The procedures suggested must comply with parameters for long sustainability and stability of production of quality fry proving their characteristics similar to the freely living populations to the maximum extent.

2. TECHNOLOGY DESCRIPTION

INTRODUCTION

The European grayling (*Thymallus thymallus* L.) belongs to the most economically significant species within tour waters of Czech Republic. However, there have been numerous factors forcing reductions of its population since 1990's. There is a whole range of causes to these circumstances. The essential prerequisite for evolution of natural population of salmonid fish is their successful reproduction in natural way ensuring the preservation of genetic variability as well as stability of these populations. The existence of a successful natural reproduction requires presence of sufficient numbers of brood fish. The numbers of brood fish (as well as fish in general) within a specific area is affected by the water stream complexity (Harsányi and Aschenbrenner, 2002; Turek et. al., 2009), hydrologic conditions (Rogers et al., 2005), the intensity of predatory pressure from fish-eating predators (Mareš and Habán, 2003; Spurný, 2000, 2003a,b), the water pollution (Kolářová et al., 2005), the previous fish keeping management as well as the numbers of fishermen (Lusk et al., 2003).

To prevent further reductions in the population of European grayling, there are several measures to be taken with respect to its protection, i.e. prevention of unsubstantiated modifications of water streams, efforts towards improvement of their complexity, reduction of predator activities, changes to the fishing rules as well implementation of the overall strategy of keeping particular trout fisheries etc.

One of the options to support the naturally living populations, especially in such areas with lack of optimal natural reproduction, is planting quality and adaptable fry – i.e. such fry, whose genetic characteristics are not very different from the naturally living populations, into which the fry is added.

On the other hand, uncontrolled planting of fry of different origin may affect the genetic characteristics of the original local populations with negative impact, which can further result in abolishment of the intra-specific diversity finally leading to reduction of such populations.

As the numbers of brood fish in open waters are still decreasing, there is a reduced yield from the fry obtained through induced spawning of fish living in genuine nature. There can be a significant increase in numbers of brood fish, as well as the fry can be achieved by means of controlled farming. However, that requires proficiency in the entire process of keeping, including the early stage rearing, juvenile and young breeding fish. When establishing a brood stock to be kept under the artificial farming conditions, the most convenient process is based on the original populations living in genuine nature within the particular area.

Photo 1. *European grayling.*

The keeping process must include such procedures that can never affect genetic characteristics of the fish in stock, not even in the long run. The very principles of technology for rearing the European grayling under controlled conditions includes procedures applied within the management of keeping brood European grayling, procedures for establishment of brood stock, technologies for rearing of particular spawn categories (fry, yearling, young breeding fish, brood fish), procedures for reproduction under artificial conditions and veterinary aspects of the entire farming process.

3. UTILISATION OF FARMING TECHNOLOGIES IN THE FIELD OF MANAGEMENT OF BROOD EUROPEAN GRAYLING KEEPING

UTILISATION OF FREE-LIVING BROOD FISH FOR THE PURPOSE OF INDUCED REPRODUCTION

The process very often used at present to obtain brood fish lies in their trapping in open waters during the pre-spawn stage (often directly within spawning areas) using electric power generators is utterly inconvenient and even devastating with respect to the impact on the free-living populations.

At this time of the year, grayling is very sensitive to the effects of electric power and rough handling. Although the fish is returned back into the water stream following the induced spawning, most of them die within the next weeks or months. Trapping of fish right at their spawning areas further damages the already spawned eggs resulting in interference with the natural reproduction at its most sensitive stage. Annual occurrence of this procedure might wipe the entire species within a very short period of time at the specific area. Trapping of fish during spring months are often complicated by large amounts of water flow that reduces its effectiveness.

As far as trapping of the brood European grayling in open waters is concerned, there might be a more convenient procedure with the brood fish trapped during autumn time (e.g. when trapping the brood river trout) with their keeping in suitable reservoirs during winter months. The most common method for trapping the European grayling is the trapping within open waters using electric power generators. The conditions in such areas should be maximally convenient for abundance of this fish species and its natural reproduction. The conditions shall further enable effective trapping of brood fish. Areas that seem very convenient for this purpose comprise the fish conservation areas (CHRO), where the occurrence of angling has been eliminated. However such CHRO area would serve the purpose if the requirements below have been met:

- *The section of CHRO shall be done with preference to water streams showing very good resemblance to corresponding natural environment, with the least effect of industrial or communal waste water.*
- *The whole of CHRO as such should preserve the option for migration of fish. In this respect, the CHRO shall be compatible with further sections of the water stream.*
- *The area size should allow for abundance with several hundred of brood fish.*
- *There are no selective measures recommended within populations of the particular CHRO. Addition of fish in such areas should only be conducted in terms of natural reproduction only, i.e. neither the very CHRO nor its tributaries shall be provided with any fry, especially descendants of fish farmed within artificial environment.*
- *To preserve effective reproduction, trapping of fish shall leave approximately 1/3 of the brood fish population in the specific CHRO. For this reason, trappings shall be conducted with respect to the recommended return of young brood fish after their first spawn, together with the brood fish of best quality.*

Handling of trapped brood fish till their spring spawn can be provided using, for example, ground and trench ponds, channels and store-ponds. Yet flow through ponds are more convenient (usually up to 1 ha) supplied with water of good quality with sufficient water column (1–3 m) allowing the fish to find their natural feed.

An example of such pond can be seen in the Figure 2. That is a flow through pond with the water surface area of 1 ha under maximum water level conditions, with the depth of 4 m at the dam body and inflow channel reaching deep into the pond. Brood fish of both genders are planted into the pond, which has been filled with water for several weeks, at the end of October, with the total number of 200–400 pcs at maximum. The pond stays full over winter months with the water level reduction following in the spring (March), by about 1.5 m together with flow through reduction. This condition allows the water warm up faster and better and to expose a certain part of the channel reaching into the pond.

This channel can be then used for observation of intensity of fish migration to the inlet to estimate the optimum time taken by trapping the fish and their relocation to the handling reservoir close to the hatchery. The channel can be also sued for installation of the trapping device.

With their natural feed available, the fish will mature better and produce more of their genital products. When keeping the fish in reservoirs with lack of their natural feed, they shall be fed with artificial feeds. However, some of the fish may reject the artificial feeds as they are never accustomed to the matter. The low level of nutrition is then reflected in ill production of genital products.

During the spring time (usually at the break of March and April), when the fish start heading towards the inlet, these ponds shall be subject to trapping and the fish relocated into smaller handling reservoirs close to the hatchery. These reservoirs feature reduced water flow allowing for temperature increase. A supply of water at constant temperature (optimal temperature would be 10–14 °C) is an advantage for the hatchery. Maturing of brood fish is then conducted under ideal conditions. The fish in under control and mature fish spawn. The usual number of cycles required for spawning of one fish is approximately 2–3, the break between cycles shall be equal to 4–7 days. The fish must be handled with the best care. Anaesthesia is recommended for fish spawning (Kolářová et al., 2007). Anaesthesia of brood European grayling in our environment would be most often conducted using the 2-phenoxyethanol (concentrated to 0.3–0.4 ml/l) or using the clove oil (concentrated to 0.03 ml/l). The preparation of anaesthetic solution requires careful blending of the agent used in water.

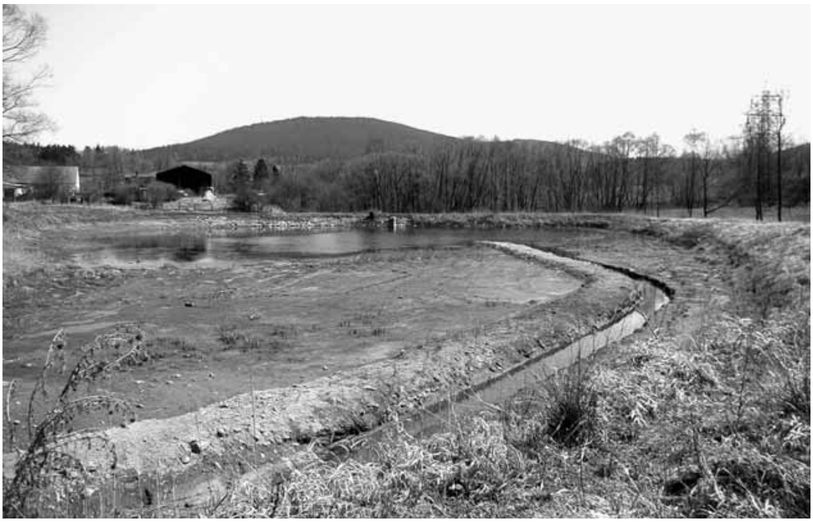

Photo 2. *Flow through pond suitable for keeping the European grayling.*

The best result can be achieved starting with a smaller volume (1 litre) of water at the temperature of approx. 20–25 °C (e.g. using a sealed plastic bottle) by means of repeated shaking. This concentrate shall be then poured into a container with water, where the process of fish anaesthesia is to be performed. Recommended containers include fish tubs with the volume of 40 litres. It is recommended to aerate the anaesthetic bath using an air diffuser (aeration stone) connected to a pipe with compressed air.

Any repeated anaesthesia of larger number of fish in the container (the time interval required for anaesthesia is subject to progressive extension) must be performed with frequent replacement of anaesthetic solution. Frequent replacement of the anaesthetic contents is required in case its aerating options are disabled. The fish shall be left in the anaesthetic solution until achievement of their total anaesthesia (they lie on side and breathe), which can be generally achieved within the interval of 2–4 minutes using the recommended anaesthetic agents of said concentration. There is no higher level of anaesthesia (resulting in stopped breath) is not required with respect to the action (induced spawning) and not even desired (potential health hazards for the fish). Spawning should be followed by application of the short bath in the potassium permanganate (Kolářová and Svobodová, 2009). The very method of reproduction has been described in special section.

The fish shall be returned back into the water stream as soon after the induced spawning as possible. Planting of fish into the natural water stream contributes towards significant reduction of their mortality after spawning (the mortality rate of brood fish, in this case, is comparable to the mortality rate observed during natural spawning) and enables their re-use during other breeding seasons. The quality of eggs obtained is very good and proven by high fertilisation rate (usually 70–90%) resulting in higher effectiveness of the entire farming process. There are no costs associated with keeping of brood fish within controlled environment.

FARMING OF BROOD EUROPEAN GRAYLING UNDER CONTROLLED CONDITIONS

Farming facilities designed for keeping of the European grayling should be provided with all-year supply of water of good quality the temperature of which should not exceed 20 °C in a long run. Farming facilities should be designed to allow flow through.

BROOD STOCK ESTABLISHMENT

Brood fish farming under controlled conditions shall start from the fry stage. When forming brood stock to be kept under controlled farming conditions, the most convenient method should be based on the natural environment with free-living populations within specific areas. The ideal case comprises obtaining genital products from brood fish originating from the greatest number of streams possible within the same catchment area under minimum influence of fish farming (especially planting fry of the European grayling) and natural reproduction environment (e.g. fish conservation areas).

In our environment, the reproduction of European grayling takes place during April till May. Water temperature belongs to the main factors affecting the fish maturing process. The optimum water temperature in this period is approximately 10 °C. Any long-term decrease of temperature below 6 °C would basically stop the maturing process.

INDUCED SPAWNING

Induced spawning of fish shall be performed in such manner that ensures potential genetic variability of the offspring gained. There is method used frequently at present, when a large quantity of eggs (even from several tens of females) is matched with sperm from a large number of males, the so called "poly-spermatic fertilisation", which is proving inconvenient with respect to preservation of the genetic variability of offspring.

Recent surveys have shown that the poly-spermatic fertilisation results in competition of sperm and most eggs would be fertilised with sperm from a small number of males only, compared to the large quantity of sperm used for eggs fertilisation. In order to preserve maximum genetic variability of offspring gained through induced spawning of brood fish, the current practice links to recommendations of different procedures (Kašpar et. al., 2008). With respect to the environment at most of our grayling hatcheries, induced spawning of the European grayling can be conducted using the procedure described below.

The most convenient method for fertilisation of eggs is the dry process, when the eggs is inserted into a dry bowl together with the ovarian fluid, or even onto a mesh used for separation of ovarian fluid droplets with subsequent relocation or eggs into a dry plastic container (Fig. 3). Female should be spawned individually, i.e. the eggs from a specific female shall be inserted into a dry container or mesh. The quality of spawned eggs shall be subject to visual inspection (i.e. assessing the presence of blood, egg clusters or evidently low quality eggs, etc.). In case of good quality, further process shall proceed with a gauge (e.g. scoop) taken to relocate a quantity of eggs of certain volume (approximately the quantity matching an average volume of eggs gained from 1 female) into a larger dry container (container A). The aim of this measure is to supply the container A with a similar quantity of eggs from every spawning female. The remaining volume of eggs (if any) shall be placed into the container B. if the quality of eggs is evidently low, the scoop shall be placed into the container B directly, or even discarded immediately.

Photo 3. *Induced spawning of European grayling female.*

Containers A and B are used to gather eggs throughout the entire spawning process. These containers shall be covered with a wet cloth and the eggs (during the spawning process) may not be exposed to sunlight, any temperature significantly different from the temperature of water used for eggs incubation, with special attention to prevent any contact of egg with water. The presence of water in eggs, prior to fertilisation results in significant reduction of the final fertilisation rate.

Following the spawning of females, the eggs in container A shall be mixed together and then divided (to the best technical extent possible) into the greatest number of portions possible (smaller containers). Particular portions of eggs will be subsequently fertilised using the sperm from various males. The number of males used for fertilisation of one portion of eggs should correspond with the number of males available, as well as the number of containers with particular portions of eggs. That means the fertilisation of eggs will be conducted using a specific make for a particular single portion of eggs. The optimum number of makes used for fertilisation of 1 particular portion of eggs should be 1–6, with the rule implying that the fewer the males used for a specific single portion of eggs, the higher the probability of achieving the maximum genetic variability. The general recommendation implies that every particular portion of eggs shall be treated with milt from at least two males, as this practice can help reduce the loss of eggs with milt from a single male only (the so called "individual fertilisation"), if the quality of such sperm is low. The sperm (milt) shall be applied onto the spawned eggs directly with the same rule as for eggs, i.e. the milt may not come into contact with water. The contact with water can be prevented prior to spawning, by wiping the urogenital apparatus of the fish being spawned (applicable to females) and its vicinity, together with the anal fin, with a piece of wet cloth (wipe cloth, etc.). Genital products shall be mingled, infused with water (from the source to be used for further incubation of eggs), which will activate gametes and initiate the very process of fertilisation, and the eggs shall be mixed with care once again. The initiation of fertilisation process can be started using a physiological solution replacing water (0.9% water solution of sodium chloride), which, according to the experience gained by some fish keepers, will increase the percentage of fertilised eggs. The temperature of such physiological solution should match the temperature of water used for incubation. In ideal case, such solution shall be prepared prior to spawning, with water used for subsequent incubation of eggs. The eggs should be mingled using a suitable tool, e.g. a clean spatula or scoop (plastic, rubber or wooden). The thickness of water mass above the eggs should not exceed 1–2 cm to prevent excessive dissolution of sperm and reduction of the fertilisation rate. Following this step, the containers with eggs shall be left to settle for approximately 2–3 minutes to complete the fertilisation process correctly. The eggs shall be further infused carefully, using the same water as the volume used for incubation, together with all the portions of eggs from the previous container A, the material shall be poured into a single container again, followed by thorough mixing. The step associated with mixing of eggs once again is important for the fact that the later stage requires particular incubation facilities provided with a homogenous mixture of eggs from various parental couples. The stage of washing eggs using clean water, the container(s) will be supplied with the maximum amount of clean water possible and left to settle the egg for the period of approximately 1–2 hours. This stage results in swelling of eggs and the eggs are very sensitive to vibration at this stage. Once the eggs have swollen, it shall be relocated into the incubation facility.

The eggs placed in container B shall be handled similarly. Fertilisation of this eggs shall be conducted using the same males as for material in the container A. Every incubation apparatus shall be labelled in such way that the eggs originally from containers A or B will be distinguished properly. If the process of spawning has been split over several days, the above mentioned procedure will be used to establish portions of eggs A and B for every spawning day. Throughout the entire spawning season, the container A will be supplied eggs from various females in the quantity (volume) similar to quantities obtained from particular females respectively (i.e. same gauge used for handling eggs within a specific spawning season).

In case of spawning taking longer time within a single day (several hours) and a large number of fish subject to induced spawning, the procedure of spawning and fertilisation shall be repeated several times during the day.

The application of anaesthesia is convenient for fish subject to spawning (Kolářová et al., 2007) with subsequent bath using the potassium permanganate solution (Kolářová and Svobodová, 2009). Upon completion of the disinfectant bath, the spawned fish shall be returned back into the natural water streams as soon as possible.

Once the brood stock has been established, the eggs from container A shall be used with preference. It shall be noted that, if the spawning was conducted within different timescales, maximum genetic variability of future brood fish requires collection of approximately the same amount of fry from every spawning stage (i.e. from every container A during a specific spawning season). The material from container B will only be used in case the quantity of eggs from the container A is insufficient.

The establishment of a brood stock shall be conducted with the estimate of 200 pcs of fertilised eggs per one future brood female. An optimum brood stock should comprise at least 100–200 females. If we are to establish a brood stock comprising 100 females and 100 males, we will need approximately 20,000 pcs of fertilised egg. These eggs should be obtained from the largest variety of parental fish as possible (at least 20–30 couples).

In order to prevent significant phenotype and genetic modifications pursuant to a long-term (for several generations) impact of artificial farming on the brood fish kept, with subsequent effect on their offspring (Fleming and Einum, 1997; Einum and Fleming, 2001; Verspoor, 1988; Hanák, 2008), every brood stock shall be formed using offspring of free-living fish in accordance with the above described procedure. In case the production of fry material is performer using the first generation of fish reared under controlled conditions, it might be assumed that the characteristics of parental populations shall be reserved to the maximum extent. In order to ensure maximum preservation of initial characteristics of offspring, the eggs from farmed females shall be fertilised to the maximum extent, using the sperm from free-living males originally from, for example, fish conservation areas (CHRO). If the specific farming facility has a reliable source of free-living males, some of them may be removed from the rearing process at the time of sexual precocity. In spite of the latter, it is recommended to keep the brood stock in such condition that the ratio versus genders equals to 1:2 to the benefit of females.

HORMONAL INDUCTION OF INDUCED SPAWNING

In case of high probability implying a negative change in conditions allowing for optimum maturing of brood fish (e.g. prior to an expected significant decrease of water temperature) the process of brood females maturing can be affected positively by means of application of hormonal agents.

The hormonal induction of ovulation represents an alternate procedure used for synchronisation between the ovulation period and increasing the number of ovulating females. It will allow us to schedule spawning of female fish within a single period, or even two periods. This procedure will help us avoid the need for multiple repeated inspections of females (focused on their ovulation) and their potential spawning. That will reduce both the amount of labour required as well as the stress and risk of mechanical damage to females, as those are usually causes for the fairly high rate of mortality of this fish species after spawning.

The hormonal induction of ovulation is performed on female fish under anaesthesia (for the anaesthesia procedure see previous section) during the pre-spawning period using injection of hormonal agents into the dorsal muscle. The date for application shall be scheduled in such way that the performance of induced spawning (once the expected ovulation process has been achieved) can be conducted on convenient day (days).

Right before the injection of hormonal agent, the assistant shall remove a female fish under anaesthesia from the bath and lay it onto a wet soft cloth (placed on a work table at convenient height). Another assistant will use a different piece of wet cloth to fix the female fish in place, while covering the head and tail part with the tail fin. Prior to this step, the person performing the very injection shall draw the solution into syringe to prepare for its application. The injection itself shall be applied intramuscularly into the dorsal muscle beneath the dorsal fin (approximately 1–2 cm below the dorsal fin) diagonally at the angle of approx. 30° in cranial position (towards the head). The needle shall be run into the depth of approx. 1–2 cm in order to prevent interference with any internal organs. The said amount of solution will be then pressed by the syringe piston to enter the muscle and the syringe shall be drawn back to pull the needle out, with the syringe subsequently placed off the work table (to avoid personal injury due to spontaneous movement of the female fish). It is recommended to disinfect the puncture point with a light amount of potassium permanganate or any other suitable agent. Once injected the female fish shall be placed into the container provided with water flow and when the anaesthetic condition has perished (usually within a few minutes), the female fish can be planted into a reservoir to await the commencement of ovulation and performance of the induced spawning.

This injecting procedure can be performed using one of the two proven agents. The first one is the Gonazon™ agent, the preparation process must comply with the instructions from manufacturer, i.e. the concentrate shall be dissolved with the solvent supplied and the final solution shall be used for injecting. The second agent is the Supergestran containing efficient substance – Lecirelin. Every packet (box) contains 10 sealed glass capsules (every capsule contains 2 ml of the efficient substance solution to produce solution with concentration equal to 25 μg/ml). The dosage instructions show 1 ml per 1 kg of fish, i.e. 25 μg/kg). The weight of fish can be estimated with the accuracy deviation of 0.1 kg and the agent dosage shall be maintained depending on the weight of female fish with the accuracy deviation of 0.1 ml. As far as the storage of both agents is concerned, the instructions from manufacturer shall be observed. Previous experiments associated with induced ovulation of female fish also employed the carp hypophysis. With respect to the general avoidance of this agent, focused on its replacement with synthetic agents in form of drugs, we do not recommend its use.

The duration of latency interval (from the injection of hormonal agent up to the commencement of ovulation, i.e. the option for induced spawning of eggs) is significantly affected by water temperature. Temperatures of 6–8 °C can induce ovulation within about 8–10 days, dropping to 6–7 days at the temperature of 8–9 °C and approximately 4–5 days at the temperature level of 10–12 °C. The induced spawning shall be then scheduled accordingly. With regard to the fact that especially lower levels of water temperature result in delayed ovulation of some females, it is desirable to wait for approximately 2–4 days (this interval shall be amended depending on water temperature, i.e. longer intervals at lower temperature and shorter intervals at higher temperature) before another inspection of the fish and spawning of remaining females.

INCUBATION OF EGGS

The most convenient facilities for eggs incubation comprise Kannengieter's vessels (Fig. 4). Every such vessel is made of two parts, whereas the volume of the internal part is usually equal to 1–1.5 litre. Such vessel can be used for incubation of approximately 20,000 pcs of eggs. There are also larger versions of these vessels available, while the incubation capacity increases in proportion with the vessel size.

Photo 4. *Incubation of European grayling egg in Kannengieter's vessels.*

Once the eggs has been fertilised and placed into incubation apparatus, attention shall be paid to regular removal of the dead (white) egg gathered on the surface of incubated eggs clusters. Prior to development of eye points (usually 80–90 °D), the eggs is fairly sensitive to vibrations, therefore such vessels shall be provided with a minimum flow through only. The eggs are also sensitive to light effects. Prior to the completion of incubation process, the eggs shall be relocated from the incubation vessels on the classic Rückel-Vacek apparatus with openings sized approximately 1–1.5 mm or even on trough inserts. Such apparatus may be used for the very eggs incubation in alternate cases. The apparatus used for incubation of eggs for establishment of the brood stock must be labelled clearly. The temperature of water used for incubation should be equal to the optimal level of 10–12 °C. The duration of incubation period depends on the water temperature and ranges within the interval 150–200 °D. The incubation process can be run with preventive baths of egg (Kolářová and Svobodová, 2009). The rate of fertilisation rate for eggs from free-living fish ranges around 70–90%. Any insensitive handling of eggs during the period prior to eye points development may result in serious losses. Hatching of fry occurs on Rückel-Vacek apparatus and trough inserts. This process requires setting the apparatus for the lower flow option and increase of the flow through to cater for greater demand for oxygen among the brood stock as well as careful removal of egg casing.

KEEPING OF FRY AND YEARLINGS UNDER CONTROLLED CONDITIONS

Once the fry has hatched, the so called "dwell time in rearing" follows with the fry lying at the bottom of incubation apparatus and feeding on the nutrients contained in the yolk sack. This period is finished after digestion of approximately 2/3 of the yolk sack and the fry starts to swim. The duration of such dwell time usually ranges around 40–60 °D (4–6 days). The feeding of fry can be started at the end of this stage, directly in the apparatus, or the fry can be planted for the purpose of extensive keeping on its natural feed.

As far as extensive and semi-intense keeping, the fry is planted immediately after its initial swimming even with the remaining part of yolk sack, or after the initial feeding on troughs, to be planted into prepared ponds or nursery reservoirs (natural swimming pools, fire protection reservoirs etc.) abundant with sufficient amount of natural feed of optimal size (fine plankton). These reservoirs shall be filled approximately 10–14 days prior to fish planting. The ideal situation occurs with sufficient inflow of clear water and firm bottom free of any thick layer of sediments. It is recommended to apply reasonable amount of organic fertiliser within (e.g. compost, litter) prior to filling of the pond, which will support evolution of natural feed for the fish. The water temperature may exceed 20 °C during the first year of keeping. The optimum size of such reservoirs is 0.5–1.5 ha each in case of extensive keeping without any extra feeding, and up to 0.5 ha as far as the semi-intense keeping with extra feeding of the stock is concerned. The size of brood stock depends on the size of fish planted (fry, fattened fry), the quantity of natural feed and the intensity of potential fattening as well as requirements related to the size of fish caught. In case of solely extensive method of keeping, it is recommended that 10 pcs of fry be planted, i.e. 2–5 pcs of fattened fry, per 1 m^2 of the water reservoir surface. With sufficient amount of feed and the increasing level of fattening will allow for extensions of the brood stock. Initial stock size would equal to approximately 50–150 pcs of fry in such case, 20–50 pcs of fattened fry per 1 m^2 of the reservoir surface respectively. These reservoirs shall be observed for the level of occurrence and size of plankton to balance any reductions by means of fattening using complete feeding mixtures. Installation of automatic feeding points at the inlet part of the keeping reservoir belongs to recommended methods. With larger brood stocks, some of the fish can be trapped during the vegetation season. The rate of loss associated with the above described methods of keeping usually range around 30–70% during the vegetation period.

As far as the intensive keeping is concerned, the starting stage of the subsequent rearing period, the so called "active rearing period", when the fry shows significant motional activity and progressive transformation from endogenous to exogenous nutrition, such fry shall be relocated onto shallow troughs (mostly sized 4 x 0,4 x 0,2 m) and the fattening stage commences. The stock within each trough comprises 40–60,000 pcs at the beginning of the fattening stage. Fattening troughs must be well screened and protected from direct exposure to sunlight. During the fattening stage and other stages of keeping, it is recommended that complete feeding mixtures be used. There were some trout feeding mixtures proven in practice, with lower fat content, made by renowned manufacturers. The size of pellets for fattening should be less than 0.3 mm. The fattening process shall be combined with manual feeding using smaller doses at high frequency (6–10 x per day) over the entire trough surface, with later application of automatic feeding points, when the fish start feeding readily (e.g. controlled with a timer), at the best rate of 2 devices per 1 trough. The feeding dose size should correspond with lower threshold of dosage recommended within feeding instructions applicable to river trout usually supplied by the feeding material manufacturer.

The pellet size in submitted feed should correspond with the size of kept fish (see feeding catalogues from the manufacturer). It is recommended that low water column is preserved within troughs at the beginning (approx. 10 cm). In case the fry struggles to accept the feeding mix, it is necessary to use the live or even frozen plankton of optimal size. The use of plankton increases the risk of disease importation into the stock and inhibits the establishment of custom for feeding on the feed mix, yet its utilisation if necessary in some cases in order to prevent mortality of the fry due to starvation. When using plankton, the convenient method includes combining the natural feed and the feeding mixture, i.e. combined dosage of both. The unit losses would be less than 20% at the initial stage of rearing.

Following 3–4 weeks of initial rearing, the fry (at the usual size of approximately 3 cm) shall be relocated into larger reservoirs (optional planting into ponds and nursery reservoirs – see above), where the rearing process continues up to the yearling stage. The usual systems for intense rearing include rectangular troughs or circular pools (Fig. 5). The size of brood stock depends mainly on the size of reservoirs and the oxygen content in water. There are usually 2,000–4,000 pcs of fry planted per 1 m^3 of water. The rate of oxygen content in water at the outlet parts of rearing reservoirs should not drop below 60%. To ensure optimal growth, with regard to the significant temperature tolerance of grayling during its first year of life, such reservoirs shall be supplied with water of higher temperature. The water temperature may reach the level of 25 °C during summer months provided that attention is paid to strict adherence to hygiene requirements and sufficient content of oxygen in water (oxygen content at the outlet exceeding 60 %). Rearing reservoirs can be fitted with aerating or oxygen supply devices to allow for adequate increase of unit count within brood stocks. Depending on the growth rate, fish shall be divided into more reservoirs during the rearing stage. The stock size of yearlings towards the end of their rearing stage should be approximately 500–1,000 pcs.m^{-3}. Unit losses during the rearing stage of yearlings would usually stay below 30%.

Photo 5. *Circular rearing reservoirs for European grayling.*

The course of fry rearing stage shall be associated with preventive inspection of fry with focus on parasitic infections (at least once in two weeks time or immediately in case of mortality increase) and strict attention to the clean environment (i.e. removal of feed leftovers, faeces and deceased pieces). During periods critical with regards to the evolution of parasitic infections (usually summer months), the use of preventive treatment baths is recommended (Kolářová and Svobodová, 2009).

The establishment of habit for consumption of granulated feeding mixtures at the very initial stage of evolution of the fry of European grayling provides for further continuation with its rearing under controlled conditions and to obtain the required brood stock in the final stage. This procedure allows for regular supply of feed at adequate amount and promotes elimination of the risk associated with imported parasitic infections to a great extent.

4. REARING OF YOUNG BREEDING FISH AND BROOD STOCK

REARING OF YOUNG BREEDING FISH & BROOD GRAYLING UNDER EXTENSIVE CONDITIONS

Rearing of young breeding fish under extensive conditions can be conducted using suitable reservoirs of pond type, channels etc. The main source of nutrition comes from the natural feed. Rearing of brood stock in such reservoir can be implemented starting with yearlings preferably, yet such environment can be planted with two years old fish – shots. The brood stocks shall be reared under extensive or semi-intense conditions, i.e. to establish the habit for feeding on their natural feed. The quality of genital products of brood grayling reared under extensive conditions is mostly good. The disadvantage of extensive rearing of brood fish is the low quantity within stock, i.e. fairly low number of brood fish reared per one unit of area of the reservoirs used (usually 100–300 pcs.ha^{-1}), depending on the specific are productivity. This system will allow for rearing of young breeding fish and brood stock together and the purpose of induced reproduction shall be served with selection within the pre-spawning period focused on mature fish with sufficient production of genital products.

REARING OF YOUNG BREEDING FISH & BROOD GRAYLING UNDER SEMI-INTENSE CONDITIONS

Semi-intense rearing of young breeding fish and brood grayling in ponds is a very effective method. A pond suitable for rearing of brood grayling should be sized between 0.5 and 1.5 ha. Its depth should not exceed 1 m all over the water surface, with one part deeper to allow for trouble free wintering of kept fish. The necessary prerequisite for this method is the supply of water by means of sufficient inlet of fresh water, ideally from a creek or river. The temperature of water inside the point should not exceed the level of 22 °C during summer months. Suitable areas include ponds with sandy bottom, preferably with a layer of gravel added onto a certain part of the bottom surface. Muddy ponds are not suitable for grayling farming. These environments can be provided with some water plants as these create suitable conditions for evolution of maggots of water insects that represent an important part of the feed for grayling. The distribution of natural feed can be further supported with supply of sufficient amount of fertiliser into the pond. Advantages concern the pond location as areas close or urban environment are easier to protect from fish eating predators. Their negative impact can be also reduced by perimeter fencing around ponds and stretching wire or meshwork barriers above the water surface.

The stock of brood grayling should include 300–500 pcs.ha^{-1}. Both genders shall be reared together. In case we do not have a reliable source of free-living males (e.g. CHRO), the number of males farmed can be reduced down to the gender ratio of 1:2. This selection should be performed once the males have matured, during the pre-spawning period, when the males become evidently recognisable (Fig. 1). Free living males required for spawning shall be trapped preferably during autumn period (end of October) to be kept in rearing pond together with females over the winter. Their presence apparently has a positive effect on females maturing. Prior to this addition of free living fish, these shall be distinguished from the farmed ones (group or individual labelling) to enable their identification at a later stage.

However, most of the free living populations of grayling have been currently reduced to such extent that trapping of a small group of fish might impose serious risk to the population's ability for natural reproduction. Therefore, the induced spawning should be rather done using farmed fish of both genders, whereas the number of reared males does not have to be reduced in such case. Wild brood fish will then remain in the water stream to proceed with natural spawning that is a decisive factor for preservation of natural populations in open waters.

Table 1. *Review of external morphological signs with significant gender dimorphism, i.e. conditions typical for particular genders during the fish maturity period.*

Sign	Male	Female
Enlarged abdominal cavity	dull	significant
Abdomen stimulation releases	white sperm ("milt")	eggs, prior to the very spawning
Urogenital opening	slit-shaped	oval, swollen
Dorsal fin	large flag-shaped, sharp end, colourful	smaller, with rounded end, less coloured
Body colour ("wedding dress")	contrast to dark purple	less significant

Some of the feeding habits of brood grayling can be catered for with the natural feed, that be both autochthonous (zoo plankton, benthos, insect maggots) and allochthonous (mainly flying insects). The outstanding needs are satisfied by means of extra feeding with granulated mixtures. These are supplied via the automatic feeding points. If the brood fish being kept has been accustomed to the granulated feeds, the pond should be fitted with one or two feeding points. The fish can get used to granulated feeds soon and are willing to accept the feeds supplied. The feed dose size and the frequency of extra feeding depend mainly on the size of brood stock and the supply of natural feed, therefore this value cannot be determined accurately and it shall be subject to alterations and changes according to the local conditions. Extra feeding for brood grayling should be purchased from renowned manufacturers with lower fat content and reasonable albumin contents. The general recommendations concern mixtures containing the maximum of 15% of fat and approximately 55% of albumin. The extra feeding should be stopped in autumn (during the second half and towards the end of October) as excessive fat would impair the quality of genital products from brood fish. The spring extra feeding shall start in the post-spawning period only, once the fish has been returned into the pond. Brood grayling is reared in such ponds throughout the whole year, with the only change during prior-spawning period, when these are trapped and fish able to spawn will be placed into smaller reservoirs until the very spawning.

Once the spawning has finished and the fish has been returned into the pond, the extra feeding should resume, where the initial feeding doses can be increased (with respect to the quantity of natural feed available) to enable the fish recover their optimal condition. The rate of unit losses incurred mainly during the post-spawning period ranges between 20–40% per year, using this rearing method.

Young breeding fish can be planted into semi-intense rearing from the first or second year of age, with the stock size of 800–1,500 pcs.ha$^{-1.}$ Suitable areas include mainly smaller ponds (0.5–1 ha) to enable easier control over the fish health condition. In case there is only one suitable pond available, the young breeding fish can be reared together with the brood stock, yet the overall stock distribution must be set accordingly. Young breeding fish should be provided with extra feed using materials featuring the same characteristics as feeding mixtures for brood fish, with granules of corresponding size. This fish is then subject to selection of pieces for completion of the brood stock in autumn months, to be planted into the pond for winter period, together with the brood stock. The combined rearing of both young breeding fish and brood stock requires separation of these groups from one another right after trapping in the pre-spawning period, with the young breeding returned back into the filled pond and suitable brood fish used for induced spawning.

Any selection conducted during sourcing the fish for completion of the brood stock is truly inappropriate, except for removal of ill or morphologically deformed pieces. The individuals that behave as "outsiders" in the farming environment (slower growth) may bear genetic characteristics essential for survival under natural conditions. With strict adherence to the principles of hygiene, unit losses among young breeding fish can be maintained within the interval of 10–20% per year.

The greatest advantage of this rearing method is satisfaction of some feeding requirements with regard to supply of natural feed, which seems to be irreplaceable in order to ensure optimal growth of the European grayling as well as development and maturing of its genital products. The disadvantages comprise the fairly strict requirements related to the quality of rearing pond and the problematic protection of fish from fish eating predators.

REARING OF YOUNG BREEDING AND BROOD GRAYLING UNDER INTENSIVE KEEPING CONDITIONS

Intensive rearing of young breeding and brood grayling can be conducted using ground ponds, concrete store-ponds, circular pools or trench ponds with the water volume equal to tens or hundreds of m^3. The essential prerequisites include sufficient inlet with fresh water the temperature of which should not exceed the level of 22 °C during the hottest months. The water temperature shall be prevented from decrease to low levels as such condition would impair fast growth of the fish. Particular age groups (1–2 years, 2–3 years, young breeding fish group) shall be kept in separate reservoirs for optimal results. Three years old fish can be included within the brood stock. Yearlings can be planted in quantities around 100–300 pcs.m^{-3}, two years old fish shall be distributed in the ratio of 30–80 pcs.m^{-3} and brood fish is usually planted in quantities of 5–15 pcs.m^{-3}. Males and females can be kept together. If there is a reliable source of free living males, the number of males inside the brood fish reservoir can be reduced. The rearing process shall be conducted with strict adherence to the hygiene rules, removals of silt, feed leftovers and deceased pieces. The rate of oxygen saturation within the reservoir, measured at the reservoir outlet, should not drop below 60% in the long run.

It is recommended that reservoirs be subject to regular drain and deep cleaning (once in 3–4 months). Maximum care shall be provided when handling the fish. Unit losses incurred during rearing of young breeding fish range around 20% per year and these occur throughout the whole year, the rearing of brood fish then suffers 20–40% mortality rate especially in the post-spawning period. The post spawn period shall be therefore managed with strict adherence to hygiene principles with timely removal of deceased pieces, any massive losses shall be consulted with a veterinary expert regarding application of treatment measures (baths, antibiotics application).

Any feeding of fish according to this rearing method shall include solely complete feeding mixtures. The fat content in mixtures used shall range up to the maximum of 15%, the albumin content should be approximately 55%. The daily feeding dose hall comprise approximately 1–1.5% of the stock weight, depending on water temperature. The most convenient practice deals with provision of feed through automatic feeding points, yet the fish can be fed manually too. The granules should always correspond with the size of fish being farmed. Young breeding fish can be fed as long as they accept feed. It is recommended to stop feeding the young breeding fish during October in order to enable absorption of fat stored within the abdominal cavity as a result of feeding with fabricated mixtures in order to improve the quality and fertilising ability of genital products. The trapping of brood fish takes place directly prior to spawning and the subsequent procedure followed is similar to the semi-intense farming methods. Induced spawning is then conducted in accordance with the procedure described in previous section.

COMPLETION OF BROOD STOCKS

The resultant quantity of young breeding fish to be included within the brood stock shall correspond with the number of brood fish lost (usually 20–40% out of the total number of brood fish planned per year + the reserve quantity equal to at least 20%). The brood stock can be completed depending on specific status conditions and the level of actual loss within one- to three-year cycles, i.e. the stock for completion of the brood stock shall be established every year for the one-year cycle, any longer cycles shall be associated with formation of such stocks accordingly.

INDUCED REPRODUCTION OF BROOD FISH FARMED

The purpose of induced reproduction of brood fish lies in sole production of fish to be planted in open water streams. The spawning period would not generally differ from conditions seen at the free living population and it usually takes place during April in local environment. During the pre-spawning period (usually till mid April, when the fish start migrating to reservoir inlets), the fish should be trapped and removed from the rearing reservoirs (ponds and special reservoirs) and the individuals suitable for spawning shall be placed into handling reservoirs provided with flow through facility (e.g. troughs). These should be supplied with water of a comparable temperature (the optimal level is 10–14 °C) and similar chemical properties as the water inside the rearing reservoir, or even with the same water, in ideal case. These troughs allow for easy control over the fish without any exposure to excessive stress. As shown by experience of the foreign farmers, males would keep close to the water inlet, while females would prefer lower part of the trough. Females can be deemed ready for spawning as soon as they start approaching males within the inlet area.

Females shall be sorted prior to the very spawning and mature fish selected for the process itself (soft abdominal area, cranially from the urogenital opening, swollen urogenital opening, pressing of the abdominal cavity will release eggs). The non-mature fish shall be returned back into the handling reservoir. The inspection of maturity of fish due to spawning shall be conducted in regular intervals of three to five days each.

The very procedure of the induced spawning must be conducted with strict attention to preservation of genetic variability to the maximum extent, following the same steps described in previous section. Nevertheless, in case the entire offspring group is sold to another party, the spawned eggs did not have to be split into groups A and B. If there are any significant negative changes to the environment within the pre-spawning period (e.g. significant drop in water temperature) the maturing of fish can be boosted by means of hormonal agent application. The fish selected for spawning shall be at least 3 years old, yet the hen fish shall be at least 4 years old, in preferred case. The quality of genital products (especially eggs) from young fish is low and the fertility rate would be very poor there. It is recommended to fertilise the eggs from females reared under controlled conditions using sperm from free living males. However, this is often impossible due to various reasons (problems associated with obtaining the fish from their natural living environment and their potential keeping through winter months, frequent cases with small amount of sperm produced or even "no sperm release" at all, etc.) and the males reared on farm shall be used.

In case of such spawning including the free living fish, these shall be handled separately from the farmed fish in order to prevent confusion. If we are to spawn females trapped in open waters as well, the eggs from such fish shall be incubated separately from the eggs originating from farmed fish, whereas attention shall be paid to preserve legible marking of incubation apparatus units.

It is very recommended to conduct the induced spawning with the aid of anaesthesia that is of significant contribution to prevent any damage to the fish and to support relieve of genitals, especially at females, with the spawning followed by a short bath in the potassium permanganate (Kolářová and Svobodová, 2009). These measures provide for important reduction of the post-spawning mortality of brood fish. Once spawned, the fish is returned into rearing reservoirs or water streams.

The estimate of future production of eggs by the kept stock can be drawn with respect to the parameters below:

- *absolute fertility (quantity of eggs spawned per one female) approx. 1,500–3,000 pcs,*
- *relative fertility (quantity of eggs per one kilogramme of female weight) 8,000–15,000 pcs.kg^{-1} of the females weight,*
- *the percentage of females achieving sexual maturity in their 3rd year of life is equal to approx. 40–60%, the remaining fish would mature in further years. Males usually mature one year ahead of females.*

The fish kept in controlled environment live longer (usually 4–7 years), compared to the situation under natural conditions. The latter allows farmed fish grow into larger size, i.e. they produce more eggs compared to the free living pieces. The unit weight of females used for induced spawning mostly ranges between 200–600 g. The number of spawn cycles completed within the life period of this fish would generally range between 2 and 4. Farmed fish can show certain morphological changes in progressive order (e.g. reduction of fin surfaces) as well as their behaviour (e.g. the loss of shyness, response to feeding).

These changes are due to the adaptation of their organism to new environment specific for certain unnatural aspects. However, the use of the technology of brood fish trapping usually does not result in genetic changes, i.e. transferable to the offspring.

Marking of fish is a very practical instrument for identification purposes, which can be applied to groups (e.g. the VIE system comprising implanting of elastomeric labels beneath the transparent top layer of skin) or individuals (microchips or the VIA systems with implanted visible pads bearing alphanumeric codes beneath the top layer of skin – Fig. 6). This marks can be applied, if the size of fish complies with requirements related to the specific marking methods (for detailed information refer to the following website: www.nmt.us), most conveniently during winter months, when the fish are not very sensitive to handling. The spawning period is not suitable for application of such markings and any such activity would increase the post-spawning mortality to a significant extent. The marking should include the use of anaesthetics. As far as the small fish are concerned, there are great loses of markings applied. For this reason, the marking process shall start with young breeding fish only. The chip systems show minimum occurrence of marking loss allowing for reliable identification of individuals.

Photo 6. *European grayling provided with VIA marking.*

The offspring from females kept under controlled conditions using the above mentioned method possess the prerequisites necessary for successful adaptability within trout river environment and reservoirs within such specific area. In ideal case, the fry shall be planted once fattened and reaching the size of 3–5 cm during the full vegetation season (summer) directly within fisheries. The methodology applied when rearing the fish until planting is similar to the one described in part about fry breeding. To ensure effective support to free living populations, it is not recommended to plant the fry kept under the controlled farming conditions for a longer period (1 year and longer). The adaptability of such fish and their survival in natural environment would be very poor.

HEALTH ISSUES IN FISH FARMING

The essential conditions required for successful rearing of the European grayling deal with the health condition. This status depends on the correct compliance with the zoo hygienic rules, provision of quality feed doses and efficient prevention against importing of infections into the stock. In spite of all that, every keeper needs to be prepared for a spontaneous outbreak of infection and able to implement adequate measures in time. Better reference has been drafted with regards to description of some most serious illnesses that the European grayling might suffer. Detailed descriptions, preventive measures and treatment of particular diseases can be found in publications by Kolářová and Svobodová (2009) and Kouřil et al. (2008).

VIRAL DISEASES

Salmonid fish can generally suffer from 4 types of viral disease rated dangerous in the Czech Republic, those are also included within the list in European legislation: **viral hemorrhagic septicaemia (VHS), infectious necrosis of pancreas (IPN), infectious haematopoietic necrosis (IHN) and the infectious salmon anaemia (ISA).** Out of the above, ISA has not been experienced at farms of salmonid fish in the Czech Republic as yet. The IPN strikes on young age categories of fish (up to 5 cm of length) and may occur at the grayling, together with VHS and IHN.

The initial symptoms of all above mentioned diseases are very similar – darkening of the body surface, exophtalmos (bulged eyeballs), dizziness, swimming disorder, loss of reflexes. Therefore any suspected occurrence of disease shall be consulted with professional veterinary experts. Accurate diagnosis of viral diseases of salmonid fish can be only achieved using laboratory procedures based on identification of the cause conducted by the National Reference Laboratory of SVS of Czech Republic for viral diseases of fish: VÚVeL Brno (the reference area of Moravia and Silesia) and the Reference of Laboratory of SVS of Czech Republic for viral diseases of fish: SVÚ Č. Budějovice (the reference area of Czech). Laboratory examinations shall be performed on ill fish showing symptoms (10–15 live pieces) and the water temperature should not exceed 10 °C during transport.

Treatment of viroses is not carried out.

The dangerous disease shall be removed in accordance with provisions of the Ordinance No. 299/2003 Coll. Any keeper, whose animals have started showing symptoms implying suspected occurrence of a dangerous disease, shall be responsible to ensure that the animals suspected of such disease and sensitive to relevant diseases shall not leave their position up until the arrival of a veterinary expert.

Prevention. The Act No. 286/2003 Coll. on veterinary prevention implies that salmonid fish keepers shall subject these and their eggs to examinations focused on potential occurrence of a dangerous disease: IHN, IPN, VHS and ISA. The relevant legislation rates the European grayling among species prone to the above mentioned diseases. Farms producing fry to be planted into open waters only shall be subject to viral examination of the ovarian fluid collected during spawning of specific brood fish species. The collection of samples shall be performed in cooperation with veterinary experts appointed to inspect the specific stock.

BACTERIAL DISEASES

All the three bacterial diseases mentioned below are rated among fish diseases within the List No. III. within the European legislation (the Ordinance No. 299/2003 –List III. and Act No. 381/2003 Coll. in the Czech Republic). In case of occurrence, the veterinary authorities will announce the nest of the relevant disease and together with the protection zone. Such disease shall be considered combated once the regular disinfecting and re-planting of the area with a sensitive species result in no evident or suspected re-occurrence of the disease. The monitoring period shall be determined by the national veterinary authorities.

The treatment of bacterial diseases is conducted via application of antibiotics (Kolářová and Svobodová, 2009). The selection and determination of the most suitable antibiotic can be simplified using the relevant guidance for bacteriological examination procedure and identification of the cause by means of bacteriological examination with attention to results of the antibiotics sensitivity test performed on the originator. Fish are tested by national veterinary authorities of Czech Republic and the veterinary and Pharmaceutical University in Brno. The examination required submission of live fish only!

Furulosis of salmonid fish (Furunculosis)

This disease is spread worldwide, often in the Czech Republic as well. All the salmonid fish species are prone to this disease, including the European grayling. The initiator of this disease is the Aeromonas salmonicida bacteria, abbreviated as salmonicida. This disease occurs mainly during higher water temperature (15–21 °C), it will be latent at temperatures below 7 °C. The course of such disease might be pre-acute without any specific symptoms (high fish mortality), whereas acute course results in nervous symptoms and prolapsus recti and the sub-acute and chronic stage of the disease will bring typical changes to the skin (abscess – furunculi).

Enteric Redmouth Disease – ERM

The initiator of this disease is the Yersinia ruckeri bacteria. The grayling fish is also sensitive to this infection. This disease occurs mainly when the water temperature is 13–15 °C during the first year of fish life. The affected fish is dark, dizzy, showing haemorrhage on fin bases, jaws, the palatum of fish mouth (giving the ground for the English name of the disease) and the opercula.

Bacterial Kidney Disease – BKD

The initiator of this disease is the Renibacterium salmoninarum bacteria. This disease affects all the salmonid fish species, including the grayling fish. This disease breaks out mainly once the water temperature has risen to 13–18 °C during spring time. The soft and alkali water impairs the treatment. The disease mainly affects fish in their second year of life. The affected fish would be dark and show nervous symptoms.

PARASITE DISEASES

The treatment of parasite occurrence is often performed using anti-parasite baths and application of anti-parasite agents added to the feed (Kolářová and Svobodová, 2009). These are differentiated as ectoparasite and endoparasite.

Ectoparasite diseases

The parasite initiator is located on the skin and gill, causing necrosis. The outbreak of infection will occur mainly within large gatherings of fish and the lack of light. Adult grayling kept in our environment often suffers from the infection caused by the *Chilodonella piscicola* (protozoon) – that can survive without a host for several days at the water temperature of 3–5 °C, while dying within one hour without a host at the temperature of 20 °C. Sunlight and light impairs its reproduction. Another parasite protozoon is the *Ichthyophthirius multifiliis* (fish skin parasite) – its evolution cycle lasts for 35 days with the water temperature of 10 °C, and 3–4 days at the temperature of 21–24 °C. If the evolved parasite released into the water out of the theront cyst fails to find a host within 2–4 hours, it will perish. Theronts die 2–3 x faster when exposed to light and they would also perish fast at the pH level of 5.5. The infestation is caused by affected fish or with water containing theronts. The skin might be also affected by parasites from members of the single fluke group (*Monogenea*), from the *Gyrodactylus* or *Dactylogyrus* family (gill worms).

Endoparasite diseases

Salmonid fish are, especially during the first year of their life, prone to the infection caused by the protozoon called *Hexamita salmonis* that lives in the rear part of intestine, the gall bladder and gall ducts. It does not adhere to organ surfaces, yet it will float within. This protozoon causes digestion disorders, the resultant emaciation and death. The intestine of salmonid fish may contain fluke worms (the *Trematoda* family), round worms (the *Nematoda* family), mostly represented by flatworms (the *Cestoda* family) and their most common representative, the *Proteocephalus neglectus* reaching the length of 100–150 mm. The intermediate host would be copepod. Mature flatworms adhere to the pyloric appendix mucosa and their body will protrude into the intestine luminal. The intra intestine mucosa is subject to mechanical damage resulting in inflammatory processes and necroses. These changes result in interference with the physiological function of intestines. The affected fish will lose weight and may even die. Massive infection can cause congestion of the intestine luminal.

5. COMPARISON OF "PROCEDURE NOVELTY"

The traditional and widespread method for obtaining brood European grayling concerns trapping of the freely living individuals in open waters during the pre-spawning period. However, the population of brood fish in open waters has been subject to dramatic reductions over the recent years. In order to achieve the required quantity of spawned eggs, electric power generators are being used for trapping of fish within progressively increasing areas of our trout rivers with the obviously negative impact on the level of their natural reproduction in water streams that actually represent the essential prerequisites for their survival and preservation of the genetic variability. The effectiveness of such trapping is usually subject to significant effect of high flow through of water during spring months. This process is often impossible due to the latter reasons. Another adverse factor is represented by the fact that the trapping of brood grayling using electric power generators during the pre-spawning period us associated with serious damage to the fish showing higher sensitivity in such period and that is reflected by their subsequent losses in high numbers. There are many cases with evident negative impact on the freely living populations due to the trapping of brood fish and their induced spawning. In extreme cases, this way of management results in total destruction of populations used for such purpose.

The production of fry obtained by implementing traditional procedures suffers from serious interim fluctuation and great dependency on seasonal conditions. Due to the instability of the traditional technology, the fry and fry customers will often source the fry material in other regions or even abroad. Planting of foreign fry, however, may infringe the stability of local populations.

Establishment of brood stock under controlled conditions will be the prerequisite for increase and stabilisation of European grayling fry at the level satisfactory to the need of entities farming on trout waters within the Czech Republic. Formation of the regional hatchery system using local populations will allow for abandonment of planting foreign populations sourced from other regions or abroad. The very keeping of brood fish and adherence to the above detailed principles will allow for compliance with the long-term sustainability and stability of production of quality fry showing the characteristics of naturally living populations to the maximum extent. There will be a simultaneous reduction in intensity of utilisation of brood fish obtained from open waters resulting in support to their essential natural reproduction.

6. DESCRIPTION OF TECHNOLOGY IMPLEMENTATION

This technology has been designed mainly for facilities dealing with reproduction of the European grayling to be planted in open waters (e.g. angling associations' hatcheries, smaller private facilities). The purpose of application of this technology in practice is to enhance and stabilise the production of quality fry of the European grayling from the original genetic predecessors, to be further planted within open waters, and the subsequent restrictions of its transfer between regions including foreign territories. Planting of fry with original genetic characteristics will provide for better effectiveness of aid to the freely living populations, preservation of intra species variability and prevention against genetic contamination due to planting of foreign fry.

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CHAPTER 6

GENERAL DISCUSSION ◆ ENGLISH SUMMARY ◆ CZECH SUMMARY ◆ ACKNOWLEDGEMENTS ◆ LIST OF PUBLICATIONS ◆ TRAINING AND SUPERVISION PLAN DURING STUDY ◆ CURRICULUM VITAE

6.1. GENERAL DISCUSSION

Brown trout (*Salmo trutta* m. *fario* L.) and grayling (*Thymallus thymallus* L.) belong to dominating and commercially most important species in trout waters of the Czech Republic. However, their populations are currently decreasing in Czech streams and this decrease is affected by several factors (Randak, 2006). To re-establish threatened or extinct populations and increase the recreational fishery, hatchery-reared fish are commonly stocked as part of enhancement and restoration programmes (Cowx, 1994).

Markedly distinct environments have indeed generated differences in many aspects of the behaviour of cultured and wild fishes, through selection of different inherited traits in the two environments and through differential experience during development. Such differences have implications for the appropriate management of aquaculture systems, for the impact of farm escapees on wild fish stocks and for the success of restocking programmes. To understand fully and to avoid the potential impacts of escapees on wild stocks, detailed information is needed on the circumstances in which farmed fishes out-compete, displace or impair the growth of wild conspecifics, in nature. It is important to assess whether and to what extent present knowledge of the developmental origin of behavioural deficits in cultured fishes can be combined with programmes of habitat improvement to make restocking programmes more effective (Huntingford, 2004).

Main goal of this thesis was to evaluate the overall performance of several age categories of brown trout and grayling, reared under different conditions after their stocking into natural river, which presents typical fishery areas in Czech Republic. The second goal was to evaluate the effects of stocking of artificially reared fish on wild population in river. Adjacent aims of my Ph.D. study were to propose measures enabling to enhance the production of good quality and adaptable stockfish of the target species for stocking in the open waters and to check the influencing factors on the wild population of salmonids.

ADAPTABILITY OF STOCKED ARTIFICIALLY REARED FISH IN THE BLANICE RIVER

Most studies, in which survival of stocked salmonids was evaluated, notified worse results of artificially reared fish than of wild resident fish of the same age (eg. Miller, 1953, 1958; Reimers, 1963; Vincent, 1960; Ersbak and Haase, 1983; Arias et al., 1995). It is consistent with results of the first chapter of presented study. Recapture rates and site ability of two-year old grayling reared in the pond (fed by natural prey only) and in the hatchery (fed by dry pellets only) stocked in addition to wild conspecifics into natural river were lower than recapture rates of wild fish in experimental (stocked) and control (unstocked) section of Blanice river. Thus, even considering the lower recapture rate, the results cannot be interpreted as a general failure of artificially reared grayling in running waters, as was suggested by Kaya (1990) based experiments with Arctic grayling (*Thymallus arcticus*, Pallas). The results of this study did not confirm the high rate of downstream migration of stocked grayling (reported by e.g. Magee and Byorth, 1994; Kaya and Jeanes, 1995; Carlstein and Eriksson, 1996; Thorfve and Carlstein, 1998), since the differences in downstream and upstream dispersal between wild and reared fish were not significant. Fish that remained within the range of the survey dispersed outside the release section to a similar extent, irrespective of their origin. On the other hand, the higher site fidelity of wild fish in the present study suggested that previous experience and knowledge of a given environment is of importance.

The different rearing technique (pond v. hatchery) did not influence recapture rates of stocked fish in this experiment. Some authors published contradictory results about influence of pre-stocking rearing technique on survival of stocked fish. Carlstein (1997) observed that European grayling reared in a natural pond showed higher post-stocking survival in a lake than fish reared in a conventional hatchery. The same results were published by Näslund (1992) in brown trout. On contrary, results published by Johnsen and Hesthagen (1990) indicate that the rearing method is not essential to the recapture rate of stocked brown trout.

In second part of this study, no differences were observed in the recapture rates and movement of adult hatchery reared brown trout in comparison with wild adult fish in stocked or control section. Better results of stocked fish in this experiment can be explain by their higher initial weight, which advanced these fish in competition with wild resident population in experimental section. This hypothesis is consistent with study of territorial competition among brown trout fry, Johnsson et al. (1999) which showed that a 30% size advantage in introduced fish can balance the advantage of ownership.

In both experiments presented in this study, growth rates of all groups of stocked fish were not significantly different in comparison with their wild conspecifics. This suggest that, as long as the stocked fish adapt to the conditions of the natural watercourse, they are able to make full use of the food available in the environment. Compensation of differences in condition factors values of artificially reared and wild grayling during experiment (chapter 1) is also the confirmation of this supposition. Published results about post-stocking growth rate of artificially reared fish are inconsistent. The result published by Weiss and Schmutz (1999b) indicated that abiotic factors in particular biotopes substantialy affected growth of stocked fish. The prey sources are evidently sufficient for increased density of fish in the Blanice river after stocking. Food competition was not a limiting factor for survival of stocked fish in presented study. Even, stocked artificially reared fish could learn to feed on novel prey by seeing a trained wild conspecific 'demonstrator' feeding on such prey as suggested by Brown and Laland (2001). The possible short-time weight loses which were observed by Thorfve (2002) could not be reviewed through the time duration of experiments in this study.

EFFECT OF STOCKING ON WILD POPULATION

In the presented study no significant effect of doubling wild population of two-year old grayling was observed. The marked wild fish in control sections showed the same values in all monitored parameters as their conspecifics in the experimental sections (with stocking of one group of artificially reared fish). These results correspond with several early studies (e.g. Bohlin et al., 2002; Baer and Brinker, 2008), which demonstrated no effect of stocking of artificially resred fish on wild resident population. Nevertheless, modestly lower recapture rate of wild grayling in experimental than in control section may suggested some negative effect of stocked fish on resident wild population.

Significantly more juvenile wild brown trout marked in experimental sections was recaptured out of this section in comparison with the same age category in control sections. Similarly, Nickelson et al. (1986) found out that stocking of hatchery reared fish may contribute to the displacement of wild fish. Growth of marked categories of wild fish was not significantly different between experimental and control sections. These results point at rather spatial than food competition between stocked and wild fish in Blanice river as well as cannot be generalized, because also biotic and abiotic factors substantialy affected interaction between resident and stocked fish in particular biotopes (Weiss and Schmutz, 1999a).

PRODUCTION OF GOOD QUALITY AND ADAPTABLE STOCKFISH

The aim of presented technologies is to provide fish farming practice with information about procedures and management in farming of brood European grayling (*Thymallus thymallus* L.) and brown trout (*Salmo trutta* m. *fario* L.) under controlled conditions. In the past, the only method to obtain the eggs was to capture the ripe, wild grayling females and males at their spawning grounds, using the electrofishing (Luczynski et al., 1986). This practise may lead to degradation of mature grayling population in natural rivers and to efficiency reduction of natural spawning, which is irreplaceable for conservation of grayling population (Harsanyi et Aschenbrenner, 2002). The same methods were used for brown trout stock production. Proposed methods of rearing can facilitate stockfish production of the target species for restocking the open waters without negative effect on resident populations and their natural spawning. The modern practice recommended by Kašpar et al. (2008) is applied for maximum preservation of genetic variability of offspring gained through induced spawning of brood fish.

CONCLUSIONS

Results of present study suggested that stocking of artificially reared salmonids can be used to strengthen weakening populations of the species in natural waters, although it is necessary to known local conditions and parameters of resident population. Hovewer, it is necessary to notice that stocked fish needn't shew the same performance as resident fish. Therefore the natural spawning facilitation should be practised and supported in most of localities.

By applying present technologies in practice is possible to improve and stabilise the production of quality genuine European grayling and brown trout fry as well as planting in open waters with subsequent restriction of fry transport between regions including purchases from abroad. Simultaneously, it will be possible to reduce exploitation of wild mature fish for stockfish production.

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6.2. ENGLISH SUMMARY

Brown trout (*Salmo trutta* m. *fario* L.) and grayling (*Thymallus thymallus* L.) belong to dominating and commercially most important species in trout waters of the Czech Republic. At present, we can observe decrease of these species populations in our waters. This decrease is influenced by many factors. The most serious ones are for example unfavourable changes of rivers, predation by otter, cormorant etc. anthropogenic pollution, overfishing and more effective fishing techniques. One of the easy ways to support growth of such populations is stocking of artificially reared stocks. Nowadays, fishery subjects try to supplement wild populations of these species by artificial stocks more intensively. The effect of these procedures is mostly very low. For improvement of these stocking programs it is necessary to evaluate the adaptability of stocked fish reared in different ways and stocked at different age stages. It is also important to verify if stocked fish do not influence wild populations in the rivers where we put our fish in a negative way.

CHAPTER 2 – POST-RELEASE GROWTH AND DISPERSAL OF POND AND HATCHERY-REARED EUROPEAN GRAYLING *THYMALLUS THYMALLUS* COMPARED WITH THEIR WILD CONSPECIFICS IN A SMALL STREAM

In this work we studied adaptability of two-year old grayling reared from the fingerlings stage in the pond (fed by natural prey only) and in the hatchery (fed by dry pellets only) after their stocking into natural river. The adaptability was evaluated based on recapture, migration and growth of individual marked fish after 168 days post stocking. These parameters were compared with the same marked wild resident grayling of the same size (age) group, which inhabit experimental sections. The population of two-year old wild grayling in these sections was doubled by stocking of one group from reared fish. Recapture rates and site ability in the stocking section were higher in wild grayling than in reared groups of fish. Specific growth rate (SGR) and migration were not different among groups of fish. The influence of rearing techniques (pond vs. hatchery) on recapture and growth of stocked artificially reared fish was not observed. Initially lower condition factors of reared grayling were equal to wild fish after recapture, suggesting adaptation of artificially reared fish that remained in the sections studied. No differences in assessed parameters were seen between wild resident graylings in the experimental (stocked) sections and control sections (without stocking). The results of our experiment suggest that the amount of food is not a limiting factor for survival of stocked fish. Shelter capacity of river as well as “prior residence effect“ of wild fish can be a limiting factor for successful adaptation of stocked artificially reared grayling.

CHAPTER 3 – RECAPTURE RATE AND GROWTH OF HATCHERY-REARED BROWN TROUT (*SALMO TRUTTA* V. *FARIO*, L.) IN BLANICE RIVER AND THE EFFECT OF STOCKING ON WILD BROWN TROUT AND GRAYLING (*THYMALLUS THYMALLUS*, L.)

In this study we examined adaptability of adult brown trout reared from the fingerlings stage in the hatchery (fed by dry pellets only) after their stocking into natural river. The adaptability was evaluated based on recapture, migration and growth of individual marked fish after 168 days post stocking and their influence on population of different age (size) categories of wild brown trout and grayling inhabiting experimental sections.

The effect of stocking on wild population was assessed based on comparison of recapture, migration and growth of individual marked fish in experimental sections with fish marked in the same way in control sections. All observed parameters of stocked adult brown trout were comparable with adult wild fish in experimental and control sections. Recapture rate of other age categories of brown trout (large and small juvenile), and of both categories of grayling (juvenile and adult) was a little lower in experimental sections that in control sections, but these differences were not statistically important. Significantly higher portion of juvenile wild brown trout marked in experimental sections was recaptured out of this section in comparison with the same age category in control sections. Growth of marked categories of wild fish was not significantly different between experimental and control sections. Acquired results, surprisingly showed good ability of adult hatchery reared brown trout to succeed in life in natural environment and also in competition with wild fish. The results point at a fact that stocking of adult hatchery reared fish, coming from different regions, may influence genetic variability of resident population in case of addition in natural spawning. Higher migration of large juvenile brown trout from experimental sections and non-influencing of growth in all categories of wild fish point at a fact, just like the previous study, of rather spatial than food competition between stocked and wild fish in Blanice river.

CHAPTER 4 – TECHNOLOGY OF BROWN TROUT CULTURE IN CONTROL CONDITIONS FOR RUNNING WATERS STOCKS PRODUCTION

The aim of the technology is to give to fishery practice information about breeding management of local brown trout (*Salmo trutta* m. *fario* L.) populations in control conditions. The technology includes methods of broodstocks founding, technologies of rearing of fry, yearlings, pre-adult fish and broodfish, methods of artificial reproduction of cultured broodfish and veterinary aspects of culture. Production of high-quality brown trout stocks with original genetic traits for restocking of running waters could be increased and stabilised using the technology in fishery practice. The suggested methods of culture and management are sustainable and suitable for production of quality stocks.

CHAPTER 5 – TECHNOLOGY OF EUROPEAN GRAYLING BROODFISH CULTURE IN CONTROL CONDITIONS FOR SUSTAINABLE RUNNING WATERS STOCKS PRODUCTION

The aim of the technology is to give to fishery practice information about breeding management of local European grayling (*Thymallus thymallus* L.) populations in control conditions. The technology includes methods of broodstocks founding, technologies of rearing of fry, yearlings, pre-adult fish and broodfish, methods of artificial reproduction of cultured broodfish and veterinary aspects of culture. Production of high-quality grayling stocks with original genetic traits for restocking of running waters could be increased and stabilised using the technology in fishery practice. The suggested methods of culture and management are sustainable and suitable for production of quality stocks.

6.3. CZECH SUMMARY – SOUHRN

Pstruh obecný (*Salmo trutta* m. *fario* L.) a lipan podhorní (*Thymallus thymallus* L.) patří k dominantním a hospodářsky nejvýznamnějším druhům tzv. pstruhových, nyní lososových, vod v ČR. V současnosti však dochází v našich tocích k poklesu jejich stavů. Tento pokles je ovlivněn řadou faktorů. Mezi nejvýznamnější patří nešetrné úpravy toků, působení predátorů, antropogenní znečištění, zvyšující se rybářský tlak a zvyšování účinnosti lovných způsobů atp. Jednou z relativně jednodušších možností, jak podpořit volně žijící populace těchto druhů ryb, je i vysazování uměle odchovaných násad. V současnosti je ze strany rybářských subjektů vidět snaha o intenzivnější doplňování volně žijících populací pomocí vysazování takto odchovaných ryb. Efekt těchto opatření je však často mizivý. Pro zlepšení výsledků těchto vysazovacích programů je třeba ověřit adaptabilitu vysazených ryb odchovaných různými způsoby a vysazených v různém věku. Důležité je také ověřit, zda vysazené ryby negativním způsobem neovlivní divoké volně žijící populace v tocích, do kterých jsou vysazeny.

KAPITOLA 2 – RŮST A MIGRACE LIPANA POHORNÍHO (*THYMALLUS THYMALLUS*) ODCHOVANÉHO V RYBNÍCE A SÁDCE PO VYSAZENÍ DO MENŠÍ ŘEKY V POROVNÁNÍ S DIVOKÝMI LIPANY STEJNÉ KATEGORIE

V této práci byla posuzována adaptabilita dvouleté násady lipana podhorního odchovaného od stádia plůdku v rybníce (krmený pouze přirozenou potravou) a v sádce (krmený výhradně suchým peletovaným krmivem) na podmínky přírodního toku. Adaptabilita byla zkoumána na základě návratnosti, migrace a růstu individuálně označených ryb po 168 dnech od vysazení. Tyto parametry byly porovnávány se stejně označenými divokými rezidentními lipany stejné velikostní (věkové) kategorie vyskytujícími se v úsecích vysazení, jejichž obsádky byly zdvojnásobeny vysazením jedné ze skupin uměle odchovaných ryb. Míra návratnosti a setrvání v úseku vysazení byly vyšší u divokých lipanů než u uměle odchováných ryb obou skupin. Specifická rychlost růstu a migrace se mezi jednotlivými skupinami ryb nelišila. Vliv podmínek odchovu (rybník v. sádka) na návratnost a růst vysazených uměle odchovaných ryb nebyl pozorován. Vyrovnání hodnot koeficientu kondice všech skupin ryb v průběhu experimentu naznačuje dobrou adaptaci znovuodlovených uměle odchovaných ryb na přírodní podmínky v případě jejich dlouhodobého přežití v toku. Nebyly zjištěny rozdíly v žádném ze sledovaných parametrů mezi divokými lipany v úsecích, do kterých byly vysazeny uměle odchované ryby, a divokými rybami stejné kategorie v kontrolních úsecích bez vysazení. Výsledky experimentu naznačily, že limitujícím faktorem pro přežití vysazených není množství potravy, ale může jím být prostorová (úkrytová) kapacita toku ve spojení s efektem dřívějšího osídlení u divokých rezidentních ryb.

KAPITOLA 3 – NÁVRATNOST A RŮST UMĚLE ODCHOVANÉHO PSTRUHA OBECNÉHO (*SALMO TRUTTA* M. *FARIO*, L.) V ŘECE BLANICI A VLIV VYSAZENÍ NA POPULACE DIVOKÉHO PSTRUHA OBECNÉHO A LIPANA PODHORNÍHO (*THYMALLUS THYMALLUS*)

V této práci byla posuzována adaptabilita dospělých pstruhů potočních, chovaných od stádia plůdku intenzivním způsobem v sádce (krmených výhradně suchým peletovaným krmivem), na podmínky přírodního toku a jejich vliv na populace různých věkových kategorií divokých pstruhů obecných a lipanů podhorních, kteří se vyskytují v experimentálních úsecích.

Adaptabilita byla zkoumána na základě návratnosti, migrace a růstu individuálně označených ryb po 168 dnech od vysazení. Vliv na původní populace byl posuzován na základě porovnání návratnosti, růstu a migrace označených divokých ryb v úsecích, kam byli vysazeni uměle odchovaní pstruzi se stejně značenými rybami v kontrolních úsecích. Všechny sledované ukazatele byly u vysazených ryb srovnatelné s dospělými divokými pstruhy obecnými v experimentálních i kontrolních úsecích. Návratnost ostatních věkových kategorií pstruhů obecných (velkých a malých juvenilních) i obou kategorií lipana (juvenilních a adultních) byla mírně nižší v experimentálních než v kontrolních úsecích, ale rozdíly nebyly statisticky průkazné. Signifikantně větší podíl juvenilních divokých pstruhů obecných experimentálních úseků byl odloven mimo úsek původního výskytu ve srovnání se stejnou kategorií v kontrolních úsecích. Růst žádné z kategorií označených divokých ryb se mezi oběma typy úseků významně nelišil. Získané výsledky překvapivě dokazují dobrou schopnost dospělých uměle odchovaných pstruhů obecných obstát v přírodních podmínkách i v konkurenci s divokými rybami. Zároveň upozorňují na možnost ovlivnění genetické struktury rezidentních populací pstruha obecného v případě vysazení dospělých ryb pocházejících z jiných regionů. Zvýšená migrace větších juvenilních pstruhů z experimentálních úseků a neovlivnění růstu všech kategorií divokých ryb poukazuje, stejně jako předchozí experiment, na spíše prostorovou než potravní konkurenci mezi vysazenými a divokými rybami v řece Blanici.

KAPITOLA 4 – TECHNOLOGIE CHOVU PSTRUHA OBECNÉHO V KONTROLOVANÝCH PODMÍNKÁCH ZA ÚČELEM PRODUKCE NÁSADOVÉHO MATERIÁLU PRO ZARYBŇOVÁNÍ VOLNÝCH VOD

Cílem technologie je poskytnout rybářské praxi informace o chovatelských postupech a managementu chovu místních populací pstruha obecného (*Salmo trutta* m. *fario* L.) v kontrolovaných podmínkách. Hlavní pozornost je věnována popisu technologických postupů zakládání generačních hejn, odchovu jednotlivých kategorií, umělé reprodukci odchovaných generačních ryb, inkubaci jiker a zdravotní problematice. Účelem zavedení technologie v praxi má být zvýšení a stabilizace produkce kvalitních geneticky původních násad pstruha obecného pro zarybnění volných vod. Navržené postupy splňují parametry dlouhodobé udržitelnosti a stability produkce kvalitních násad.

KAPITOLA 5 – TECHNOLOGIE CHOVU GENERAČNÍCH LIPANŮ PODHORNÍCH ZA ÚČELEM UDRŽITELNÉ PRODUKCE KVALITNÍHO NÁSADOVÉHO MATERIÁLU PRO ZARYBŇOVÁNÍ VOLNÝCH VOD

Cílem technologie je poskytnout rybářské praxi informace o chovatelských postupech a managementu chovu místních populací lipana podhorního (*Thymallus thymallus* L.) v kontrolovaných podmínkách. Hlavní pozornost je věnována popisu technologických postupů zakládání generačních hejn, odchovu jednotlivých kategorií, umělé reprodukci odchovaných generačních ryb, inkubaci jiker a zdravotní problematice. Účelem zavedení technologie v praxi má být zvýšení a stabilizace produkce kvalitních geneticky původních násad lipana podhorního pro zarybnění volných vod. Navržené postupy splňují parametry dlouhodobé udržitelnosti a stability produkce kvalitních násad.

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6.5 LIST OF PUBLICATIONS

2010

PEER-REVIEWED PAPERS AND THEIR IF

Turek, J., Randák, T., Horký, P., Žlábek, V., Velíšek, J., Slavík, O., Hanák, R. 2010. Post-release growth and dispersal of pond and hatchery-reared European grayling *Thymallus thymallus* compared with their wild conspecifics in a small stream. Journal of Fish Biology 76, 684–693.

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2009

PEER-REVIEWED PAPERS AND THEIR IF

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2008

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2006

OTHERS

Turek, J., 2006. Semiindustrial intensive rearing of Eurasian perch (*Perca fluviatilis* L.) in a recirculation system (in Czech). Dipl-Ing. thesis, University of South Bohemia, Faculty of Agriculture, České Budějovice, 67 pp.

6.6. TRAINING AND SUPERVISION PLAN DURING STUDY

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Aplikovaná hydrobiologie (Applied hydrobiology)	2007
Ichtyologie a systematika ryb (Ichthyology and systematics of fish)	2009
Anglický jazyk (English language)	2009
Ekologie ryb (Ecology of fish)	2010

Other courses	**Year**

Scientific seminars	**Year**
	2006
	2007
	2008
	2009

International conferences	**Year**
1st International Workshop of Aquatic Toxicology and Biomonitoring, Vodňany, Czech Republic	2008
Aquaculture Europe 08, Krakow, Poland	2008

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