CHAPTER 5

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.

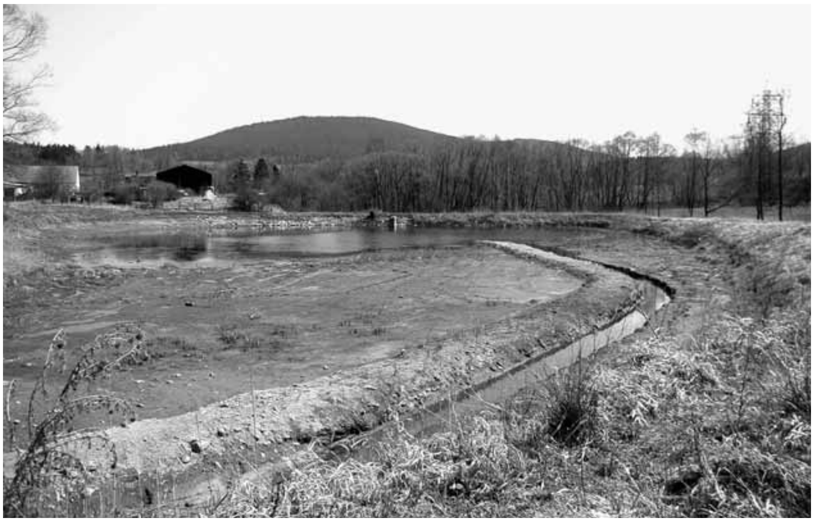

Photo 2. *Flow through pond suitable for keeping the European grayling.*

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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